

**IDENTIFICATION OF SALMONID FISHES
FROM TRIBUTARY STREAMS AND LAKES
OF THE MID-COLUMBIA BASIN**

FINAL REPORT - 1998

BY

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EXECUTIVE SUMMARY

This study was initiated to obtain baseline information regarding the status of native salmonid fishes in tributary streams and rivers of the Mid Columbia River basin (MCRB). The study focused on westslope cutthroat trout (*Oncorhynchus clarki lewisi*), interior redband rainbow trout (*O. mykiss gairdneri*), and bull trout (*Salvelinus confluentus*). Specimens were collected from the Methow subbasin in 1992 and the Entiat and Wenatchee subbasins in 1993, frozen on dry ice, and sent to Colorado State University for taxonomic analysis. The survey included 142 collection sites in the MCRB: 80 in the Methow subbasin, 18 in the Entiat subbasin, and 44 in the Wenatchee subbasin. A similar taxonomic study of westslope cutthroat trout and interior redband trout from the Wenatchee and Yakima river subbasins was conducted in 1998 and is included in Appendix D.

The primary conclusion from the baseline information collected in this study is that populations of native westslope cutthroat, interior redband rainbow trout, and bull trout do remain in the Mid-Columbia basin (Appendix G, Table 1). Westslope cutthroat and bull trout were generally found in small, isolated populations in headwater reaches above some kind of physical barrier. While populations of interior redband rainbow were also found in the MCRB, extensive stocking of both rainbow trout and steelhead confounds identification with absolute certainty.

Evaluation included classical taxonomic methods (Behnke 1992), mitochondrial DNA analysis (Shiozawa and Evans 1994), and osteological analysis for bull trout (Cavender 1980). All specimens were analyzed for classical taxonomic characters, and a taxonomic diagnosis was made based on the results. A population was rated "pure" if all diagnostic criteria were in the expected range for the subspecies. A rating of "essentially pure" denotes a population considered to be representative of the subspecies, but one or more of the criteria were slightly outside the expected range. It is difficult to assess, in some cases, whether this is natural variability or due to multiple sources of the population.

Mitochondrial DNA analysis was performed on all bull trout, and most of the brook trout specimens, to evaluate the extent of hybridization between these taxa in the samples collected. Mitochondrial DNA analysis was also completed on populations judged morphologically "pure" or "essentially pure" westslope cutthroat and interior redband rainbow trout. In addition,

several "hybrid" populations, and difficult specimens were also evaluated by mtDNA analysis. For evaluation of rainbow and cutthroat trout, restriction fragments of the cytochrome b and ND-1 genes proved to be most diagnostic and consistent. The cytochrome b was digested with HinfI, MboI, and RsaI ; and the ND-1 with MboI, DdeI, and MspI. All bull trout and brook trout were also analyzed for polymorphisms produced by RFLP patterns of the cytochrome b and ND-1 genes. Restriction enzymes used in bull trout evaluation were: AluI, DdeI, HinfI, RsaI, and Sau3AI (isoschisomer with MboI) for the cytochrome b region, and AluI, RsaI, and MspI for the ND-1 region. In some specimens of bull trout, ND-2 was also amplified and cut with HaeIII, HinfI, MspI and RsaI.

The impacts of historic stocking of salmonids in the MCRB are extensive. Mainstem rivers and larger tributaries are dominated by trout that were stocked or show effects of hybridization with hatchery fish. The general pattern of altitudinal distribution observed in several streams in the MCRB was rainbow trout in the lower elevation, low gradient reaches, followed by a hybrid zone and finally cutthroat and bull trout in the steeper and colder headwater reaches. In our sampling, however, the hybrid zone tended to be generally longer than described by Mullan et al. (1992), and often extended to the uppermost sampling locations. It is assumed that natural barriers to hybridization have been affected by stocking.

Tributary streams of the MCRB are unique, when compared to most of western North America, in that there are presumed naturally occurring hybrid zones between two taxa of indigenous trout (westslope cutthroat and interior redband rainbow trout). Therefore, the presence of hybrids in a population must be viewed in the proper perspective. Hybridization is generally considered to be "genetic contamination" where it is the result of stocking of non-native trout. In the MCRB, however, this may be part of a natural evolutionary process. While we were able to detect hybridization at many collection sites, distinguishing between natural occurring and anthropogenic hybrid zones proved to be problematic. This remains an important consideration with regard to populations of salmonid fishes in the MCRB. Given the current capabilities of various molecular methods, this question would best be addressed by protein electrophoresis, which can evaluate multiple loci expressed from the nuclear genome, and for which there is a substantial amount of information already in place from the taxa of concern.

There was strong concordance between classical taxonomic methods and mtDNA analysis. While mtDNA is an effective tool for questions of taxonomic concern, it is important to recognize some constraints (Hillis and Moritz 1990). Mitochondrial DNA is located within the cytoplasm of the cell, and is maternally inherited. It is haploid and does not undergo recombination every generation, as is the case with nuclear DNA. Populations in which hybridization has occurred will generally have multiple mtDNA haplotypes, while "pure" populations have only one haplotype. Due to the maternal inheritance of mtDNA, results must be interpreted with proper caution.

Populations identified as "pure" westslope cutthroat by classical methods all had only one mtDNA haplotype indicative of *lewisi* for the restriction enzymes used in the diagnosis. Several populations identified as introgressed by meristic analysis had two mtDNA haplotypes within the population, containing both cutthroat and rainbow diagnostic RFLP patterns. Introgression of this type is usually more recent (within the last 50 years), as closed populations tend to have a common mitochondrial haplotype.

The molecular methods employed in this study were designed to address taxonomic concerns at the species and subspecies level. We found specific diagnostic markers to be very useful for this purpose, but not sufficient to provide insight to population level questions. For population genetic considerations, more restriction enzymes would be required to show variation among and between populations of interest. Further investigations into the genetic structure of populations surveyed in this study could also incorporate allozyme information which is very effective in identifying subtle genetic differences between and among populations.

While it is difficult to ascertain the source of some "pure" populations, their persistence in the MCRB is important. All three species of interest in this study are thought to be in decline throughout their ranges. Considering the paucity of large populations of westslope cutthroat, interior redband rainbow trout, and bull trout in the MCRB, special attention should be given those identified as "pure" in this baseline study.

Westslope Cutthroat Trout

"Pure" or "essentially pure" westslope cutthroat trout were found in 21 collection sites in the MCRB, including 9 in the Methow subbasin, 3 in the Entiat subbasin (two of these were in the same

stream: Tommy Creek), and 10 in the Wenatchee subbasin. These populations are listed in Appendix G. In this survey of the MCRB, two general phenotypic patterns emerged. Many of the populations identified as "pure" westslope cutthroat represent the "ideal" *lewisi* phenotype: classic westslope spotting and coloration; 170-180 scales in the lateral series; 18-19 gill rakers on the first gill arch; 30-40 pyloric caeca. A second phenotypic pattern (variable spotting, several spots on the heads, higher lateral series scale counts of 190-200+; and higher gill raker numbers: 19-20) was also observed in specimens from a number of locations in the MCRB. This second pattern is presumed to represent localized variability characteristic of the MCRB. All of these populations had the same mtDNA haplotypes in the cytochrome b and ND-1 genes (for the restriction enzymes used in diagnosis), which would indicate that the restriction sites used to distinguish westslope cutthroat from rainbow trout and Yellowstone cutthroat are relatively old (> 70,000 ybp) and do not reflect differentiation within *lewisi*.

Interior Redband Rainbow Trout

"Pure" or "essentially pure" interior redband rainbow trout were found in 4 collection sites in the MCRB, including 3 in the Methow subbasin and 1 in the Entiat River subbasin (Appendix G). Identification of native redband rainbow trout was confounded by the extensive stocking history in the MCRB and the lack of distinct mitochondrial DNA markers available at this time. The most reliable characters for the specimens collected in this study were a combination of meristic elements (scales, pyloric caeca), spotting patterns, and presence of primitive parr marks. Mitochondrial DNA restriction patterns were able to clearly identify rainbow trout, but confident separation of coastal and redband rainbow, based on DNA alone, awaits further research. Additional efforts with regard to interior redband rainbow populations in the MCRB should include information from protein electrophoresis.

Bull trout

Bull trout were collected in 15 locations in the MCRB, including 11 in the Methow subbasin, 2 in the Entiat subbasin and 2 in the Wenatchee subbasin. In the Wenatchee system several known populations were not sampled. In addition to documenting populations of bull trout in the Mid-Columbia basin to provide a current reference as to their distribution in this region, we attempted to evaluate to what extent, if any, hybridization with brook trout is occurring in the MCRB and address the lingering

taxonomic uncertainty (particularly in Washington) concerning bull trout and Dolly Varden.

Diagnostic haplotypes that distinguish bull trout and brook trout selected from restriction digests of the Cytocrome b and ND-1 genes include the following enzymes: AluI, DdeI and SAu3AI. All populations of bull trout and most populations of brook trout were assessed for diagnostic haplotypes that differ between species. Two hybrids were diagnosed by morphological characters and both specimens had brook trout mtDNA haplotypes. These hybrids were collected from Beaver Creek in the Methow River subbasin, and from Chickamin Creek in the Wenatchee River subbasin. We found bull trout in the Methow subbasin to be similar to bull trout throughout the Columbia basin, with some exceptions. A rare haplotype, previously found in single specimens from two populations in the Columbia basin (Gold Creek and Clark Fork (Williams et al. 1996)) was observed in specimens from Goat Creek and the Twisp River, indicating this haplotype is more broadly distributed than previously thought.

When this study was initiated, there was some question, particularly in the State of Washington, as to the taxonomic status of bull trout. In particular, distinction between bull trout and Dolly Varden (*Salvelinus malma*) was assumed to be problematic. There should be no doubt as to the taxonomic identity of bull trout in the MCRB. In this study, three independent lines of taxonomic information: osteological analysis, morphological and meristic evaluation, as well as molecular genetic data generated from mitochondrial DNA all point to the expected conclusion that the MCRB bull trout are clearly distinct from Dolly Varden.

The present survey included specimens from 142 sites in the Methow, Entiat, and Wenatchee river subbasins. Information on interior redband rainbow trout from several locations in the Okanagan subbasin is also included in Appendix B. The Lake Chelan subbasin was not sampled, and may contain populations of bull trout, and westslope cutthroat trout (interior redband rainbow were not native above Chelan Falls). This project was intended to provide a broad overview of the distribution of native trout in the MCRB, and should serve as a starting point for continued monitoring and investigation.

IDENTIFICATION OF SALMONID FISHES FROM TRIBUTARY STREAMS AND LAKES OF THE MID-COLUMBIA BASIN

BACKGROUND

This project was initiated to obtain baseline data for inventory of species diversity of salmonid fishes of the Mid-Columbia River Basin (MCRB), and to provide technical assistance in taxonomic identification. The primary focus is on three species of resident salmonids native to the Methow, Entiat, and Wenatchee river subbasins that have often been "overlooked" due to extensive agency efforts focusing on anadromous salmon and steelhead. Of concern is validation of the extensive stream survey and inventory of the Mid-Columbia River tributaries and streams (Mullan et al. 1992), examination of additional locations for the occurrence of native salmonids, and the establishment of baseline taxonomic data.

The Mid Columbia River subbasin management area is bounded to the west by the North Cascade Mountains and to the east by the Columbia Plateau. This portion of the Columbia basin refers to the mainstem and its tributaries between the Grand Coulee Dam, near the Canadian border, and the confluence of the Columbia and Snake Rivers. The project included sites in the northern part of the Columbia basin: the Methow, Entiat, and Wenatchee subbasins.

The upper tributaries and streams of the Mid-Columbia basin historically supported five species of non-anadromous or resident salmonids: bull trout (*Salvelinus confluentus*), westslope cutthroat trout (*Oncorhynchus clarki lewisi*), interior redband rainbow trout (*O. mykiss gairdneri*), mountain whitefish (*Prosopium williamsoni*), and possibly pygmy whitefish (*Prosopium coulteri*). In general, these species partition the aquatic habitat along an altitudinal gradient, with bull trout and cutthroat trout in the uppermost reaches and resident interior redband rainbow trout in the lower gradient reaches. Mountain whitefish are relatively ubiquitous in all but the higher gradient headwater reaches representing the most abundant salmonid in terms of biomass for an individual species (Mullan et al. 1992). Pygmy whitefish have only two records of occurrence in Washington (Diamond Lake-near Spokane and Chester Morris Lake-near Seattle, but could possibly be in deep lakes such as Lake Wenatchee or Lake Chelan in the MCRB) (Scott and Crossman 1973). For the purpose

of this study, whitefish (Coregoninae) will not be considered further.

Anadromous salmonids in the Mid-Columbia basin lakes, streams, and rivers include chinook salmon (*O. tshawytscha*), steelhead (*O. mykiss*) and sockeye salmon (*O. nerka*). Historical natural populations of coho salmon (*O. kisutch*) in the Mid-Columbia had been effectively extirpated by 1943 (Mullan et al. 1992). The primary focus of the present study is non-anadromous (resident), native salmonines; therefore, consideration of chinook, sockeye, and coho salmon will be limited. Steelhead are included in this research project in terms of their relationship to resident interior redband rainbow trout, as there is a body of circumstantial evidence that supports the hypothesis that anadromous runs of steelhead may be generated from "resident" populations of redband trout (Mullan et al. 1992).

Stocking of various non-native trout in the MCRB was rather extensive in the early part of this century (Ken Williams, Washington Dept. of Wildlife, personal communication). In the early 1900's, streams and lakes were stocked by "pioneers" and several different early resource agencies, leaving little or no records. Naturally occurring trout in accessible reaches of these watersheds were commonly believed to be depleted by the 1930's. In response to a perceived recreational need, extensive stocking programs were initiated. Rainbow trout of various origins have been released in the MCRB since the early 1930s (Chapman et al. 1994). Although stocking of resident trout in streams is not currently common in the State of Washington, except in a few select areas, stocking of mountain lakes continues as a major program. There is little doubt that most of the lakes and streams in these subbasins have been impacted to some extent by stocking. In some cases "pure" native fish were planted. Thus, the populations identified as "pure" in this study may be the result of historic stocking activity in originally fishless waters or may be relict indigenous populations.

Bull Trout

Bull trout, recognized as a "good" species separate from Dolly Varden since 1978, has a broad disjunct distribution throughout interior western North America (Cavender 1978, 1980; Bond 1992). Its distribution in the MCRB is mostly limited to smaller, colder headwater streams, though larger migratory bull trout inhabit large rivers and lakes during most of the year.

Several authors have discussed the different life history strategies for bull trout, dividing them into four types (e.g. Leary et al. 1983, Brown 1992, Rieman and McIntyre 1993). Three of these types were present historically in the MCRB. The adfluvial form is found throughout the range of *S. confluentus*. It resides in lakes and migrates to tributaries where juveniles remain for one to three years before returning to the lake environment (Fraley and Shepard 1989). Resident bull trout remain in a stream for their entire life, while fluvial bull trout make extensive spawning migrations, moving between mainstem rivers and smaller tributaries. The fluvial and adfluvial forms attain a much larger sizes (up to 10 kg) than the resident stream form. Although not found in the MCRB, occasional anadromous bull trout have been reported in other areas of the species range (Ted Cavender, Ohio State University, personal communication; Gordon Haas, University of British Columbia, personal communication). Very little is known about the genetic basis for these life history differences, or how these strategies relate to the genetic structure of local populations or metapopulations. Rieman and McIntyre (1993) provided valuable information on the role of the metapopulations for the conservation of bull trout.

Bull trout have been the object of considerable attention in recent years. Once thought to be a serious predator to important sport and commercial species, it was often the object of extensive overharvest and exploitation. When the present survey was initiated, bull trout was designated a Category 2 species by the US Fish and Wildlife Service (in need of review for the purposes of the Endangered Species Act (ESA)). The bull trout is included on the list of species of special concern by the American Fishery Society (Williams et al. 1990).

In October, 1992, a petition requesting the listing of bull trout as endangered throughout its range (including Washington, Oregon, California, Idaho, Nevada, and Montana) was submitted by three conservation organizations in Montana (Swan View Coalition, Friends of the Wild Swan, and the Alliance for the Wild Rockies, Inc.). In addition, based upon documented declines in the state of Oregon, the Oregon chapter of the American Fisheries Society, under the auspices of the Natural Production Committee (NPC) also petitioned the US Fish and Wildlife Service to list bull trout in the Klamath River Basin in Oregon. In a June 1994 finding, the US Fish and Wildlife Service rendered a decision which found bull trout warranted for listing under the Endangered Species Act but precluded the listing due to the need to address higher priority species. Klamath River and Columbia River bull trout distinct

populations were listed as threatened under the Endangered Species Act on June 10, 1998.

Accordingly, two of the primary goals of the present study were to document the distribution of bull trout in the Mid-Columbia basin and to establish a base-line reference for future considerations regarding both its status and future management decisions. Objectives of this study with respect to bull trout were to: 1) locate and identify populations of bull trout in the Mid-Columbia basin to provide a current reference as to their distribution in this region; 2) evaluate to what extent, if any, hybridization with brook trout is occurring in the MCRB. and 3) address the lingering taxonomic uncertainty (particularly in Washington) concerning bull trout and Dolly Varden. Determination of the extent of overlap in distribution of brook trout and bull trout and extent of hybridization between these species in the Mid-Columbia basin is important, as brook trout often replace bull trout where they come into contact (Leary et al. 1983; Markle 1992).

Westslope Cutthroat Trout

The westslope cutthroat trout is native to South Saskatchewan, and the upper and middle Columbia and upper Missouri basins. It is assumed from karyological evidence (Thorgaard 1983) that westslope cutthroat ($2N=66$) are intermediate between the primitive coastal cutthroat trout (*O. c. clarki*) ($2N=68$) and the more derived interior subspecies: Lahontan (*O. c. henshawi*) and Yellowstone cutthroat trout (*O. c. bouvieri*) ($2N=64$).

Very little has been published regarding westslope cutthroat trout in the MCRB. For example, the symposium held by the American Fisheries Society (Gresswell 1988) concerning the status of interior stocks of cutthroat trout produced several papers that focus on westslope cutthroat trout, but none mention the presence of this subspecies in the Methow, Entiat, or Wenatchee subbasins. Behnke (1988) documented the occurrence of westslope cutthroat trout in the Chelan drainage, and noted the occurrence of isolated populations throughout the Mid-Columbia region (Behnke 1992). Surveys conducted in the late 1940s and early 1950s have brief references to the occurrence of cutthroat trout in the Mid-Columbia basin (Bryant and Parkhurst 1950). Surveys by Mullan et al. (1992) documented the occurrence of "cutthroat trout" throughout the Mid Columbia headwater tributaries. We assume that westslope cutthroat were present in the upper and mid

Columbia by mid-Pleistocene (Behnke 1992), but it appears that sporadic distribution of *O. lewisi* in this portion of the Columbia basin is the result of late glacial or postglacial dispersion. Dymond (1931) described the mountain cutthroat (*O. c. alpestris*) from disjunct populations of *O. lewisi* in the upper Columbia basin in British Columbia (Behnke 1992).

The presence of westslope cutthroat trout in the Mid-Columbia basin was clearly affected by one of the most catastrophic geological events in the history of western North America. Glacial Lake Missoula was a massive body of water (about the size of Lake Erie and Lake Ontario combined, some 500 cubic miles of water) created by glacial ice dams where the Clark Fork River enters Lake Pend Orielle in Idaho. It is now known that these ice dams broke about 100 times during the Pleistocene (Parfit 1995), initiating a series of mammoth flood events that shaped the topography of the Columbia plateau. These floods presumably carried Lake Missoula westslope cutthroat trout into the upper and middle Columbia basins. Staggering volumes of water (more than 600 million cfs- equaling ten times the volume of all the present rivers in the world) raged through the scablands of Washington (Parfit 1995), creating massive backwater pools that presumably allowed westslope cutthroat to colonize the drainages of the east slope Cascades. These massive floods occurred repeatedly, and it is likely that trout coming in the various flood events were able to find some refugia. How these "naturally introduced" cutthroat interacted with the westslope we assume were already present is unclear.

Later invasions by redband rainbow trout replaced cutthroat in most drainages, but "pure" westslope cutthroat populations remained above barriers (e.g. Lake Chelan) and in some headwaters where temperature regimes favored cutthroat.

Based on collections described by Mullan et al. (1992) and our collections in the summers of 1992 and 1993, it is obvious that both redband rainbow trout and westslope cutthroat trout often occur in the same system. They may be allopatric or sympatric, but rainbow trout generally occupy the lower reaches. There is a transition zone where both species and/or intermediates occur; and cutthroat trout (along with bull trout) dominate the upper, higher gradient sections where annual temperature units are considerably less (Mullan et al. 1992). Confident identification is often difficult, particularly where low levels of introgression have occurred. Natural zones of sympatry between redband rainbow and westslope cutthroat trout are known to occur in the John Day

River drainage in northern Oregon and southern Washington and in the Yakima River subbasin (Yakima Fisheries Project 1995). Given the thermal regimes of the region, it is quite possible that similar zones of sympatry existed in the study area prior to settlement and subsequent translocation of trout. The extensive planting of both native and non-native rainbow and cutthroat subspecies that has occurred in the last century has undoubtedly affected natural mechanisms of reproductive isolation, adding another layer of complexity to correct identification.

Westslope cutthroat in the MCRB are either indigenous relict populations, or introduced *lewisi* from two Washington broodstock lakes (Twin Lakes- introduced population from Lake Chelan or local indigenous stocks in the Wenatchee subbasin; Kings Lake- introduced population from Priest Lake, Idaho (Behnke 1992)). The Washington Department of Fish and Wildlife (WDFW) also lists Nelson Lake, Ford Hatchery, and Washoe Park Trout Hatchery, Montana, as sources for westslope cutthroat trout in Washington (unpublished WDFW data). In most cases, Twin Lake progeny were used for cutthroat stocking in the Methow, Entiat and Wenatchee drainages (Ken Williams, WDFW, personal communication). Records of stocking from 1952-1993 in the MCRB list only Twin Lakes as a source, with the exception of one stocking in 1984 of Lake Lenore (Lahontan) cutthroat in Big Twin Lake. Lahontan cutthroat from Lake Lenore were not stocked in streams of interest here. Non-native cutthroat trout in the MCRB would most likely be from Yellowstone Lake cutthroat that were introduced many years ago, or coastal cutthroat (*O. c. clarki*) that were transported within the state of Washington from the western slope of the Cascades to the MCRB. The State of Washington received many millions of eggs from Yellowstone Lake but very few of the shipments were made directly to locations in the study area (Varley 1979) (see Appendix A). Two shipments of 100,000 eggs were received in "good" condition at Peteros by the Methow Hatchery in 1930, and the Okanagon County Game Commissioner in 1932. Chelan State Fish Hatchery received over 700,000 eggs between 1930 and 1941, Colville received 550,000 eggs in six years between 1917 and 1938, Leavenworth Hatchery received over 500,000 eggs in 1951, and the Wenatchee Hatchery received one shipment of 100,00 eggs in 1932 (Varley 1979).

Interior Redband Rainbow Trout

There has been considerable confusion in regards to natural variability and historic distribution of rainbow trout in the

Columbia basin. Based on many years examination of museum collections and field collections, Behnke (1992) summarized the taxonomy of interior redband trout of North America. A review of this subject is presented in Appendix B (Behnke and Proebstel 1992).

Long standing perceptions that resident rainbow trout and anadromous steelhead are always genetically distinct and reproductively isolated may be suspect (e.g. Currens et al. 1988; Mullan et al. 1992; Chapman et al. 1994). It is likely that in some situations, steelhead contribute to the genetic structure of "resident" populations. Conversely, based on circumstantial evidence in the Methow River, "resident" rainbow trout may contribute to steelhead runs (Mullan et al. 1992). Gene flow occurs to prevent complete separation, and gene flow is likely stimulated by introduction of both non-native hatchery rainbows and steelhead. Genetic mixing of steelhead from stocking in the study area may have begun as early as 1918 and has been extensive. Non-native coastal steelhead contributed to the gene pools of various hatchery stocks in the Mid-Columbia basin (Mullan et al. 1992, Chapman et al. 1994), and have potentially impacted populations of native resident interior redband rainbow trout. In the 1930's and 1940's, "rainbow trout" were reared in the Leavenworth, Chiwaukum and Methow Hatcheries for stocking of waters in the MCRB. In more recent years, between 1961 and 1973, a "common" Mid-Columbia broodstock of steelhead was developed and used in all Mid-Columbia River hatcheries. This broodstock was derived from commingled stocks gathered below Priest Rapids Dam (Mullan et al. 1992). In 1974, another broodstock was developed for the Wells Dam Hatchery and "residual" fish from this broodstock have been stocked in the rivers and streams of the Mid-Columbia basin (Ken Williams, WDFW, personal communication). In addition, the Winthrop National Fish Hatchery, and its historic efforts toward propagation of the "Winthrop-strain rainbow trout" has certainly impacted the rivers and streams in at least the Methow River system. Chapman et al. (1994) summarized the available information concerning the origin and number of rainbow trout stocked in the MCRB from 1949-1994: a total of 12,626,274 rainbow trout from at least 15 different brood sources. Recent records of the WDFW indicate stocking rainbow trout in the MCRB relied heavily on coastal hatchery rainbow trout from the Glendale, South Tacoma, Spokane, and Tokul Creek Hatcheries. Furthermore, intentional hybrid crosses were made between rainbow and cutthroat trout very early in Washington (Cranford 1912) and were widely stocked.

The accurate identification of relict populations of interior redband rainbow trout in the Mid-Columbia basin, is clearly confounded by likely genetic interaction with non-native steelhead, rainbow trout, and cutthroat trout. In light of these factors, there is little doubt that very few uncontaminated indigenous native rainbow trout populations remain.

Taxonomic Concerns

The focus of the present survey is taxonomic identification. Simply stated, this concerns identification of species, subspecies, and local forms of salmonid fishes. There should be a distinction made between this and systematic purposes which would include evolutionary (phylogenetic) and ecological relationships; or population genetic questions which focus on genetic variability within and among populations. Due to the number of sites sampled and populations assessed, we have not attempted to address phylogenetic or population genetic questions in this report. Accordingly, characters used for the analysis in the present study are those which have shown to be diagnostic for identification. For example, molecular genetic analysis has emphasized those regions of DNA that this and previous research have identified as containing polymorphisms among the taxa of interest that may be useful as taxonomic markers. An attempt has been made to restrict the number of characters and methods to only those which address the specific scope of the study.

METHODS

Collection of Specimens and Preservation of Tissue

During 1992, the first year of the study, specimens of bull trout, cutthroat, rainbow, and suspected hybrid trout were collected by hook and line and electrofishing from preselected sites in the Methow subbasin. In 1993, specimens of cutthroat, rainbow, and suspected hybrid trout were similarly collected in the Entiat and Wenatchee subbasins. Appendix C includes maps depicting sampling location sites in the three subbasins and purity ratings of populations for each species. A continuation of this survey was initiated and completed in 1998 and analyzed separate from the 1992-1993 data; the 1998 report has been included in Appendix D for easy reference.

In 1993 due to a statewide concern over the decline of bull trout in Washington and the then pending ruling on the petition to list this species under the Endangered Species Act, a non-lethal technique was used to sample bull trout. A clip of the upper caudal fin was taken and frozen on dry ice for genetic analysis. Each bull trout was anesthetized and a series of morphometric and meristic measures were made, in addition color photographs of ventral and dorsal profiles were taken. Once measured, bull trout were returned to the stream unharmed.

Preservation of tissue samples for various forms of DNA analysis is normally accomplished by freezing tissue, or whole specimens on dry ice or in liquid nitrogen (Dessauer and Hafner 1984). For the purposes of the present study, specimens were frozen on dry ice upon collection, and stored at -80° C prior to extraction of DNA. As we isolated DNA for the present molecular evaluation, duplicate samples of tissue were taken and stored at -80° C for future genetic studies. The specimens were then permanently fixed in 10% formalin, stored in 40-50% isopropyl alcohol, and will be incorporated into the collection of salmonid fishes at the University of Colorado Museum to serve as a permanent reference collection. A small subset of specimens from several sites in the Methow subbasin were not fixed in formalin, and are cryopreserved at -80° C for future studies.

Classical Taxonomic Data- Basis of Diagnosis

Cutthroat and Rainbow Trout

All populations were evaluated on the basis of morphological and meristic characters. Characters useful in identification both westslope cutthroat trout and interior redband rainbow trout are summarized in Behnke (1992) and include: number of scales in the lateral series and above the lateral line, number of pyloric caeca, number of basibranchial teeth, number of gill rakers on the first gill arch, number of gill rakers on the posterior side of the first gill arch (presence or absence of posterior gill raker development), spotting pattern, coloration, size and shape of parr marks, and number of vertebrae. Appendix E includes measurement statistics of morphological and meristic characters for cutthroat and rainbow trout captured in this study.

A series of spotting indices was used to evaluate the observed variability in spotting patterns. Individual specimens were rated for their overall spotting phenotype (Spt) from 1 to 5, where

1 represents a typical "pure" cutthroat spotting pattern, and 5 represents a typical "pure" rainbow spotting pattern. The size of spots (Ssz) was rated from 1 to 3 with 1 being relatively small and 3 greater than or equal to the diameter of the pupil of the eye. The total number of spots on the head (SptH) was counted for each specimen, and each sample was rated for uniformity among the specimens, or sample spot index (SSI). A parr mark index (PMI) was used to help distinguish redband rainbow and cutthroat trout (which have primitive (oval) parr marks with secondary rows visible above and below the larger more obvious parr marks associated with the lateral line region), from coastal rainbow trout (which have more rounded parr marks, generally lacking secondary rows, with parr marks absent or greatly reduced in mature fish). A PMI rating of 1 refers to derived (coastal rainbow) parr marks, and 3 refers to primitive (cutthroat or redband rainbow) parr marks.

Cutthroat Trout

It may be assumed that all native cutthroat trout are *lewisi* (though a remote possibility of native *bouvieri* -similar to relict populations in Crab Creek and Waha Lake does exist- see Behnke (1992) for a discussion of origins and distributions of Yellowstone and westslope cutthroat trout). Non-native cutthroat would be either Yellowstone or coastal cutthroat trout.

There is substantial overlap in many of the meristic characters between westslope and Yellowstone cutthroat. Only spotting and coloration of mature male fish are consistently different, but not complete (e.g. Yellowstone cutthroat from McBride Lake in Montana have westslope-like spots, and *lewisi* from the John Day drainage have larger spots, more similar to typical *bouvieri*). Westslope cutthroat typically have 150-200 scales in the lateral line, with means generally 165-180. Specimens from the closest regions to the MCRB (Salmon and Clearwater drainages and some British Columbia populations) tend to have much higher scale counts, with averages up to 200 or more. Pyloric caeca numbers are typically 25-50, with mean values usually 30-40. Numbers of gill rakers on the first arch are typically 17-21, with means of 18-19. Differences in coloration, spotting, number of basibranchial teeth, and posterior gill raker development allow clear separation from Yellowstone Lake cutthroat (assumed to be the source of possible introductions to the MCRB) by an experienced observer (e.g. Marnell et al. 1987).

Coastal cutthroat may be identified by their lower scale counts (lateral series: 140-180, 120-140 for some resident stocks;

above the lateral line: 30-40); gill raker morphology and number (short, blunt gill rakers averaging 17-18), and spotting pattern (evenly distributed on the body- many spots below the lateral line, on abdomen, and on the head). Spotting patterns are most useful in distinguishing coastal and westslope cutthroat (Qadri 1959).

Rainbow Trout

As discussed, widespread stocking of hatchery rainbow trout and coastal or mixed steelhead throughout the region makes correct identification of indigenous native rainbow trout a difficult task. Virtually all hatchery rainbows are derived from coastal rainbows (*O. mykiss irideus*). Clear separation between coastal rainbow and interior rainbow can be made on the basis of several meristic characters (scale counts and pyloric caeca numbers, spotting pattern, and shape of parr marks including presence or absence of supplemental dorsal and ventral rows) (Behnke 1992). Interior redband rainbow typically have 130-170 scales in the lateral series with means from 135-160, and typically 30-50 pyloric caeca with means about 40 (Behnke 1992). Coastal rainbow typically have 120-140 scales in the lateral series, with means around 130, and much higher pyloric caeca numbers (40-70 with averages about 55). Also, most interior redband rainbow trout retain primitive parr marks as adults while coastal rainbow do not.

Hybrids between rainbow and cutthroat trout were identified on the basis of meristic and morphological characters (scales in the lateral series and above the lateral line, pyloric caeca numbers, gill raker numbers, basibranchial teeth counts), types of parr marks, and spotting patterns) (Behnke 1992).

Bull Trout

Classical characters that are useful in taxonomic study of bull trout include: osteological: supraethmoid, vomer, hyomandibulae, gill rakers, and maxilla; morphological characters: width of head, eye placement, general shape of the mouth, flatness of the head, and position of adipose fin; meristic characters: mandibular pores, branchiostegals, gill rakers, pectoral fin rays, and vertebrae.

Morphological and meristic data for bull trout were collected for comparison to Cavender (1978, 1980), and Bond (1992). Measurements on specimens collected in the 1992 field season were made on preserved specimens. Live specimens captured and released in 1993 were anesthetized with appropriate dosages of Tricain Methane Sulfonate (MS-222), and the following meristic

counts and morphometric measurements taken: number of branchiostegal rays on left and right sides, anal fin ray counts, mandibular pore counts on left and right sides, standard length, fork length, length of maxillary bone, head width, predorsal length and weight. In addition, a color photograph was produced of several specimens, with careful attention taken to clearly depict the coloration and spotting pattern of the dorsal fin, as well as the profile of the head.

Discrimination of hybrids between bull trout and brook trout (*Salvelinus fontinalis*) has been discussed by Leary et al. (1983, 1985) and summarized by Markle (1992). The spotting pattern on the dorsal fin (and caudal fin in specimens greater than 140 mm in length) is diagnostic (Markle 1992). Pigmentation of the pelvic, pectoral and anal fins is also very different between bull trout and brook trout. Brook trout have a tri-colored (black-white-brown) leading edge of these lower fins whereas bull trout have only a white leading edge.

Osteological Studies

Several bones of the skull were examined for comparison with Cavender (1978, 1980). Osteological preparations of disarticulated bones of the skull were made according to methods outlined in Mayden and Wiley (1984), with some modifications. Five grams of trypsin was added per 100 ml of saturated sodium borate solution, resulting in a much higher concentration of both trypsin and sodium borate. This reduced the time for initial clearing per specimen from approximately 2-3 weeks to about 5-6 days. For several of the larger specimens, the enzyme buffer solution was changed after 3-4 days and replaced with a solution of concentrations described by Snyder and Muth (1990) (30% sodium borate and 1-2% trypsin). Cleared bones were allowed to dehydrate slowly in 70% isopropyl alcohol to minimize cracking or other distortions.

Disarticulated bones from specimens were screened, sorted and stored at constant temperature and humidity. Bones that have been used in previous studies as diagnostic characters were analyzed with the aid of a computer enhanced imaging process (Optimas). These include: vomer, hyomandibula, premaxillary, and supraethmoid. Gill raker morphology was also examined using disarticulated gill rakers lightly stained with alizarin red solution as described in Snyder and Muth (1990). Additional bones that contribute to the unique head shape of *S. confluentus* (ethmoid, frontale, parietal, supramaxilla, dentary, glossohyal,

and basibranchial) were also evaluated for potential use as diagnostic characters.

Molecular Genetic Data

DNA Isolation and Analysis

Methods described in Current Protocols in Molecular Biology (Ausubel et al. 1991), abridged by P. Evans, Brigham Young University, and D. Proebstel, Colorado State University, were used for isolation of total DNA.

Total DNA was extracted from ground tissues by digestion in a 400 μ l solution of 10 mM Tris-HCl, pH 8.0/ 25 mM EDTA/ 100 mM NaCl/ 0.5% SDS/ proteinase K (0.5-0.1 μ g/ml) for 8-10 hours at 55 C. The DNA was purified by two extractions with phenol/chloroform/isoamyl alcohol (25:24:1; vol/vol/vol) and precipitated in two volumes of 100% ethanol after making the solution 300 mM in sodium acetate, pH 5.4. An alternative purification procedure involved precipitation proteins and desalting with 200 μ l of 5 M potassium acetate, followed by one extraction with phenol/chloroform/isoamyl alcohol (25:24:1; vol/vol/vol) and precipitation in two volumes of 100% ethanol and a final wash with 70% ethanol. The DNA was then resuspended in 10 mM Tris and 1.0 mM EDTA (pH 7.2, TE buffer) or sterile distilled water. DNA concentration was determined by absorbency at 260 nm and by estimations from ethidium bromide-stained gels. A negative control with no tissue was taken through the entire procedure.

Polymerase Chain Reaction

Mitochondrial DNA

Amplification was performed in a 25-40 μ l volume reaction containing 67 mM Tris (pH 8.8), 6.7 mM MgSO₄, 16.6 mM (NH₄)₂SO₄, 10 mM 2-mercaptoethanol, four dNTP'S (dATP, dTTP, dGTP, and dCTP) at 1 mM, two primers at .2-1 μ M, total DNA (10-500 ng), and 0.2-0.5 units of Taq polymerase (Perkin Elmer). Negative controls with no added DNA and the supernatant from the tissue extraction control were included in each reaction. Primers 765 and 766 (LGL Ecological Genetics Inc.) were used in the amplification of a 1.3 kb region of the mitochondrial genome encompassing the cytochrome B gene sequence. Larger fragments of mtDNA including the ND1 (NADH dehydrogenase), ND2 (NADH dehydrogenase), and ND5/6 (NADH dehydrogenase) regions were also amplified by the polymerase chain

reaction (PCR). The PCR cycle consisted of 32 cycles of denaturation for 45 seconds at 95°C, annealing for 40 seconds at 50°C, and extension for 2.5 minutes at 70°C.

Restriction Enzyme Digestion

The PCR amplification products of mtDNA were initially screened by digestion with nine restriction enzymes to determine which of the enzymes produced diagnostic differences between the taxa of salmonids addressed in this study. These enzymes were AluI, CfoI, HaeIII, HinfI, MboI, MseI, MspI, RsaI, and Sau3AI, all of which recognize and cut four or five base pair sequences of the PCR product. Digestion reactions were performed in 10 ul volumes in 10X TAE buffer (1:10 volume), 1-2 units of digestion enzyme, 5 ul PCR product and SDW. Reaction mixture was incubated for 1-3 hours at 37 C, and reconstituted with 5 ul SDW and 1 ul dye. Digested fragments were then separated according to size on 2-3% agarose gels containing ethidium bromide. Gels were illuminated under ultraviolet and photographed to identify restriction fragment patterns. Different patterns were assigned a letter designation according to a standardized database compiled at BYU (Evans and Shiozawa, unpublished data).

For evaluation of rainbow and cutthroat trout, restriction fragments of the cytochrome b and ND-1 genes proved to be most diagnostic, and consistent. The cytochrome b was digested with HinfI, MboI, and RsaI ; and the ND-1 with MboI, DdeI, and MspI. All bull trout and brook trout were also analyzed for polymorphisms produced by RFLP patterns of the cytochrome b and ND-1 genes. Restriction enzymes used in bull trout evaluation were: AluI, DdeI, HinfI, RsaI, and Sau3AI (isoschisomer with MboI) for the cytochrome b region, and AluI, RsaI, and MspI for the ND-1 region. In some specimens of some bull trout, ND-2 was also amplified and cut with HaeIII, HinfI, MspI, MboI, Sau3AI and RsaI.

RESULTS

All specimens were analyzed for classical taxonomic characters, and a taxonomic diagnosis was made based on the results. Mitochondrial DNA analysis was performed on all bull trout and most of the brook trout specimens to evaluate the extent of hybridization between these taxa in the samples collected. Mitochondrial DNA analysis was also completed on populations judged morphologically "pure" westslope cutthroat or interior redband rainbow trout. In addition, several "hybrid" populations and difficult specimens were also evaluated by mtDNA analysis.

Purity ratings were given to each for trout population included in the survey. The ratings were assigned as follows: 1) "pure" - considered genetically pure based on morphological and genetic diagnostic criteria; 2) "essentially pure" - one or more of the diagnostic criteria was outside the expected range for the species or subspecies (this may be due to natural variability or multiple sources of the population; additional evidence -e.g stocking history or allozyme data is considered); and 3) "good" - the specimens are phenotypically representative of the subspecies but are not considered to be genetically pure. Results are presented by site and summarized in appendices A, B, and C and Tables 1-6.

Westslope Cutthroat and Redband Rainbow Trout

In this survey of the MCRB, two general phenotypic patterns emerged. Many of the populations identified as "pure" westslope cutthroat represent the "ideal" *lewisi* phenotype: classic westslope spotting and coloration; 170-180 scales in the lateral series; 18-19 gill rakers on the first gill arch; 30-40 pyloric caeca. A second phenotypic pattern (variable spotting, several spots on the heads, higher lateral series scale counts of 190-200+, and higher gill raker numbers: 19-20) was also observed in specimens from a number of locations in the MCRB. All of these populations had the same mtDNA haplotypes in the cytochrome b and ND-1 genes (for the restriction enzymes used in diagnosis), which would indicate that the restriction sites used to distinguish westslope cutthroat from rainbow trout and Yellowstone cutthroat are relatively old (> 70,000 ybp) and do not reflect differentiation within *lewisi*.

Select populations identified as "pure" or westslope cutthroat and interior redband rainbow were screened for diagnostic mitochondrial markers to confirm morphological findings. Following protocol developed at Brigham Young University (Shiozawa and Evans 1994), the polymerase chain reaction was used to amplify five regions of the mitochondrial genome (D-loop, ND-1, ND-2, ND-5/6, and cytochrome b), which were digested with up to 9 restriction endonucleases. The D-loop region amplified consistently, but contained very little polymorphism between species and was not used in the diagnosis. The cytochrome b and ND-1 regions had better consistency in amplifications than the ND-2 and ND 5/6, and consistent differences in haplotypes between species. Several enzymes that produced distinct haplotypes between rainbow and cutthroat trout were recommended from an extensive data base compiled at Brigham Young University (Shiozawa and Evans 1994), and were compared to

results of initial screening. In the cytochrome b gene, HinfI, MboI and RsaI were useful as diagnostic markers, having different haplotypes between cutthroat and rainbow trout, as were MboI, DdeI and MspI for the ND-1 region. HinfI and RsaI digests of the cytochrome b gene also produce different haplotypes between westslope and Yellowstone cutthroat, allowing for identification of Yellowstone haplotypes in cutthroat specimens. No Yellowstone cutthroat haplotypes were observed in any of the specimens evaluated.

Identifying haplotypes specific to interior redband rainbow proved to be problematic. In the initial screening, we were unable to unambiguously distinguish redband from coastal rainbow haplotypes. Specimens could be identified as rainbow trout, to support morphological findings, but judgement as to purity (i.e. lack of coastal rainbow alleles) could not be made on molecular information alone.

Results for PCR amplification, and restriction digests for select populations of westslope cutthroat, interior redband rainbow, and putative hybrid populations are summarized in Table 1. Mitochondrial haplotypes are given letter designations intended to correspond with a standardized *Oncorhynchus* data base compiled at BYU (Evans and Shiozawa, unpublished data).

Bull Trout

Brown (1992, pp. 12-13) lists 8 populations of bull trout in the MCRB and assigns a risk level factor for each population. We sampled 15 populations of bull trout in the 1992 and 1993 field seasons, and assume that several additional populations were not surveyed (e.g. Icicle Cr. and Panther Cr.). We have made no attempt to address the risk factor associated with any of these populations but note that, in general, bull trout were found to be persisting in small headwater populations. Many of these populations were above barriers to movement, effectively cutting them off from larger metapopulations (Rieman and McIntyre 1993). Location and river kilometer of bull trout populations in the MCRB are listed in Table 2.

Mitochondrial DNA Analysis

Bull trout specimens were analyzed using molecular techniques to address two distinct questions. First, we compared selected populations of bull trout of the Methow subbasin with bull trout collected throughout their range in the United States. In addition to data collected at CSU, portions of this analysis were

completed in the laboratory of Evans and Shiozawa at BYU with valuable assistance from R.P. Evans. Previous studies have demonstrated significant genetic differences between bull trout of the Columbia and Klamath basins (Leary et al. 1991; Williams et al. 1996). We found bull trout in the Methow subbasin to be similar to bull trout throughout the Columbia basin, with some exceptions. A rare haplotype, previously found in single specimens from two populations in the Columbia basin (Gold Creek and Clark Fork (Williams et al. 1996)) was observed in specimens from Goat Creek and the Twisp River, indicating this haplotype is more broadly distributed than previously thought. Composite haplotypes for select populations from the Methow drainage compared to published haplotypes from the Columbia and Klamath basins and Dolly Varden from Alaska are presented in Table 3.

Diagnostic haplotypes that distinguish bull trout and brook trout selected from restriction digests of the Cytochrome b and ND-1 genes include the following enzymes: AluI, DdeI and SAu3AI for cytochrome b and AluI, MspI and RsaI for the ND-1 gene. All populations of bull trout and most populations of brook trout were assessed for diagnostic haplotypes that differ between species. Two hybrids were diagnosed by morphological characters and both specimens have brook trout haplotypes. Results are summarized in Table 4.

Taxonomy

Morphological and Meristic Characters

Several morphologic and meristic characters were discussed by Cavender (1980) as diagnostic for bull trout. The form of the head and position of the mouth cleft when the jaws are closed help distinguish between bull trout from Dolly Varden. The form of the head is characterized by a flat skull roof and elongated jaw (articulating well behind the posterior margin of the eye) which is relatively broad when viewed from above. Upper and lower jaws are of equal length bisecting the anterior profile below the midline. Larger bull trout (greater than 100 mm) from the Methow were consistent with all the above characters. Though this pattern was readily apparent in larger specimens, it proved to be ambiguous in specimens smaller than 100 mm. The position of the mouth cleft was not obvious in all specimens, especially smaller individuals. In addition, the jaw (maxilla) did not extend past the posterior margin in smaller individuals. The flat skull roof, formed where the frontals join, including the region of the supraethmoid, was also useful in separating bull trout from brook

trout. The position of the eye, also discussed by Cavender (1980), proved to be ambiguous in this study.

Data for meristic characters for preserved specimens of bull trout and brook trout from the MCRB including gill rakers on the first gill arch (upper, lower, and total), branchiostegal rays on the right and left side, pyloric caeca numbers, and mandibular pores (left right and total), are presented in Appendix F and summarized in Table 5, along with comparative values published by Cavender (1980).

Additional data from bull trout collected in the 1993 field season were taken on anesthetized fish. We intended to apply the Haas-McPhail linear discriminant function (LDF), and interpret additional characters to the specimens sampled. Accumulation of reliable data for this purpose proved to be problematic. Specifically, accurate counts of branchiostegal rays and mandibular pores were not possible on anesthetized specimens, particularly smaller ones (> 100 mm). Measurements (especially length of jaw) were also difficult to make with the kind of accuracy needed to obtain dependable results and still maintain concern for the well being of the anesthetized fish. Successful application of the Haas-McPhail LDF, in our opinion, requires preserved specimens and a very experienced taxonomist. Our experience in this regard concurs with findings in British Columbia where professional field biologists were given preserved specimens and asked to apply the LDF. A misidentification of approximately 80% was observed (Gordon Haas, University of British Columbia, personal communication).

Osteological Characters

Osteological preparations were made on a subset of the specimens collected in the 1992 field season. Individual bones were analyzed with the aid of Optimas Image Analyzer. Individual specimens from 8 locations in the Methow River subbasin are listed in Table 6.

Premaxilla, Supraethmoid, Vomer, Hyomandibulae

The degree of development of the ascending process of the premaxilla differs greatly among species of *Salvelinus* (Cavender 1980). All of the premaxillae examined were congruent with descriptions and illustrations of Cavender (1980), having a long process tapered toward the dorsal end. The posterior margin of the premaxillae were also consistently broad as is expected in specimens of *S. confluentus* and *S. fontinalis* (Figure 1). This broad posterior margin of the bone articulates with the rounded

head of the supraethmoid. Superaethmoids examined from all osteological preparations were consistent in that they all had an elongated "head region" with a poorly developed lateral process (Figure 2). This extended region distinguishes bull trout from Dolly Varden, which have a more rounded "head region", with strongly developed lateral processes. All specimens examined were consistent in this characteristic feature that serves to form the relatively broad "flatheaded" snout of the bull trout.

The vomers of the specimens examined showed a relatively limited range of variability, particularly in the positioning of the vomerine teeth. Cavender (1980) describes a gap between the vomerine teeth and the palatine teeth on either side, which was observed in Methow River subbasin bull trout. This is in contrast to Dolly Varden in which the vomerine teeth form a transverse ridge in a curved row that is continuous with the palatine teeth. All of the vomers examined had only a slight posterior positioning of the vomerine teeth, which was more pronounced in specimens of *S. malma* from Alaska (personal observation) and depicted in Cavender (1980).

The hyomandibulae proved to be the most diagnostic bone assessed. All specimens were consistent in the morphology of hyomandibulae, having a broad, rather concave lateral face and complete lack of development of the adductor ridge that is present in Dolly Varden and several other species of *Salvelinus* (Figure 3). This was consistent in the smallest specimens and afforded complete separation between bull trout and Dolly Varden.

Gill Raker Morphology

Cavender (1980) pointed out that the presence or absence of well developed tiny marginal teeth on the gill rakers was useful in separating *Salvelinus* into two distinct groups, one containing bull trout, the other Dolly Varden. With proper staining, these teeth were observable in all specimens and proved to be of value in diagnosis of bull trout.

Analysis of Specimens by Subbasin

Methow River Subbasin :

Lower Methow River (Mouth to Winthrop)

Black Canyon Creek (Sites 1 & 2, N=10, 115-198 mm)

Black Canyon Creek is a 2nd order tributary of the Methow River. Specimens collected from site 1 (river kilometer 0.3) and site 2 (river kilometer 4) are rainbow trout. Respectively, the number of gill rakers (range= 18-20, mean=19.0), (18-20, 19.0); scales in the lateral series (135-169, 149.4), (129-172, 145.2); scales above the lateral line (28-31, 29.6), (25-31, 26.6); and numbers of pyloric caeca (37-54, 44.2), (36-42, 38.4) are within the expected range of interior redband rainbow trout. None of the specimens have basibranchial teeth, and most of the specimens have primitive parr marks with secondary rows associated with redband rainbow trout. Within the samples, there is variability in spotting pattern, and size of spots, suggesting multiple sources of the population, likely the result of historic stocking. Several specimens lack spots on the head. Steelhead use portions of Black Canyon Creek and have potentially impacted resident rainbow in the system. The trout in Black Canyon Creek are judged to be primarily interior redband rainbow with influences from historic stocking, but not a "pure" native population.

Methow River, mainstem (Site 3, N=1, 269 mm)

The single specimen from river kilometer 6 of the Methow River makes it difficult to make definitive judgement concerning this location. The specimen examined appears to be a "good" example of redband rainbow trout. The meristic counts are all within the expected range (scales in the lateral series- 150; scales above the lateral line 29; pyloric caeca 42) and lack of basibranchial teeth and spotting characteristics are consistent with diagnostic characters for redband rainbow trout. Anadromous steelhead and stocked rainbow trout are present in the lower portions of the Methow River and are assumed to affect the population. However, more fish would be needed to give a conclusive statement about this site.

Gold Creek (Site 6, N=5, 149-203 mm)

Gold Creek is a 4th order tributary of the Methow River entering at river kilometer 35.1. Specimens sampled from river kilometer 8.7 of Gold Creek are phenotypically "good" representatives of interior redband rainbow trout. Primitive parr marks are present in all specimens, including larger fish. The sample spotting index and general character of spotting indicates a slight cutthroat trout influence in the population, but scale counts (136-147 (141.4), 25-31 (27.6)), counts of pyloric caeca (37-52 (44.2)), and lack of basibranchial teeth show

that cutthroat trout gene flow has been minimal. Existing information on the stocking history of Gold Creek indicates that cutthroat trout were present in the drainage prior to 1937 and rainbow trout have been stocked in several sections of the creek (Mullan et al. 1992). In addition, anadromous steelhead have access to at least the lower portions of the system in the North Fork. Historic stocking in the drainage has either been with interior redband trout, or has not affected the phenotype of the native trout. While this population is not "pure" native redband rainbow, it is one of the better representatives of the native rainbow phenotype in the lower Methow drainage, and should be managed as a native population, pending further investigation.

South Fork Gold Creek (Site 5, N=6, 160-184 mm)

The South Fork of Gold Creek is a low gradient (0.8%) 3rd order tributary of Gold Creek. Specimens collected from river kilometer 11.6 of South Fork Gold Creek are judged to be mostly coastal rainbow trout and presumably derived from historic stocking. While the sample was very uniform in meristic counts and spotting index, most of the characters indicate a this is a non-native population. Specifically, low scale counts 122-128 (126.0) for lateral series, 24-27 (26.0) for scales above the lateral line; consistent lack of primitive parr marks; absence of basibranchial teeth and general character of profuse smaller spots are indicative of coastal rainbow trout. The numbers of pyloric caeca 35-46 (40.3) are low for pure coastal rainbow, but not unexpected from a stocked population, or one in which there was some historic gene flow with interior redband rainbow trout.

Foggy Dew Creek (Sites 7 & 8, N=11, 134-212 mm)

Specimens collected from Foggy Dew Creek show a gradation from cutthroat-rainbow hybrids in the lower portions of the creek to "essentially pure" westslope cutthroat trout in the upper reaches. Specimens from site 7 (river kilometer 5.6) show a range of phenotypes. Some individuals are excellent examples of cutthroat trout, while others are obvious hybrids. Higher scale counts (156-182 (169.6) for lateral series, 33-40 (36.2) for scales above the lateral line) and pyloric caeca numbers (34-51, 39.6) are in the range of those expected from westslope cutthroat but overlap with interior redband rainbow trout. One specimen lacks basibranchial teeth (0-12, 4.6), and several spots were noted on the heads of all but one of the specimens examined, indicating gene flow with rainbow trout. In addition, the sample spot index, spot size index, and parr mark index show that the

population has been influenced by fish from multiple sources. Rainbow trout contribution to the genetic structure of the population appears to be from interior redband rainbow trout (parr marks and spotting pattern).

Specimens collected from site 8 at river kilometer 8.7 are "essentially pure" westslope cutthroat trout. All fish have basibranchial teeth (2-10, 5); scale counts are high (175-193 (182) for lateral series, 38-44 (41.2) for scales above the lateral line) and pyloric caeca numbers relatively low (36-45 (39.2)). Spotting characteristics are consistent with those expected of westslope cutthroat trout. One larger specimen (212 mm) has several large spots on the head, which is likely the result of limited historic gene flow with rainbow trout. Management activities that may have a tendency to break down natural barriers to reproductive isolation (i.e. stocking of non-native strains) should be avoided in Foggy Dew Creek, as this collection indicates cutthroat trout are maintaining a relatively pure population in the upper reaches of the stream.

Reports of cutthroat trout introduction from downstream stocking in 1917 (Mullan et al. 1992) should be further investigated to determine the parental source of the cutthroat trout, as well as the stocking history of Cooney Lake. This will facilitate gaining a clearer understanding of the taxonomic affinities of this population of "essentially pure" westslope cutthroat trout.

Crater Creek (Sites 9 & 11, N=36, 124-234 mm)

Crater Creek is a 2nd order tributary of Gold Creek entering at river kilometer 8.4. Specimens collected from two sampling locations on Crater Creek indicate this stream contains an introgressed population of cutthroat and rainbow trout in the lower reaches of the stream, and an introduced population of rainbow trout in the upper sections. Cutthroat trout were introduced from an unknown source in 1924 (Mullan et al 1992). Specimens collected at site 9 (river kilometer 1.9) appear to be primarily westslope cutthroat and interior redband rainbow (based on primitive parr marks and spotting characteristics in a majority of the specimens). The wide range of diagnostic characters (scales in the lateral series: 149-196 (180.8.0); above the lateral line: 32-43 (38.2); pyloric caeca 33-44 (39.0); gill rakers 18-24 (20.5); and basibranchial teeth (0-12, 10/36 specimens without teeth)) and full range of spotting patterns indicate a hybrid swarm at this location. Mitochondrial DNA

analysis confirms this conclusion. Both rainbow and westslope cutthroat trout haplotypes were observed in this population.

Site 11 (river kilometer 7.7) was previously reported to have only rainbow trout, and it was assumed to be the result of stocking headwater lakes (Mullan et al. 1992). Our collection was consistent with this finding and fish are judged to be mostly non-native coastal rainbow trout. Low scale counts (124-143 (131.0) for lateral series, 24-31 (27.3) for scales above the lateral line); low numbers of pyloric caeca (32-49, 39.4); spotting pattern; and lack of primitive parr marks in any of the specimens are the basis of this conclusion. None of the specimens have basibranchial teeth. Phenotypic characteristics of this population do not indicate any influence of cutthroat trout, but spotting patterns and pyloric caeca numbers indicate a slight influence of interior redband rainbow. Whether this is due to interaction with trout native to Crater Creek or an artifact of the source population used in stocking is speculative.

Libby Creek (Site 12, N= 5, 125-206 mm)

Libby Creek is a 3rd order tributary of the Methow River entering at river kilometer 42.5. The mainstem of Libby Creek was sampled at river kilometer 5.6 (site 12). Specimens collected from the lower site indicate a population that is of multiple origin. The predominate phenotype is that of interior redband rainbow trout, but influences of both coastal rainbow and cutthroat trout are apparent. Specimens have meristic elements reflective of redband trout (scales in the lateral series: 133-187: 152.0; scales above the lateral line: 30-37, 32.4; pyloric caeca numbers: 32-42, 38.4). The irregular spotting pattern, and shape of parr marks, infers the presence of coastal rainbow trout in the common gene pool. One of the specimens had basibranchial teeth, high scale counts (187, 37) and a spotting pattern generally associated with westslope cutthroat trout. The headwater lakes in Libby creek are known to support mostly rainbow and some cutthroat trout, and it is likely that most of the drainage has potential gene flow between these two species.

North Fork Libby Creek (Site 13, N=8, 123-190 mm)

The North Fork of Libby Creek (site 13) was sampled at river kilometer 13.7. It contained a hybrid population, but predominately cutthroat trout. Approximately half of the specimens examined have a "good" westslope cutthroat trout phenotype, with high scale counts (166-181), basibranchial teeth

(2-6), lower pyloric caeca counts (37-42), and primitive parr marks. The other half of the specimens are slightly hybridized with rainbow trout, based on their spotting pattern and lack of basibranchial teeth. Mullan et al (1992) reported only cutthroat trout above river kilometer 1.6, but noted that no barriers to upstream migrants existed. Libby Lake, source of the North Fork of Libby Creek, is reported to have only cutthroat trout but was not sampled. While it is possible that pure cutthroat may yet remain in Libby Lake (elevation 2,322 meters), at least the lower portions of this stream now shows the influence of gene flow with rainbow trout.

Beaver Creek (Site 78, 79 & 199 N= 13, 81-350 mm)

Beaver Creek is a 3rd order tributary to the Methow River entering at river kilometer 56.6. Rainbow trout were collected at site 78 (river kilometer 12.4) along with one brook trout. Anadromous steelhead are reported to use the lower portion of the stream and are likely contributing genetically to the population of rainbow trout at site 78. Meristic characters are fairly typical of redband rainbow (scales in the lateral series: 128-153, 137.0; scales above the lateral line: 24-34, 29.5; pyloric caeca numbers: 41-48, 44.0; total number of gill rakers in the first gill arch: 19-21, 20.0; none of the specimens have basibranchial teeth). Parr marks suggest some contribution from non-native rainbow trout (rounded parr marks, secondary rows are weak or absent), likely the result of historic stocking or interaction with steelhead.

Brook trout were reported to have replaced bull trout at all higher elevations in this stream (Mullan et al. 1992). Specimens collected from river kilometer 8.0 of the South Fork Beaver Creek (site 79) are all brook trout. No indication of hybridization with bull trout was detected in this collection (all specimens have tri-colored fins, spotting on the dorsal fin, and general head morphology which distinguish brook trout from bull trout). Mitochondrial DNA haplotypes derived from RFLP patterns of the cytochrome b and ND-1 genes are diagnostic for brook trout, concurring with morphological determination (Table 4).

Possible bull trout were identified in the headwaters of Beaver Creek (site 199). One specimen (a bull trout X brook trout hybrid), and 2 additional fin clips were collected. The hybrid is diagnosed by obvious white spotting in the dorsal fin (not black as with brook trout). Tri-colored pigmentation is present in the anal and pelvic fins. The overall spotting is that of a bull

trout, but vermiculations on the head and front half of the dorsal region are typical of brook trout (some vermiculations of this sort have been noted in small (> 90 mm.) "pure" bull trout, but not in mature adults- personal observation). Three basibranchial teeth are present which is a diagnostic character for bull trout. The specimen is a male, which agrees with Leary et al. (1983). Mitochondrial DNA haplotypes from RFLP patterns of the cytochrome b and ND-1 genes are that of brook trout, inferring a maternal brook trout contribution to the specimen (Table 4). It is very possible that this is an F-1 hybrid, based on conclusions of Leary et al. (1983, 1991), inferring that there are still "pure" bull trout in the Beaver Creek drainage.

Twisp River Watershed :

Twisp River, mainstem (Site 14, N=10, 183-251 mm)

The Twisp River is a major (4th order) tributary of the Methow River entering at river kilometer 64.7). Specimens collected at site 14 are rainbow trout, most likely influenced by stocking. Meristic characters are fairly typical for redband rainbow, but some historic influence from coastal rainbow in the source population is likely. Specimens have 124-151 (137.0) scales in the lateral line, 24-33 (28.3) scales above the lateral line, 32-56 (44.2) pyloric caeca, and 19-22 (20.3) gill rakers on the first arch. Parr marks are not visible in most specimens, and none have basibranchial teeth. Low numbers of scales in the lateral series and high numbers of pyloric caeca in individual specimens indicate genetic input from coastal rainbow. Variability within the sample in spotting patterns also indicates the population is from multiple sources. This portion of the Twisp River has been stocked extensively over many years (including Wells Hatchery residuals in recent years), and the rainbow trout collected from this site reflect this history.

Twisp River, mainstem (Sites 31 & 32, N=5 & 10 151-264mm)

The upper portion of the Twisp River was sampled at river kilometer 43.5 (site 31) and river kilometer 48.3 (site 32). These locations straddle a barrier falls at river kilometer 45.1. The bull trout (N= 5) collected at site 31 show no signs of hybridization with brook trout. All of the specimens lack any spotting in the dorsal fin, do not have tri-colored ventral fins characteristic of brook trout, and have the general head morphology expected of bull trout. Osteological analysis of diagnostic bones of the skull (specimens JMR 305, JMR 307b) found hyomandibula, supraethmoid, vomer and premaxilla to be typical of

bull trout. Mitochondrial DNA haplotypes derived from RFLP data from the ND-1, and ND-2 genes are the common haplotypes found throughout the Columbia basin. Restriction digests of the cytochrome b gene with AluI produced a rare haplotype previously found only in individual specimens from the Clark Fork River, and Gold Creek in the Yakima River drainage (Williams et al. 1996).

Previous collections report only cutthroat trout above the falls at river kilometer 45.1. Mullan et al. (1992) speculated that the fish originated from stocking of Twisp Lake, and subsequently colonized the creek above the barrier falls. All of the specimens from site 32 have basibranchial teeth (4-23, 8.7) and high scale counts (lateral series: 172-199 185.6; above the lateral line: 37-43, 41.2), which are expected in pure populations of cutthroat trout. However, there is a wider range of spotting variability in the population than would be expected if the source of the stocked fish was pure westslope cutthroat trout. Half of the collected specimens have a distribution of spots that is more typical of cutthroat trout with at least some influence of rainbow trout. There is also some degree of variability in the size of the spots, and number of spots on the head. Gill raker numbers are somewhat high for westslope cutthroat trout (20-22, 21.1). The lack of any development whatsoever on the posterior side of the first gill arch rules out the possibility of influence from Yellowstone Lake cutthroat trout. The population in the Twisp River above the falls at river kilometer 45.1 is judged to be westslope cutthroat trout, but with slight rainbow trout influence. No further stocking of rainbow trout would be recommended in this stream above the barrier even though these fish are not "pure" westslope cutthroat trout.

Little Bridge Creek (Site 15, N=5, 112-191 mm)

Little Bridge Creek, a small 3rd order high gradient stream, was found to have a "good" population of "pure" interior redband rainbow trout. All specimens have diagnostic primitive parr marks with secondary dorsal and ventral rows of smaller parr marks, spotting pattern, lateral series and scales above the lateral line (150-162, 155.2; 32-33, 32.6, respectively), and pyloric caeca numbers (35-43, 39.2) associated with the primitive rainbow (redband) phenotype. There was a slight irregularity in spotting pattern of one specimen (it had no spots on the head) but the overall sample uniformity was within the range expected from a single source population. All specimens have mtDNA haplotypes from RFLP products of the cytochrome b and ND-1 genes diagnostic for rainbow trout. Because the Twisp system has anadromous steelhead and has been stocked rather extensively in the past, the

presence of "pure" interior redband rainbow trout are the result of one of two likely scenarios. It is possible that the trout in Little Bridge Creek have remained largely unaffected by historic stocking, or that the fish used to stock this stream were propagated from "essentially pure" interior redband rainbow trout.

Buttermilk Creek (Site 16, N=11, 131-185 mm)

The specimens collected at river kilometer 5.3 on Buttermilk Creek are interior redband rainbow, with a slight influence from cutthroat and coastal rainbow trout. The pyloric caeca counts (27-49, 36.8) and primitive parr marks on most of the specimens are consistent with characteristics of redband rainbow. One of the specimens has basibranchial teeth and spotting characteristics associated with cutthroat trout. The wide range of scale counts (lateral series: 129-168, 144.7; above the lateral line: 25-36, 30.4), with one individual below the reported range of redband rainbow (129, 25), along with spotting variability indicates a slight influence of coastal rainbow trout in the population. The sample is judged to be "good", but not "pure", redband rainbow trout.

West Fork Buttermilk Creek (Site 17 & 18, N=20, 169-219 mm). The sample taken at river kilometer 2.7 (site 17) on the West Fork of Buttermilk Creek is judged to be "pure" interior redband rainbow trout. All of the specimens have well defined primitive parr marks, scale counts (lateral series: 134-149, 144.0; above the lateral line: 28-31, 29.2), and pyloric caeca numbers (30-44, 36.4) within the expected range of redband trout, and redband spotting patterns with clear primitive parr marks that were very consistent within the sample. The presence of partial barriers to movement (Mullan et al. 1992) may have a role in the persistence of the native rainbow and bull trout in this stream. The persistence of bull trout in the upper portion of the stream (above an apparent barrier falls) makes this a unique location, with two native species of salmonids present in the system.

Site 18 (river kilometer 5.5) is above a barrier falls of 3.0-3.6 meters. No rainbow trout were collected in the sample, but they were reported to be "scarce" by Mullan et al. (1992). Bull trout (N= 10) were collected at this location. All of the specimens collected were found to be "pure" bull trout, having no spots in the dorsal fin, lack tri-colored leading edges of the ventral fins, and other expected morphological characters. An

osteological preparation was made on specimen #MAR 090. Osteological analysis of diagnostic bones of the skull found hyomandibula, supraethmoid, vomer and premaxilla to be typical of bull trout. Restriction digests of the cytochrome b region of mtDNA (AluI, DdeI, and Sau3AI) and the ND-2 gene (AluI, MspI, and RsaI) which distinguish bull trout from brook trout were all found to produce RFLPs typical of bull trout of the Columbia basin.

East Fork Buttermilk Creek (Sites 19 & 20, N=34, 132-204 mm). The East Fork of Buttermilk Creek, a 3rd order tributary to the Methow River, was sampled at river kilometer 1.9 (site 19) and river kilometer 5.2 (site 20). Both locations showed evidence of hybridization between interior redband rainbow and westslope cutthroat trout. The sample from site 19 had a wide range of phenotypes. Based on spotting patterns alone, some specimens were representative of "good" westslope cutthroat trout, while others appeared to be "good" redband rainbow trout. The high scale counts (lateral series: 136-184, 159.6; above lateral line: 26-42, 34.5) and relatively low caeca numbers (31-42, 35.1) do not show signs of non-native coastal rainbow trout in the gene pool. The population at site 19 is judged to be a hybrid swarm. Cutthroat trout are possibly being recruited from the barrier falls (located at site 20), and are assumed to have been stocked (Mullan et al. 1992).

The sample from site 20 (river kilometer 5.2) is predominately westslope cutthroat trout, but also shows evidence of hybridization in spotting patterns (several specimens with spots on the head), and basibranchial teeth counts (0-6, 3- several specimens without teeth). Scale counts (lateral series: 156-188, 173.6; above the lateral line: 41-48, 44.3), and pyloric caeca numbers 24-39, 32.3) are typical for westslope cutthroat. The orange-red coloration of several fish was also typical of westslope cutthroat. Though this stream does contain trout with obvious signs of introgression, it is also possible that this is a "hybrid zone", with essentially pure cutthroat above these sample sites (Ken Williams, WDFW, personal communication). Available information regarding the stocking history indicates Twin Lakes westslope cutthroat trout have been stocked in the past.

Eagle Creek (Sites 21, 22, & 23, N=28, 136-204 mm)

Eagle Creek may be divided into three sections. Below major barrier falls at river kilometer 0.8, represented by site 21 (river kilometer 0.3), the fish are hybrids between westslope cutthroat

trout and rainbow trout. The overall phenotype suggests a significant influence of cutthroat trout (spotting pattern), but meristic characters (relatively low scale counts (lateral series: 136-178 (149.3), above the lateral line: 29-40 (32.8)), lack of basibranchial teeth in all specimens, and numerous spots on the head (up to 52 in one specimen) clearly indicate that the population is derived mostly from rainbow trout. It is difficult to speculate about the ancestry of the rainbow trout, based on meristic analysis. The spotting pattern, relatively low pyloric caeca counts (32-39, 35.5), and presence of primitive parr marks suggests interior redband rainbow influence. The population is likely the result of multiple stocking events and represents a combination of several sources of rainbow and westslope cutthroat trout.

The middle section of Eagle Creek (above barrier falls, and below the confluence with Oval Creek) is represented by Site 22 (river kilometer 3.2). This hybrid population is interesting in that it has fish that exhibit a diverse array of phenotypes, but few "intermediates" were observed. Specimens tended to look more like either westslope cutthroat or redband rainbow trout. Higher average scale counts (lateral series: 151-210, 183.4; above the lateral line: 32-48, 38.7), and primitive parr mark pattern in the majority of the specimens indicates a stronger interior redband rainbow trout influence, but coastal rainbow trout characteristics (spotting pattern and lack of primitive parr marks) also appear in the population. One specimen, in particular (J 66), is a perfect westslope cutthroat phenotype, giving rise to the possibility of movement from an upstream source, or at least partial reproductive isolation. Most specimens have evenly distributed spots (anterior to the dorsal fin and below the lateral line, a spotting pattern more characteristic of Bonneville cutthroat trout (*O. c. utah*); and, in general, have larger spots (larger than the diameter of the eye).

Upper Eagle Creek (site 23), above the confluence with Oval Creek, has an "essentially pure" population of westslope cutthroat trout. High scale counts (lateral series: 173-212, 185.3; above the lateral line: 37-44, 39.1), basibranchial teeth counts (2-15, 4.7; all specimens with teeth) and low pyloric caeca numbers (26-35, 30.7) show little sign of influence from rainbow trout. There is some slight variability in the spotting pattern, which could be attributed to a very slight historic gene flow with rainbow trout. Two specimens had one small vestigial gill raker on the posterior side of the gill arch. This characteristic was rarely observed throughout the Mid-Columbia tributaries examined

in this study. All of the specimens have mitochondrial DNA haplotypes derived from RFLP patterns of the cytochrome b and ND-1 genes that are diagnostic for westslope cutthroat.

Middle Oval Lake (Site 24.1, N=5, 147-322 mm). Specimens collected from Middle Oval Lake are rainbow-cutthroat hybrids. Although the scale counts are somewhat high (lateral series: 149-189, 171.2; above the lateral line: 28-40, 34.8), two of the specimens lacked basibranchial teeth. Lack of basibranchial dentition and higher pyloric caeca counts (35-50, 40.2) coupled with many spots on the head of one specimen (12), clearly indicate an introgressed population. None of the specimens collected have the primitive parr marks, indicating the source of stocking was most likely influenced by a coastal variety (hatchery) rainbow trout.

West Oval Lake (Site 24.2, N=5, 97-233 mm). Collections from West Oval Lake indicate a population of "essentially pure" westslope cutthroat trout is in the lake. Specimens have meristic characters that are all in the expected ranges for *lewisi* (scales in the lateral series: 162-171, 166.8; scales above the lateral line: 40-44, 42.0; pyloric caeca 33-41, 36.2, and total number of gill rakers on the first gill arch: 18-20, 18.8). All of the specimens have basibranchial teeth (4-12, 8) and spotting patterns are very uniform, with spots well concentrated toward the caudal region. Size and shape of the spots is very typical for pure populations of *lewisi* throughout its range (small, irregular in outline). The specimens also have a very deep red coloration on the ventral surfaces, also typical of westslope cutthroat. Mitochondrial DNA haplotypes from restriction enzyme digests of the cytochrome b and ND-1 genes of the mitochondrial DNA are diagnostic for westslope cutthroat, concurring with morphological conclusions. West Oval Lake was certainly stocked, as it lies above major barriers to movement. Apparently, the stocking was from a "pure" westslope cutthroat broodstock or population (likely Twin Lakes).

War Creek (Sites 25 & 26, N=36, 20-245 mm)

War Creek may be divided into two major sections, above and below a large barrier falls located at river kilometer 2.9. Lower War Creek (site 26) has multiple species of trout, including brook trout, phenotypically "good" westslope cutthroat trout, interior redband rainbow trout, and rainbow cutthroat hybrids. The single brook trout collected showed no signs of hybridization with bull

trout (all fins tri-colored, and rows of spots on the dorsal fin, higher gill raker number (20), mtDNA haplotypes from RFLP products of the ND-1 and cytochrome b genes- diagnostic for brook trout). Two of the specimens examined (MSR 71, JMR 32) represent excellent westslope cutthroat phenotypes. All meristic and spotting characteristics (scales in the lateral series: 172,191; above the lateral line: 40,41; basibranchial teeth- 4,6; pyloric caeca 35,45; no spots on the heads, primitive parr marks; spots concentrated posteriorly and above the lateral line) are in the ranges expected for westslope cutthroat trout. Two specimens (MSR 143, MSR 145) appeared to be "good" representative interior redband cutthroat trout with primitive parr marks, expected scale counts (lateral series: 136,147; above the lateral line: 27,33); no basibranchial teeth, pyloric caeca (36-50), and numerous spots on the head. Several specimens are clearly hybrids (rainbow X cutthroat) and depict evidence of influence from non-native coastal rainbow trout (lower scale counts- e.g. lateral series: 123, above the lateral line: 24; high caeca numbers- e.g. 52, 56; and derived (rounded) parr marks). It is not unexpected to find a full range of phenotypes in an introgressed population. The ratio of seemingly pure phenotypes to hybrids should be somewhat of a normal distribution with the bulk of the specimens intermediate. Morphological and meristic analysis suggests that at least some of the individuals in lower War Creek are resisting hybridization or are moving from a relatively pure source population.

Above the barrier falls (river kilometer 2.9), War Creek was sampled at river kilometer 3.1 (site 25) and is very similar to the section below the falls with respect to the specimens collected. Three specimens (MSR 127, MSR 137, MSR 139) are clearly brook trout showing no outward signs of hybridization with bull trout (fin pigmentation, spotting of the dorsal fin, and general head morphology, mt DNA haplotypes). Four specimens (MSR 130, MSR 131, MSR 136 & JMR 30) are excellent westslope cutthroat phenotypes. All of these fish have high scale counts (lateral series: 167-190, 171.2; above the lateral line: 36-44, 40.2), basibranchial teeth (6-12, 8), and spotting and parr mark characteristics that are typical for westslope cutthroat. Two specimens (MSR 124, MSR 129) are phenotypically representative of interior redband rainbow trout, with 143 & 155 scales in the lateral line, 31 and 32 scales above the lateral line, no basibranchial teeth, and pyloric caeca numbers (49, 49) in the expected range of interior redband rainbow trout, as well as primitive parr marks and spotting pattern seen in pure redband rainbow populations. The remaining fish are clearly rainbow X

cutthroat hybrids with signs of influence from non-native coastal rainbow trout (parr marks; pyloric caeca numbers (e.g. 53, 52, 51); and spotting patterns). It is assumed that all of the trout above the barrier falls were introduced (Mullan et al. 1992), but it appears that some degree of reproductive isolation is occurring in the system. Evidence from analysis of mtDNA confirms this is a hybrid population. Two different haplotypes, both rainbow and westslope cutthroat trout, were observed in the cytochrome b and ND-1 genes. This is typical of a population in which females from two different species are contributing to the common gene pool.

Reynolds Creek (Site 27, N=5, 147-201 mm)

Five specimens of bull trout were collected from Reynolds Creek. Osteological preparations were made on two of the specimens (JMR 301 & JMR 303). No sign of hybridization with brook trout was observed in these individuals (no tri-colored ventral fins, lack of spotting in the dorsal fins, characteristic morphology of the head). Osteological analysis of diagnostic bones of the skull (specimens JMR 301, JMR 303) found hyomandibula, supraethmoid, vomer, and premaxilla to be typical of bull trout. Mitochondrial DNA haplotypes from restriction digests of the cytochrome b, ND-1 and ND-2 genes are diagnostic for bull trout of the Columbia basin (Tables 3 and 4).

South Creek (Sites 28 & 29, N=31, 120-235 mm)

South Creek is a 3rd order tributary of the Twisp River entering at river kilometer 39.3. Specimens collected from near the confluence at site 28 (river kilometer 0.2) are rainbow X cutthroat hybrids, but mostly resemble interior redband rainbow trout. Scale counts in the lateral series (152-183, 164.9), and above the lateral line (31-46, 38.1); as well as numbers of pyloric caeca (33-48, 39.5) are indicative of redband X cutthroat hybrids by the rather wide ranges. Seven of 20 specimens have basibranchial teeth, which is also a clear indication of hybridization. Spotting patterns are variable, with a complete range of phenotypes. Although two specimens (JMR 37 and JMR 38) are phenotypically "good" westslope cutthroat and are possibly from an upstream source, there is obvious gene flow between rainbow and cutthroat occurring at this site.

One bull trout were collected from South Creek. No sign of hybridization with brook trout was observed in this individual (no tri-colored ventral fins, lack of spotting in the dorsal fins, characteristic morphology of the head). Mitochondrial DNA

haplotypes from restriction digests of the cytochrome b, and ND-1 genes are diagnostic for bull trout of the Columbia basin (Table 4).

Specimens collected from an upstream location (site 29- river kilometer 4.0) are "good" westslope cutthroat trout. They have similar scale counts (lateral series: 157-180, 167.6; above the lateral line: 31-39, 34.6), and pyloric caeca numbers (32-44, 36.9), compared to specimens from site 28, but spotting pattern and shape of spots are more typical of *O. c. lewisi*. There is, however, some variability within the sample in size of spots and number of spots on the head. One of 10 specimens lacks basibranchial teeth, which is not expected in a "pure" population of cutthroat trout. Despite the lack of basibranchial teeth in one individual and spotting variability, hybridization has been minimal. The population is a "good" representative of westslope cutthroat trout in the Twisp drainage. It is possible this population has been affected by historic stocking of South Creek above the falls or in headwater lakes.

North Creek (Site 30, N=8, 100-214 mm)

Specimens collected in North Creek at river kilometer 1.0 are judged to be "essentially pure" westslope cutthroat trout. They have very high scale counts (lateral series: 191-216, 203.0; above the lateral line: 41-49, 43.7) which is extremely high for westslope cutthroat trout, but not outside the range observed in other populations (Behnke 1992). These high scale counts are, however, outside of the range of probable hatchery sources (Twin and Kings lakes). This is evidence that they are more typical of indigenous Methow drainage westslope cutthroat. All specimens have basibranchial teeth (4-12, 8.0), and relatively low pyloric caeca counts (27-45, 36.4). Of particular interest is the spotting pattern, which is somewhat unusual for westslope cutthroat trout in general. The spots are relatively larger than the typical westslope cutthroat, and all specimens had at least one spot on the head (not uncommon in a pure westslope population). Gill raker numbers are relatively high in this population (19-23, 20.4), and three of the specimens examined had very weakly developed posterior gill rakers on the first gill arch. All of the specimens have mitochondrial DNA haplotypes from RFLP patterns of the cytochrome b and ND-1 genes that are diagnostic for westslope cutthroat.

Upper Methow River (Winthrop to Headwaters)

Methow River, mainstem (Sites 36, 47 & 48, N= 19, 184-254 mm)

The West Fork of the Methow River was sampled at river kilometer 98.2 (site 36), river kilometer 125.5 and 130.8 (site 47), all below a barrier falls at river kilometer 133.4. Site 48 was above the falls at river kilometer 133.9. Bull trout were collected at site 47 and were observed throughout the upper reaches of this stream. No indication of hybridization with brook trout was observed in any of the bull trout (no spots in the dorsal fin or tri-colored dorsal fins, mtDNA haplotypes from RFLP patterns of the cytochrome b and ND-1 genes diagnostic for bull trout (Table 3 and 4)). Mullan et al. (1992) reported collecting 61 bull trout in 1989 at river kilometer 15.4 (corresponding to river kilometer 132.9 in the present study), indicating a strong population of bull trout in the upper (West Fork) Methow.

Specimens collected at site 36 are rainbow trout. Based on meristic characters (scales in the lateral series: 155-168, 161.6; scales above the lateral line: 33-37, 35.4) and presence of primitive parr marks in all of individuals, the sample is interior redband rainbow. None of the specimens have basibranchial teeth, and pyloric caeca are slightly high for "pure" redband rainbow (38-54, 43.4). No evidence of hybridization with cutthroat trout was detected in mtDNA analysis (all specimens with rainbow trout haplotypes from RFLP patterns of the cytochrome b and ND-1 genes). Based on the proximity of this site to other non-native rainbow samples, and the extensive stocking history of the Methow River, it is not likely that this is an indigenous "pure" population.

Cutthroat trout collected at site 47 (river kilometers 125.5 and 130.8) show indications of hybridization with non-native coastal (hatchery) rainbow trout. Scale counts have a range that is too wide for a population of "pure" westslope cutthroat (lateral series: 139-180, 159.0; above the lateral line: 28-40, 34.0), and pyloric caeca numbers (37-53, 43.5) also reflect rainbow influence in the population. Two of 4 specimens lack basibranchial teeth. Spotting characteristics, and lack of primitive parr marks in some individuals, also indicate coastal rainbow influence in these populations.

Specimens collected above the barrier falls at site 48 (river kilometer 134.9) show slight signs of hybridization with rainbow trout but are "good" representatives of the native westslope cutthroat phenotype. Meristic characters are indicative of westslope cutthroat trout. Scale counts for these fish are very high (lateral series 197-229, 211.7; scales above the lateral line 44-51, 48.0); and pyloric caeca numbers (30-42, 35.3) are well

within the range expected for cutthroat trout. All of the specimens have basibranchial teeth 6-12, 8.8), as would be expected in a "pure" population of cutthroat. However, characteristics of the spotting pattern, especially distribution on the body and number and size of spots on top of the head, suggest genetic input from rainbow trout. It is extremely unusual, however, to find hybrids with such high scale counts. This population, while not completely "pure" native westslope cutthroat trout, is judged to be a "good" westslope cutthroat population, and should be considered worthy of protection. Further investigation into the stocking history of this upper portion of the Methow would be helpful.

Chewuch River Watershed

Chewuch River, mainstem (Sites 60, 63, 64, 76, & 77, N=34)

The Chewuch River is the major tributary of the Methow River in the upper subbasin, entering the Methow River at river kilometer 80.6. It is a 4th order stream draining the northern part of the upper Methow subbasin. There is a barrier falls at river kilometer 55.2, above which it is assumed rainbow and cutthroat trout were introduced, but not bull trout (Mullan et al. 1992). No brook trout were collected above this barrier. The Chewuch was sampled at several sites above river kilometer 48.3. Collections indicate the typical trend of species composition (rainbow trout in the lower reaches and cutthroat in the headwaters) occurs in this river.

Specimens collected at site 60 (river kilometer 48.8) are interior redband rainbow with some degree of hybridization with cutthroat trout. Meristic characters are indicative of a hybrid influence (scales in the lateral series: 133-178, 159.6; scales above the lateral line: 25-39, 34.3; pyloric caeca numbers: 39-49, 43.9; total number of gill rakers on the first gill arch: 18-22, 19.3). None of the specimens have basibranchial teeth, but spotting patterns infer historic cutthroat gene flow at this location.

Collections made from between river kilometer 49.9 and 53.6 (1.6 kilometers below Chewuch Falls, site 63) are predominantly "good" westslope cutthroat trout, with slight influence from rainbow trout. Number of scales in the lateral series (150-195, 168.4) and scales above the lateral line (33-53, 41.2) are closer to values associated with "pure" westslope cutthroat, but variation in pyloric caeca numbers: 30-51, 39.8 show a possible

genetic input from rainbow trout. Total number of gill rakers on the first gill arch (18-22, 20.3) are also more typical of rainbow. All of the specimens have basibranchial teeth, (just the opposite from site 60) and spotting patterns are fairly uniform, but several individuals depart from classic *lewisi* spotting (many spots on the head), again implying influence from rainbow trout.

Samples taken from river kilometer 67.9 (site 64) are rainbow trout that are a mixture of interior redband and coastal rainbow, likely the result of stocking. Scale counts are lower (lateral series: 129-136, 131.4; above the lateral line: 27-32, 28.8); and pyloric caeca numbers higher (37-58, 45.2) than typical for "pure" interior redband trout. None of the specimens have basibranchial teeth, and spotting patterns do not indicate hybridization with cutthroat at this location. This is unusual in that "good" cutthroat trout were collected below this site. A high degree of reproductive isolation appears to be occurring in the river. Mullan et al. (1992) attribute the persistence of rainbow in higher elevations of the Chewuch River to warming effects of 5 alpine lakes and insolation from southern exposure.

Collections made from river kilometer 73.5 (site 76) are westslope cutthroat with slight influence from rainbow trout, and from river kilometer 74.3 (site 77) are "essentially pure" westslope cutthroat. This appears to be the end of a hybrid zone that dominates most of the upper Chewuch River. Respectively, meristic characters are: scales in the lateral series: 166-191, 179.6; 175-192, 185.6; scales above the lateral line: 36-46, 40.2; 36-46, 40.0; pyloric caeca numbers: 39-47, 42.2; 29-41, 34.3; total number of gill rakers in the first gill arch: 20-21, 20.6; 18-20, 19.1; and basibranchial teeth: 0-8, 4.2 (one without); 2-12, 5.1 (all with). Specimens from site 77 all have mtDNA haplotypes from RFLP products of the cytochrome b and ND-1 genes diagnostic for westslope cutthroat. Several lakes in the headwaters of the upper Chewuch drainage have been stocked with Twin Lakes westslope cutthroat and may have contributed to this population.

Cub Creek (Site 68, N=10)

Cub Creek is a 2nd order tributary to the Chewuch River entering at river kilometer 10.6. Brook trout were stocked above a barrier falls by 1937. Specimens show no signs of hybridization with native bull trout. All of the specimens have tri-colored ventral fins, clear spots in the dorsal fin, and low numbers of branchiostegal rays (20-24, 22.3). Characteristics of the head

morphology (rounded skull roof where the frontale bones meet, interorbital distance and rounded appearance of the premaxillary region) are also typical of brook trout. Specimens from site 68 all have mtDNA haplotypes from RFLP products of the cytochrome b and ND-1 genes diagnostic for brook trout (Table 4). It is unlikely bull trout were ever present above the barrier falls in Cub Creek.

Boulder Creek (Sites 73, 74, 75, N=13)

Boulder Creek is a 3rd order tributary of the Chewuch River entering at river kilometer 14.2. Rainbow, cutthroat, and brook trout have all been stocked in the past (Mullan et al. 1992). Rainbow trout were collected at site 73 and brook trout at sites 74 and 75. Rainbow trout collected at site 73 are "good" interior redband rainbow, but extensive stocking of rainbow, cutthroat and brook trout in this stream has likely had an affect on the purity of this population. Meristic characters are typical of redband rainbow (scales in the lateral series: 132-162, 149.5; scales above the lateral line: 34-46, 39; pyloric caeca numbers: 41-48-44.0; total number of gill rakers in the first gill arch: 17-19, 18.3; none of the specimens have basibranchial teeth). All of the specimens have primitive parr marks associated with redband rainbow and cutthroat trout. Spotting patterns suggest a slight historic input from cutthroat in the past (lack of spots on the head, spots concentrated posteriorly in individual specimens).

Brook trout specimens from sites 74 and 75 show no signs of hybridization with native bull trout. All of the specimens have tri-colored ventral fins, clear spots in the dorsal fin, and low numbers of branchiostegal rays (21-25, 22.3). Characteristics of the head morphology (rounded skull roof where the frontale bones meet, interorbital distance and rounded appearance of the premaxillary region) are also typical of brook trout. Mitochondrial DNA haplotypes produced from restriction digests of the cytochrome b and ND-1 genes were diagnostic for brook trout (Table 4).

Eight Mile Creek (Sites 49 & 51, N=16, 133-210 mm)

Eight Mile Creek was sampled near its confluence with the Chewuch River (site 49) at river kilometer 1.6, and at an upstream location (site 51) at river kilometer 12.9. All specimens collected at site 49 were rainbow trout, and all specimens at site 51 were brook trout. Meristic characters of the rainbow trout

from site 49 (scales in the lateral series: 126-153, 137.8; scales above the lateral line: 27-30, 28.2; and pyloric caeca numbers 31-51, 40.3) indicate that the population is not native redband rainbow trout. Spotting characteristics and the presence of derived parr marks (rounded with no supplementary rows) also are typical of non-native coastal rainbow trout. A general lack of uniformity of spotting in the specimens collected is an indication that the population is derived from multiple sources. Available information on stocking indicates Eightmile Creek has been stocked in recent years with rainbow trout.

Specimens collected at site 51 (river kilometer 12.9) are all brook trout with no signs of introgression with native bull trout. Spotting of dorsal fins in all specimens, as well as the tri-colored edges of ventral fins are all typical of brook trout. Mitochondrial DNA haplotypes produced from restriction digests of the cytochrome b and ND-1 genes were diagnostic for brook trout (Table 6).

Falls Creek (Site 50, N=21, 119-208 mm)

Falls Creek was sampled at river kilometer 7.1 above a barrier falls located upstream (0.6 kilometers) of its confluence with the Chewuch River. Both brook trout and cutthroat trout were collected. Brook trout specimens show no signs of hybridization with native bull trout. All of the specimens have tri-colored ventral fins, clear spots in the dorsal fin, and low numbers of branchiostegal rays (21-25, 22.3). Characteristics of the head morphology (rounded skull roof where the frontale bones meet, interorbital distance and rounded appearance of the premaxillary region) are also typical of brook trout. Mitochondrial DNA haplotypes produced from restriction digests of the cytochrome b and ND-1 genes were diagnostic for brook trout.

Cutthroat specimens collected are very "good" examples of westslope cutthroat trout. They have scale counts (lateral series: 152-173, 164.6; above the lateral line: 33-45, 36.9), pyloric caeca numbers (31-40, 35.1), spotting pattern, and size and shape of spots that are typical of *O. c. lewisi*. One of nine specimens lacks basibranchial teeth, which is not expected in a "pure" population of westslope cutthroat trout. The collection shows a high degree of uniformity within the sample which is typical of small isolated populations, populations that are stocked from the same source, or those founded from a low number of individuals. Despite the lack of basibranchial teeth in one individual, the population is a "good" representative of westslope

cutthroat trout in the Chewuch watershed and should be managed as a "good" native cutthroat population.

Twentymile Creek (Site 71, N=13, 137-210 mm)

Twentymile Creek was sampled at river kilometer 5.8 (site 71). One brook trout was collected. The specimen has tri-colored fins, spotting on the dorsal fin, and a very straight maxillary bone which distinguish brook trout from bull trout. No evidence of hybridization between bull trout and brook trout was observed in this individual. Mitochondrial DNA haplotypes derived from RFLP patterns of the cytochrome b and ND-1 genes are diagnostic for brook trout, concurring with morphological determination (Table 4).

Rainbow trout collected from Twentymile Creek show slight signs of historic stocking. Meristic characters are indicative of interior redband rainbow (scales in the lateral series: 146-160, 155.5; scales above the lateral line: 29-33, 31.3; pyloric caeca numbers: 40-46, 44.0; total number of gill rakers in the first gill arch: 18-20, 19.0), but average pyloric caeca numbers are somewhat higher than expected from an indigenous redband rainbow population. None of the specimens have basibranchial teeth, and all individuals have primitive parr marks. There is enough variability of spotting patterns within the sample to suggest some degree in influence from non-native (hatchery) rainbow trout. The sample is judged to be "good", but not "pure" interior redband rainbow trout.

Lake Creek (Sites 52, 53, 54, & 55, N= 25, 92-246 mm)

Lake Creek, a 3rd order tributary of the Chewuch River, was sampled at four locations. Lake Creek flows through Black Lake which has been stocked with both rainbow and cutthroat trout. At river kilometer 8.0 (site 52) specimens collected were rainbow trout with a slight degree of hybridization with cutthroat trout. The specimens show signs of historic stocking. Scale counts (lateral series: 148-162, 156.5; above the lateral line: 30-33, 31.4) are in the range of interior redband rainbow (or rainbow X cutthroat hybrids), but pyloric caeca numbers (39-65, 51.5) are higher than cutthroat or redband rainbow and must be the result of genetic input from coastal rainbow trout. Most specimens have primitive (redband or cutthroat) parr marks, but there is considerable variation in the spotting patterns, and obvious cutthroat spotting characteristics are present in several specimens. The plausible explanation for the phenotypes observed

in the specimens collected from site 52 is that they are a mixture of both native and non-native rainbow trout that have hybridized to some extent with cutthroat trout.

One bull trout was collected at site 53 (river kilometer 12.4). No indication of hybridization with brook trout was observed in this specimen. Branchiostegal rays (27), diagnostic lack of spotting in the dorsal fin, and pigmentation in the ventral fins are clearly characteristics of *S. confluentus*. Mitochondrial DNA haplotypes from the cytochrome b and ND-1 genes are also typical of bull trout in the Columbia basin (Table 4).

Specimens collected at river kilometer 13.5 (site 54) are similar to those taken 5.5 kilometers below at site 52, but there is a greater influence from cutthroat trout. In comparison to downstream fish, scale counts are higher (lateral series: 137-191, 168.3; above the lateral line: 28-38, 33.5), pyloric caeca numbers lower (36-42, 48.3) and half of the sample has basibranchial teeth. Spotting patterns and parr marks are variable as is generally the case in a hybridized population. The trend of altitudinal gradation from rainbow trout in the lower elevations, followed by intermediate (hybrid) trout, and cutthroat trout in the higher elevations and headwaters is apparent in Lake Creek, but rainbow appear to dominate below Black Lake.

Site 55 (river kilometer 15.3) is located above a possible barrier (11% gradient- described as "falls" in our collection notes). Rainbow trout from this location all have high scale counts (lateral series: 174-202, 185.0; above the lateral line: 36-51, 41.2), and relatively low pyloric caeca numbers (35-41, 37.7). All specimens have basibranchial teeth, but in two individuals the teeth on the basibranchial plate are vestigial or weakly developed. The spotting pattern is not typical of "pure" westslope cutthroat trout in two specimens, as several large spots are observable on the head. The size of the spots in general is more typical of cutthroat trout in the "Yellowstone cutthroat" clade, but this type of spotting has been observed in specimens from several locations in the Mid-Columbia basin where hybridization with rainbow trout has been apparent. Gill raker numbers are also quite high (20-22, 21.0) for westslope cutthroat (typically 18-21, with mean values 18-19). It is possible that these fish may also be affected by movement of individuals down from Fox Lake, which was stocked with Twin Lakes westslope cutthroat.

Andrews Creek (Sites 56, 57, 58, & 59, N= 27, 150-211 mm)

Andrews Creek was sampled at river kilometers 0.5, 1.8, 4.0, and 5.3. The specimens collected from all four locations are phenotypically very similar, and due to the lack of any physical barrier should be considered one population. The sample indicates the stream is dominated by interior redband rainbow trout, with a few cutthroat trout present in the system, resulting in a slightly introgressed population. Near the mouth of the stream (sites 56 & 57), redband rainbow phenotypic characters are dominant. None of the specimens have basibranchial teeth, and scale counts are within the expected range of redband rainbow (respectively, lateral series: 133-169, 149.3; 138-160, 144.6; above the lateral line: 31-37, 33.2; 29-36, 32.8); as were pyloric caeca numbers (37-48, 43.0; 36-43, 39.8, respectively). All of the specimens have primitive parr marks, and most of the collection has typical rainbow spotting pattern. Two individuals show a slight influence of cutthroat trout in the spotting pattern, with few spots on their head and lack of spots anterior to the dorsal fin and below the lateral line. Gill raker numbers (19-21, 19.8; 18-22, 18.8, respectively) are also more typical of redband rainbow trout.

Sites 58 and 59 (river kilometers 4.0 and 5.3) produced specimens similar to the downstream collections, but one of six fish collected had a very typical cutthroat spotting pattern. None of the specimens collected from site 58 have basibranchial teeth, as compared to site 59 (river kilometer 5.3) where two of ten specimens have basibranchial teeth development. Other characters were also similar to the downstream locations (scales in the lateral series: 138-176, 154.4; above the lateral line: 29-39, 33.3; pyloric caeca numbers: 31-47, 39.1; all but two specimens with primitive parr marks). There are clear signs of hybridization between rainbow and cutthroat in Andrews Creek, but in the lower portion of the stream, near its confluence with the Chewuch River, the fish are "good" representatives of native interior redband trout.

Tungsten Lake (Site 66, N=10, 167-238 mm)

Tungsten Lake, in the upper Chewuch River Drainage, has a trout population that is a "good" example of westslope cutthroat. It is likely that the lake was stocked presumably by a source that was founded by "essentially pure" westslope cutthroat. Meristic elements are fairly typical of *lewisi* (scales in the lateral series: 168-199, 182.1; scales above the lateral line: 39-46, 42.6; pyloric caeca numbers: 29-44, 37.6; total number of gill rakers in the first gill arch: 17-21, 19.6), but gill raker

numbers are somewhat high for typical westslope cutthroat. Since Twin Lakes cutthroat do have relatively high gill raker numbers, findings suggest these fish may have originated from Twin Lakes progeny. All specimens have basibranchial teeth (2-8, 5) as expected in a population of "pure" westslope cutthroat. Spotting patterns are relatively consistent within the sample, with a minor degree of variability in the concentration of spots toward the caudal region. A few individuals have sparsely distributed spots toward the anterior portion of the body, and 2 individuals have many spots on the head (20, 10). Size and shape of spots are "classic" *lewisii* characteristics (small, irregular in outline). Mitochondrial DNA haplotypes derived from RFLP patterns of the cytochrome b and ND-1 genes are diagnostic for westslope cutthroat, concurring with morphological analysis.

Wolf Creek (Sites 33, 34, & 35, N=22, 135-244 mm)

Wolf Creek was sampled at three locations below an impassable barrier falls at river kilometer 16.6. Fish collected at the lower site 33 (river kilometer 2.7) are rainbow trout from multiple sources, with some degree of hybridization with cutthroat trout. All of the specimens have primitive parr marks and relatively low pyloric caeca numbers (38-48, 42.0), which is an indication of interior redband rainbow trout and cutthroat trout, but low scale counts (lateral series: 125-156, 140.2; above the lateral line: 27-32, 29.6) and erratic spotting pattern imply more than one source for the rainbow trout in lower Wolf Creek. None of the specimens have basibranchial teeth, but spotting patterns indicate a limited amount of genetic contribution from cutthroat trout. The specimens collected at 9.5-9.7 (site 34.1 and 34.2) are predominately westslope cutthroat trout, with some hybridization with rainbow trout. The higher scale counts (lateral series: 130-198, 172.2; above the lateral line: 26-43, 37.5) would not be present in a population that was highly introgressed, but about half (5 of 12) of the specimens lack basibranchial teeth, and there is considerable range in spotting patterns, number of spots on the head, and size of spots. Variation of this kind usually indicates that the stream has been stocked with fish of different sources over the years.

Fish collected from below the falls at site 35 (river kilometer 16.1) are considered "good" westslope cutthroat trout. Specimens from this upstream site have relatively high scale counts (lateral series: 162-193, 173.9; above the lateral line: 31-41, 36.4), pyloric caeca numbers (32-47, 38.8), somewhat high gill raker numbers (16-23, 19.7), and all specimens have well

developed basibranchial teeth (1-14, 6.7). Spotting patterns are inconsistent within the sample and are not typical for westslope cutthroat (large irregular spots with few spots anterior of the dorsal fin below the lateral line). Several specimens have unusually large spots on their heads, which indicate some gene flow with rainbow trout.

While bull trout were expected to be present in this stream (many were collected in 1989 by Mullan et al. (1992)) none were captured in this study. The present status of bull trout in Wolf Creek requires further investigation.

Goat Creek (Sites 37, 38, 39, 69, 70; N= 43, 131-243 mm)

Goat Creek is one of the most important streams in the upper Methow River drainage (river kilometer 103.0) because of its strong population of bull trout, relatively pure westslope cutthroat and interior redband rainbow trout, and unique hydrology. The stream is effectively cut off from the Methow River in the summer and fall due to irrigation diversion; although Mullan et al. (1992) speculate that the stream would mostly disappear into glacial till at its mouth before reaching the Methow even without diversion. Distribution of salmonids in Goat Creek is along an altitudinal gradient, with interior redband rainbow in the lower portions of the stream, and bull trout and cutthroat in the upper reaches. The headwaters appear to have only cutthroat trout. Bull trout were collected at sites 38 and 39. Individual bull trout were found to have a unique mtDNA haplotype for the cytochrome b gene that is found only in a few populations throughout the species range, including the Twisp River in the Methow River subbasin (Williams et al. 1996 and Paul Evans Brigham Young University, unpublished data). Mitochondrial DNA haplotypes for the ND-1 and ND-2 genes are diagnostic for bull trout, and typical of bull trout in the Columbia basin (Table 4). No outward signs of hybridization with brook trout were observed in any of the specimens (no tri-colored ventral fins, lack of spotting in the dorsal fins, characteristic morphology of the head). Osteological analysis of diagnostic bones of the skull (specimens JMR 277, JMR 284) found hyomandibula, supraethmoid, vomer and premaxilla to be typical of bull trout.

Specimens collected at site 37 (river kilometer 2.1) are judged to be "essentially pure" interior redband rainbow trout. All of the specimens have primitive elliptical parr marks and supplementary rows of spots that distinguish redband from coastal rainbow trout. The scale counts (lateral series: 146-166 154.3;

above the lateral line: 31-35, 33.0), pyloric caeca numbers (38-49, 41.8), and gill rakers (18-20, 19.5) are within the expected range for redband rainbow, and none of the specimens have basibranchial teeth. This population is likely to be interacting with anadromous steelhead that have access to the lower portions of the creek.

Specimens collected at site 38 (river kilometer 10.5) and site 39 (river kilometer 12.9) are redband rainbow X cutthroat hybrids, but many of the individual specimens are excellent *lewisi* phenotypes. This appears to be a transition zone between rainbow and cutthroat in Goat Creek. Specimens from site 38 were more like interior redband in spotting characteristics, and have lower scale counts than site 69 (respectively: lateral series: 175-193, 185.7; 175-219, 191.4; above the lateral line: 36-42, 39.0; 36-43, 39.2). Pyloric caeca numbers are similar (29-41, 34.0; 25-36, 31.4, respectively). Half of the specimens lack basibranchial teeth from site 38, and 2 of 10 from site 69 have no basibranchial dentition. There appeared to be two distinct spot sizes on specimens (some large and round, some more typical irregular *lewisi* spots). Cutthroat trout were stocked in the early 1980's at river kilometer 14.5. It is likely that the aberration in spotting is due to hybrid interaction.

The headwaters of Goat Creek, sampled at river kilometer 14.7 (site 70) was found to have "good", but not completely "pure" westslope cutthroat. Meristic characters are similar to other cutthroat populations in the MCRB (scales in the lateral series: 163-209, 188.3; scales above the lateral line: 35-43, 39.7; pyloric caeca numbers: 25-40, 30.3; total number of gill rakers on the first gill arch: 17-20, 18.6). Two of the specimens lack basibranchial teeth, and have intermediate spotting patterns. This was one of the only sites in the study area where posterior gill raker development of any sort was observed. Most specimens (8 of 10) had one or two weakly developed gill rakers, or vestigial rakers, on the posterior side of the first gill arch. These were not similar to Yellowstone Lake fish, which have well developed posterior gillrakers, but may be an indication of a unique population of *lewisi* in the MCRB.

Early Winters Creek (Sites 40 & 41, N= 17, 121-237 mm)

Early Winters Creek was sampled at river kilometer 6 (site 40) and river kilometer 20.9 (site 41). There is a major barrier falls (approximately 7 meters high) at river kilometer 12.1 which blocks upstream movement of all fish. Specimens collected from below the barrier have a full range of phenotypes: from "good"

interior redband rainbow trout to excellent westslope cutthroat trout, as well as intermediates. Based on meristic counts and the presence of primitive parr marks in all of the specimens examined, there does not appear to be any great influence from stocking of non-native coastal rainbow trout (pyloric caeca numbers: 36-45, 39.7; scales in the lateral series: 134-194, 163.9; scales above the lateral line: 29-39, 35.2). There is obviously some degree of gene flow occurring between rainbow and cutthroat trout in this portion of Early Winters Creek (3 specimens with basibranchial teeth), but the presence of excellent representative specimens of both westslope and redband rainbow gives the impression that there is a possibility of partial reproductive isolation, or movement of "pure" trout from another source. Early Winters Creek has been stocked with rainbow trout in the past.

Above the barrier falls (site 41), Early Winters Creek is dominated by bull trout. This is one of the most stable populations in the Methow drainage. It is possibly the result of colonization before the barrier falls were created because bull trout were not traditionally stocked. Due to its separation from migratory bull trout, this isolated population is predicted to be vulnerable to decline or extinction (Rieman and McIntyre 1993), but if native, it must have been above the barrier falls for many thousands of years. No indication of hybridization with brook trout was observed in either the morphological characters (no spots on the dorsal fin, lack of tri-colored edges of the ventral fins, branchiostegal rays 26-27, 26.9) or mitochondrial DNA haplotypes (all specimens with expected Columbia basin bull trout haplotypes for RFLP analysis of ND-1, and cytochrome b genes (Table 4)).

A single large (237 mm) cutthroat trout specimen (MJR 310) was collected in the upper sampling location, and additional cutthroat trout were observed in this part of Early Winters Creek and were also collected in earlier studies (Mullan et al. 1992). It is an excellent representative of westslope cutthroat trout. Meristic and morphological characters are all in the expected range (scales 193, 42; pyloric caeca 31; 9 basibranchial teeth; and westslope spotting pattern). Mullan et al. (1992) suggested that cutthroat trout in the upper reaches of Early Winters Creek were the result of movement of fish down from Cutthroat Creek which is a tributary at river kilometer 17.2. While this is indeed a possibility, the presence of bull trout above the barrier falls at river kilometer 12.1 also allows for the possibility of native cutthroat trout already being present in Early Winters Creek before the stocking of Cutthroat Lake.

Cedar Creek (Sites 80, 81, & 82, N=15, 119-176 mm)

Cedar Creek is a 3rd order tributary to Early Winters Creek (see sites 40 and 41). There is a falls at river kilometer 3.9 which serves as a barrier to movement of trout. One specimen (rainbow X cutthroat hybrid) was collected below the barrier falls (site 82) in an attempt to find bull trout. No bull trout were observed in this effort. Specimens were collected at river kilometers 8.9 and 10.6 (sites 80 and 81). Site 81 is 0.3 kilometers up an unnamed tributary of Cedar Creek flowing from the north side of Snagtooth Ridge. Mullan et al. (1992) reported that rainbow trout were stocked above the falls in 1939 and cutthroat trout in the 1960's. Morphological characters indicate that the population above the falls is now phenotypically westslope cutthroat. All of the specimens at sites 80 and 81 except one have basibranchial teeth (respectively: 1-9, 5.3; 0-7, 5.2); scale counts are in the expected range for westslope cutthroat (respectively: lateral series scales: 167-187, 177.3; 164-189, 176.2; scales above the lateral line: 37-43, 40.0; 35-42, 38.4); as are pyloric caeca numbers (respectively: 33-45, 37.6; 32-45, 38.0). There is some variability in spotting pattern within the sample. No evidence of influence from Yellowstone Lake cutthroat is observable in the population (spotting pattern and posterior gill raker development). Several specimens have spots on the head but are not indicative of extensive hybridization. The population is either the result of a slight amount of gene flow between the rainbow trout and cutthroat trout stocked at different times, or the product of stocking from a slightly hybridized source. In either case, this population is a "good" example of westslope cutthroat (probably 90% "pure"), and is worthy of protection from future stocking.

Cutthroat Creek (Site 42, N= 6, 125-138 mm)

The population of "pure" westslope cutthroat trout in Cutthroat Creek is one of the best representatives of this subspecies in the Methow River system. Cutthroat Lake was stocked (Mullan et al. 1992) from a source that was presumably native fish, but the exact source is unclear (Ken Williams, WDFW, personal communication). All the specimens have a very uniform phenotype, which is a good indication of a single source of the population. Meristic characters (scales in the lateral series: 158-175, 166.8; above the lateral line: 32-42, 36.7; pyloric caeca 34-42, 38.0; basibranchial teeth 2-16, 9.8; gill rakers 18-23, 20.2) are all within expected ranges for westslope cutthroat trout (though gill rakers are somewhat high), and spotting patterns and uniformity are excellent. Based on meristic and spotting characteristics of

specimens from Twin Lakes (Behnke, unpublished data), it is likely this population was founded from this broodstock. All of the specimens have mitochondrial DNA haplotypes from RFLP patterns of the cytochrome b and ND-1 genes that are diagnostic for westslope cutthroat. While the cutthroat trout appear to be relatively abundant in Cutthroat Creek (personal observation), Mullan et al. (1992) suggested that the growth rate is quite slow due to extremely low average water temperatures and annual thermal budgets. Excessive harvest could easily affect the status of the population. When considered together with Early Winters Creek, this upper portion of the Methow River system is extremely important with respect to native fishes. Since two species of "pure" native salmonids are present in strong numbers, any further stocking of trout in these waters would be ill-advised.

Lost River (Sites 43.1 & 43.2, N= 14, 171-257 mm)

Specimens were collected at river kilometer 1.3 and 6.3. Both locations are dominated with rainbow trout that are not native redband. One large (257 mm) bull trout was collected at river kilometer 6.3, just below a barrier falls. No indication of hybridization with brook trout was detected (no spotting in the dorsal fin, branchiostegal rays at 27, lack of tri-colored fins, and mtDNA haplotypes of RFLP patterns of the cytochrome b and ND-1 genes are typical Columbia basin bull trout (Table 4)). Due to the relatively large size of this specimen and the presence of a barrier falls just above the collection site, it is probable that this was an adfluvial fish moving up from the Methow River.

Rainbow trout collected at both locations are likely the result of stocking. Their meristic characters (scales in the lateral series: 122-147, 137.2; scales above the lateral line: 26-35, 30.5; and pyloric caeca: 31-52, 44.1) along with attributes of the spotting pattern and lack of primitive parr marks in all specimens are indicators of a predominance of coastal rainbow influence. None of the specimens collected have basibranchial teeth, and other signs of hybridization with cutthroat trout were not detected. The population is judged to be mostly non-native coastal (hatchery) rainbow trout. Lost River has been stocked extensively in recent years with rainbow trout, which is reflected in the specimens collected in this survey.

One bull trout was collected from the upper collection location site (43.2) of Lost River. No sign of hybridization with brook trout was observed in this individual (no tri-colored ventral fins, lack of spotting in the dorsal fins, characteristic morphology of the head). Mitochondrial DNA haplotypes from

restriction digests of the cytochrome b, ND-1 and ND-2 genes are diagnostic for bull trout of the Columbia basin (Table 4).

Cougar Lake (Site 44, N= 8, 224-255 mm)

The population of salmonids in Cougar Lake and its outlet stream is dominated by bull trout, with some slightly hybridized westslope cutthroat trout present. All the bull trout collected showed no indication of hybridization with brook trout (no spots on the dorsal fin; lack of tri-colored ventral fins; branchiostegal rays 26-28, 27.4; mtDNA haplotypes of the cytochrome b, and ND-1 and ND-2 genes typical for bull trout of the Columbia basin (Tables 5 and 6)). Osteological preparations of bones of the skull were made on 2 specimens (JMR 083, JMR 085). Hyomandibula, supraethmoid, vomer and premaxillary were found to be typical of bull trout throughout their range.

Cutthroat trout collected at site 44 have high scale counts (lateral series: 186-218, 198.0; above the lateral line: 37-53, 43.0) and pyloric caeca numbers (35-41, 38.7) within the expected range for westslope cutthroat trout. One specimen lacks basibranchial teeth. Spotting pattern variability within the sample indicates some degree of historic gene flow with rainbow trout. Specimens are judged to be primarily cutthroat trout with some influence from rainbow trout.

Monument Creek (Site 83, N=5)

Monument Creek is a small tributary to Lost River entering at river kilometer 11.4. Only bull trout were collected at site 83. Mitochondrial DNA haplotypes for the cytochrome b, ND-1 and ND-2 genes are diagnostic for bull trout and typical of bull trout in the Columbia basin (Tables 5 and 6). No outward signs of hybridization with brook trout were observed in any of the specimens (no tri-colored ventral fins, lack of spotting in the dorsal fins, branchiostegal rays: 25-27, 26.6; characteristic morphology of the head).

West Fork of the Methow River

Robinson Creek (Site 45, N= 14, 120-218 mm)

Robinson Creek is the first tributary to the West Fork of the Methow River. There is a barrier falls near the mouth of this small third order stream. Above the falls, at site 45 (river kilometer 1.9) only westslope cutthroat trout were collected.

The population is characterized by high scale counts (lateral series: 177-217, 192.6; above the lateral line: 38-48, 40.8) and relatively low pyloric caeca numbers (32-45, 37.7). All specimens collected have basibranchial teeth, and primitive parr marks. Spotting pattern is typical of westslope cutthroat trout, but there is a slight amount of variation within the sample. Mitochondrial DNA haplotypes from restriction enzyme digests of the cytochrome b and ND-1 genes of the mitochondrial DNA are diagnostic for westslope cutthroat, concurring with morphological conclusions. Stocking information indicates the stream was stocked in the 1960's with Twin Lakes westslope cutthroat. Though likely a result of stocking, this population is judged to be "pure" or "essentially pure" native cutthroat trout and should be protected from future stocking of non-native salmonids.

Trout Creek (Site 46, N= 5, 117-204 mm)

Trout Creek was sampled near its confluence with the West Fork Methow (river kilometer 0.8). The specimens collected were all rainbow trout. Low scale counts (lateral series: 132-158, 139.0; above the lateral line: 25-32, 28.2), spotting characteristics, and lack of primitive parr marks in all of the specimens are indicative of influence from non-native coastal rainbow trout, likely the product of stocking. Pyloric caeca numbers are more typical of redband rainbow (31-46, 41.6). None of the specimens have basibranchial teeth. The high degree of variability in spotting characteristics, and distribution of spots on the head, indicate the population is founded from more than one source. There is no indication of hybridization with cutthroat trout, but it is likely this stream has been stocked on several occasions with rainbow trout.

Entiat River Subbasin_

Entiat River, mainstem (Sites 101 & 110, N=19, 129-218 mm)

Specimens collected from the Entiat River at river kilometer 69.2 are non-native brook trout, and rainbow trout with a slight influence from cutthroat trout. All of the brook trout have tri-colored ventral fins, characteristic spots in the dorsal fin, and dome shaped ridge where the ethmoid bones meet. In addition, the 5 specimens have typical brook trout patterns of restriction enzyme digests of the cytochrome b and ND-1 genes of the mtDNA molecule (Table 4). No sign of hybridization with bull trout was detected in this collection.

The remainder of the specimens are rainbow trout, but two individuals have basibranchial teeth, indicating a limited amount of gene flow with cutthroat trout. Relatively high pyloric caeca numbers in one individual (34-54, 41.6) and irregularities in the spotting patterns indicate an influence from non-native rainbow trout in the population, while the presence of primitive parr marks, and generally high scale counts (lateral series: 142-189, 166.7; above the lateral line: 28-33, 29.6) denote interior redband rainbow trout and cutthroat trout. The fish in this region of the Entiat River show clear signs of stocking of non-native trout and charr, and it is unlikely that any native genotypes would be found near this collection site.

Collections made at river kilometer 46.7 (site 110) are rainbow trout with a slight influence from cutthroat trout, similar to the sample from site 101. Meristic characters indicate both coastal and redband rainbow genetic input, and one specimen is indicative of cutthroat trout (scales in the lateral series: 146-216, 163.7; scales above the lateral line: 28-45, 32.9; pyloric caeca numbers: 39-58, 44.7; gill rakers: 17-22, 19.1). All but one specimen (the one with very high scale counts) lack basibranchial teeth. There is some variability in the spotting patterns (spots on the head), and both primitive and derived parr marks are present in the sample. Specimens are judged to be rainbow trout of multiple sources (including non-native coastal rainbow), with cutthroat trout also potentially affecting the gene pool. It is possible that the "good" westslope cutthroat specimen was stocked or recruited from an upstream source.

Roaring Creek (Site 122, N=6, 113-138 mm)

Roaring Creek is a small (2nd order) tributary of the Entiat River (river kilometer 9.8) with the confluence approximately 0.8 kilometers below the Entiat National Fish Hatchery. The lower portion of Roaring Creek is intermittent (Mullan et al. 1992). Roaring Creek was sampled from river kilometer 3.2 at the National Forest Service boundary up to private land at river kilometer 4.3. Specimens collected appear to be "essentially pure" interior redband trout, but historic stocking information would be needed to determine whether the population is indigenous. Scale counts are slightly high for interior redband rainbow (lateral series: 152-171, 163.5; above the lateral line: 27-30, 28.7;). Pyloric caeca are within the expected range of redband rainbow (31-45, 35.8), as are gill rakers (18-20, 19). All specimens have primitive parr marks; well developed supplementary rows and spotting patterns are very consistent within the sample. No

specimens have basibranchial teeth. The population appears to be derived from a single source (consistency of spotting characteristics, and relatively small standard deviations of other characters). While it is indeed possible that these are indigenous redband rainbow trout, the close proximity of the stream to the Entiat National Fish Hatchery does prompt speculation that stocking has occurred in this stream or that the population has been affected by past hatchery activities.

Mad River Watershed

Mad River, mainstem (Sites 112, 116, & 117, N=27)

Mad River in the Entiat River drainage showed the altitudinal pattern of distribution of salmonids observed in several other locations in the Mid-Columbia basin. Rainbow trout dominated the lower reaches of the stream along with bull trout. As elevation increases, there is a hybrid zone with rainbow X cutthroat trout, in addition to bull trout. At the upper reaches of Mad River, only cutthroat trout were sampled. Specimens were collected at river kilometers 16.4 (site 117), 18.3 (site 116) and 23.9 (site 112). Information concerning the stocking history of Mad River indicates extensive stocking in the lower reaches.

Bull trout and interior redband rainbow trout were collected at the lower site. None of the bull trout showed signs of hybridization with brook trout (no spots in the dorsal fin and lack of tri-colored ventral fins- from photographs) and had typical RFLP patterns for the ND-1 and cytochrome b mtDNA genes that have been observed for bull trout throughout the Columbia basin (Table 4). Rainbow trout are judged to be "good" interior redband, but not "pure".

Collections made at river kilometer 29.5 (site 116) included bull trout and westslope cutthroat trout that showed signs of hybridization with rainbow trout. Bull trout were similar to the specimens collected at site 117, showing no signs of hybridization with brook trout in diagnostic characters (no spots on dorsal fins, lack of tri-colored ventral fins, typical Columbia basin mtDNA haplotypes for ND-1 and cytochrome b gene). The remaining specimens are cutthroat X rainbow hybrids. Scale counts and pyloric caeca numbers are in the expected ranges of westslope cutthroat trout (scales in the lateral series: 155-212, 187.1; scales above the lateral line: 30-41, 37.4; pyloric caeca: 28-38, 32). Two of seven specimens lack basibranchial teeth, and several specimens have many spots below the lateral line and in front of

the dorsal fin, giving a clear indication of hybridization with rainbow trout. The population is judged to be mostly westslope cutthroat, with gene flow from rainbow trout occurring.

At the upper sampling location (river kilometer 38.5- site 112), only cutthroat trout were collected. The population of trout at this headwater site are "good", but not "pure", westslope cutthroat. Scale counts are very high (lateral series: 195-218, 202.0; above the lateral line: 41-46, 42.6). Pyloric caeca numbers are low (27-32, 29.6), and all of the specimens collected have numerous basibranchial teeth (5-14, 9.6). The general shape of the spots is typical of westslope cutthroat (small, irregular in outline-somewhat "X-shape"). Gill raker numbers are somewhat higher than expected for westslope cutthroat (19-22, 20.2), and all of the specimens have far too many spots on the head (23-44, 33) for typical "pure" westslope cutthroat. The spotting patterns indicate historic gene flow with rainbow trout, but meristic characters do not reflect this. It is possible that this phenotype (high scale counts and spots on the head) is the "native" westslope cutthroat (i.e. not stocked from Twin or Kings lakes), but the presence of rainbow X cutthroat hybrids and rainbow trout in the lower portions of this stream, along with an extensive stocking history, suggest that anthropogenic factors may have contributed to the aberrant spotting pattern in this stream.

Tillicum Creek (Site 121, N=5, 105-157 mm)

Tillicum Creek was sampled at river kilometer 4.8 (site 121) at its confluence with Indian Creek. The population is determined to be rainbow trout, but not "pure" native redband rainbow trout. Specimens have consistently high scale counts (lateral series: 166-182, 170.8; above the lateral line: 31-33, 32.0) and pyloric caeca numbers (39-45, 41.8) that are indicative of interior redband rainbow trout. None of the specimens from this location have basibranchial teeth. Primitive parr marks are present in only two of the specimens, and there is a considerable amount of variation in the spotting pattern, with some specimens having cutthroat-like spotting. Spotting characteristics of the trout collected from Tillicum Creek are typical of hatchery (stocked) fish, with a possible limited amount of gene flow with cutthroat trout, but are not typical of a relict indigenous redband population.

Hornet Creek (Site 119, N=5, 148-160 mm)

Hornet Creek is another small (2nd order), high gradient (10%) tributary of Mad River in the Entiat River drainage. It was sampled at river kilometer 0.2 near its confluence with Mad River. Specimens collected are judged to be mostly interior redband rainbow trout, but not "pure" indigenous fish. Scale counts (lateral series: 152-159, 155.4; above the lateral line: 28-31, 29.4); gill raker numbers (18-21, 19.0); and pyloric caeca numbers (30-43, 38.6) are typical of redband rainbow. None of the specimens have basibranchial teeth. Over half of the specimens from Hornet Creek do not have primitive parr marks (oval shape, with distinct supplementary rows), and variability in the spotting pattern was observed within the sample. Subtle variation in the spotting pattern of this nature is usually the result of multiple sources of the population, as is the case when the stream has been stocked with hatchery fish.

Cougar Creek (Sites 114 & 115, N=12, 111-200 mm)

Cougar Creek is a small (2nd order), high gradient (9%) tributary of Mad River. It was sampled at river kilometer 0.3 (site 115), and river kilometer 3.2 (site 114). Bull trout were collected from the lower portion of the creek only (site 115). None of the specimens show phenotypic signs of hybridization with brook trout (no spots in the dorsal fin and lack of tri-colored ventral fins). Two of the smaller specimens (11502, 11505) have mottled parr marks. All bull trout have mtDNA haplotypes in the ND-1 and cytochrome b genes that are typical of *S. confluentus* throughout most of the Columbia basin (Table 4).

The remaining fish collected at site 115 are judged to be rainbow/cutthroat hybrids. Relatively high scale counts (lateral series: 157-177, 168.3; above the lateral line: 31-41, 36.0) and low pyloric caeca numbers (32-48, 40.1) indicate that rainbow influence is more likely from interior redband as opposed to coastal rainbow. Spotting indices show a complete range of spotting patterns, but most fish have spotting characteristics associated with rainbow trout. The presence of basibranchial teeth in two of the specimens (1, 7) is also evidence of cutthroat genotypes in the population.

Collections at river kilometer 3.2 (site 114) are judged to be "essentially pure" westslope cutthroat trout. Specimens from this upstream site have high scale counts (lateral series: 178-203, 188.4; above the lateral line: 39-41, 39.8), low pyloric caeca numbers (25-34, 30.4), typical westslope gill raker numbers (17-19, 17.8), and all specimens have well developed basibranchial

teeth (7-10, 8.4). Spotting patterns are consistent within the sample and are typical for westslope cutthroat (small irregular "x-shape" spots with few spots anterior of the dorsal fin below the lateral line). All of the specimens have mtDNA haplotypes from RFLP products of the cytochrome b and ND-1 genes diagnostic for westslope cutthroat. Several spots were observed on the heads of all the specimens (3-13, 8) which may indicate a small amount of gene flow with rainbow trout in the past or may be characteristic of this population.

Miners Creek (Site 111, N=0)

Miners Creek was electrofished at river kilometer 3.5; no specimens were collected.

Preston Creek (Site 109, N=0)

Preston Creek was electrofished above falls, but no specimens were collected at this location.

Tommy Creek (Sites 107 & 108, N=6, 113-222 mm)

One specimen was collected from Lake Louise (site 107), which flows into Tommy Creek, and at river kilometer 1.9 of Tommy Creek (site 108). The single specimen from site 107 appears to be a "pure" westslope cutthroat trout. Meristic characters are all very typical for westslope cutthroat (scales in the lateral series and above the lateral line: 194, 35; pyloric caeca: 33; basibranchial teeth: 5) and spotting pattern, as well as general shape of the spots, are classic westslope cutthroat. It is difficult with only one specimen to give any definitive statement about the population of trout in Lake Louise, but the one fish collected is judged to be "pure" westslope cutthroat.

Specimens collected at site 108 (river kilometer 1.9, at the trailhead for trail 1424) are also judged to be "pure" westslope cutthroat trout. Basibranchial teeth were found to be deeply imbedded below the surface of the skin on the tongue, but all of the fish collected have basibranchial teeth (1-12, 6.2). Scale counts for this population are very high (lateral series: 197-208, 202.8; above the lateral line: 40-45, 43.0). Pyloric caeca numbers are very low (29-34, 30.6), as would be expected for "pure" westslope cutthroat trout. Gill raker numbers are also low (16-18, 16.4) which is somewhat lower than most populations of westslope cutthroat but not entirely unusual for a stream population (Behnke 1992). No development of the posterior side

of the first gill arch was noted in any of the specimens, which is also expected of westslope cutthroat trout. Mitochondrial DNA haplotypes for the ND-1 and cytochrome B gene are consistent with morphological findings. All of the specimens had RFLP patterns that are diagnostic for westslope cutthroat trout.

Lake Creek (Site 106, N=6, 131-152 mm)

Lake Creek was sampled at river kilometer 6.0 above its confluence with the Entiat River. Specimens collected at this location are fairly typical of interior redband rainbow trout in their meristic characters (scales in the lateral series: 150-174, 160.7; scales above the lateral line: 30-32, 31; pyloric caeca numbers: 36-42, 39.0). None of the collected specimens have basibranchial teeth, and gill raker numbers are low but consistent (17-18, 17.8). There are subtle variations within the sample in spotting pattern and primitive parr marks that suggest multiple sources of the population. The population is judged to be a "good", but probably not "pure" redband rainbow. Information concerning the stocking history in this stream and the nearby portion of the Entiat River would be valuable regarding this conclusion.

North Fork Entiat River (Site 102, 103, & 105; 138-231 mm)

The North Fork of the Entiat River was sampled at river kilometer 0.2 (site 105), at river kilometers 5.2-7.2 (site 103), and at river kilometer 11.6 near the confluence with Grouse Creek just below a barrier falls (Site 102). The sample of fish from site 105 are rainbow trout from multiple sources. None of the specimens have basibranchial teeth. Low pyloric caeca numbers (29-43, 37.2) and relatively high scale counts (lateral series: 147-160, 153.0; above the lateral line: 26-27, 27.2) indicate the specimens are primarily interior redband rainbow trout, but variations in spotting patterns, and the lack of primitive parr marks in 2 of the specimens show an influence from non-native hatchery (coastal) rainbow trout in the population. The brook trout collected at this location shows no indication of hybridization with bull trout. The dorsal fin has typical brook trout spotting, and the ventral fins have the characteristic tri-colored marking on their lower edge of the fins. The mtDNA haplotypes from RFLP patterns of the cytochrome b and ND-1 genes are also those expected from brook trout (Table 4).

Specimens collected from site 103 (river kilometers 5.2-7.2) are mostly interior redband rainbow trout, with some degree of hybridization with cutthroat trout. One specimen is a "good"

westslope cutthroat phenotype (211 scales in the lateral series, 36 scales above the lateral line, 2 basibranchial teeth, with typical cutthroat spotting pattern). Three specimens are "good" redband rainbow phenotypes (spotting pattern and primitive parr marks with obvious secondary rows), and two specimens are intermediate between rainbow and cutthroat (spotting pattern and parr marks).

Specimens collected at site 102 are "good" examples of westslope cutthroat trout, but not "pure" cutthroat. One of the specimens lacks basibranchial teeth, and the spotting patterns indicate some limited gene flow with rainbow trout (many spots in front of the dorsal fin and below the lateral line, and numerous spots on the head (38 & 25) in 2 specimens). Scale counts are very high (lateral series: 185-212, 200.2; above the lateral line: 35-38, 36.6), and pyloric caeca numbers low (28-36, 33.2), which indicates that the population is only slightly hybridized. While not completely "pure", this is a "good" phenotypic representative of the native cutthroat in the Mid-Columbia basin and no further stocking is recommended in this portion of the North Fork Entiat.

South Pyramid Creek (Site 104, N=5, 153-196 mm)

Pyramid Creek was sampled at river kilometer 1.9 where trail 1439 first crosses the creek. Specimens collected have most meristic characters typical of interior redband rainbow trout (no specimens with basibranchial teeth; scales in the lateral series: 142-156, 149.4; pyloric caeca numbers: 33-41, 36.6) The numbers of gill rakers (15-18, 17.2) and scales above the lateral line (25-28, 26.2) are lower than expected from a "pure" population of redband rainbow trout, and there is variability in the spotting patterns and parr marks that are likely due to gene flow with non-native hatchery rainbow trout. The population is judged to be "good", but not "pure" redband rainbow trout.

Wenatchee River Subbasin

Lower Wenatchee River (Mouth to Icicle Creek) :

Mission Creek (Site 307, N=10, 121-181 mm)

Mission Creek was sampled at river kilometer 16.1 (site 307) above its confluence with East Fork Mission Creek. Brook trout and interior redband rainbow trout were collected. Brook trout showed no signs of hybridization with bull trout (distinct rows of spots in the dorsal fin, and tri-colored pectoral, pelvic, and

anal fins; all specimens have mtDNA haplotypes specific to brook trout in the cytochrome b and ND-1 genes (Table 4)).

Rainbow trout from this location are interior redband. Specimens have typical redband rainbow meristic characters (scales in the lateral series: 162-170, 165.0; scales above the lateral line: 31-33 32.0; pyloric caeca: 33-48, 43.0). All specimens had primitive parr marks, but secondary rows are not clearly developed. The spotting patterns are different from other populations of "pure" indigenous redband rainbow trout observed in the Mid-Columbia sub-watersheds (e.g. Toats Creek and Sweat Creek in the Okanagan subbasin- see Appendix B.). No basibranchial teeth were detected in any of the specimens. While this population is judged to be interior redband rainbow trout, it is not likely a native population. Stocking history of Mission Creek is similar to other streams in the Wenatchee subbasin, and multiple plantings (including brook trout) have likely had some affect.

East Fork Mission Creek (Site 308, N=5, 105-133 mm)

Only brook trout were collected at river kilometer 2.1 of the East Fork of Mission Creek. None of the specimens show signs of hybridization with bull trout. All fish have distinct spots in the dorsal fin, and tri-colored ventral fins. Mitochondrial DNA RFLP patterns from the cytochrome b and ND-1 genes are typical of brook trout sampled in the Columbia basin and elsewhere (Table 4).

Peshastin Creek Watershed

Ingalls Creek (Site 210, N=5, 119-148 mm)

Ingalls Creek was sampled just above its confluence with Peshastin Creek. Specimens collected are primarily interior redband rainbow trout, but there is some indication of a slight influence of coastal rainbow in the population. Scale counts and gill raker numbers are similar to other nearby Peshastin Creek tributaries (lateral series: 151-166, 159.8; above the lateral line: 29-33, 30.8; total gill rakers: 18-20, 18.8), but there is a greater range for pyloric caeca counts (28-55, 41.2). While the mean number of caeca is typical for redband rainbow, it is not expected to observe 55 caeca in an individual unless there has been some gene flow with non-native coastal (hatchery) rainbows. There is also a fair amount of variation within the sample in spotting patterns and secondary rows of primitive parr marks,

which also imply different sources of rainbow trout in the population.

Negro Creek Site 206, N=6, 125-145 mm.

Negro Creek enters Peshastin Creek at river kilometer 16.1 and was sampled just above the confluence (river kilometer 1.1). Specimens collected are interior redband rainbow, very similar to rainbow trout collected about 8.0 kilometers upstream in North Schaser Creek. Lateral series counts are slightly high for redband rainbow (146-172, 162.8) and scales above the lateral line are 31-36 (33.7). Pyloric caeca numbers are quite typical for redband rainbow (33-48, 40.0), as are gill raker numbers (18-21, 19.2). One specimen has 7 vestigial gill rakers on the posterior side of the first gill arch, which is rare in interior redband rainbow trout. None of the specimens have basibranchial teeth, and spotting patterns are variable within the sample (a range of spots on the head was observed, similar to fish in other Peshastin Creek tributaries). There are two distinct types of parr marks observed in the population, indicating that this is probably not an indigenous "pure" native population of rainbow trout. Mitochondrial DNA RFLP patterns of the cytochrome b and ND-1 genes are diagnostic for rainbow trout, but could not unambiguously identify interior redband from coastal rainbow trout.

North Schaser Creek (Site 203, N= 5, 115-165 mm)

Schaser Creek enters Peshastin Creek at river kilometer 22.9. Specimens collected at river kilometer 1.9 of North Schaser Creek (site 203) are judged to be "good" interior redband rainbow trout. Scale counts are slightly high for redband trout (lateral series: 157-173, 164.6; above the lateral line: 31-32, 31.4), as are pyloric caeca numbers (37-49, 42.6). No specimens have basibranchial teeth and gill raker numbers are typical for redband trout (19-21, 20.0). All of the specimens have primitive oval parr marks, but the secondary rows of parr marks are vague in several specimens. The population is fairly uniform in overall spotting pattern, with some degree of variability in the number of spots on the heads of specimens collected. While all characters are within the expected range for interior redband trout, there is just enough variability in spotting characteristics within the sample to question if this is indeed an indigenous "pure" population. Stocking history of North Schaser Creek would help to clarify the status of this population.

Upper Wenatchee River (Icicle Creek to Headwaters) :

Chiwaukum Creek (Site 301, N=4, 131-180 mm)

Chiwaukum Creek was sampled at river kilometer 4.8 (approximately 1.6 kilometers above the end of the dirt road). There is a barrier falls at river kilometer 6.9 of Chiwaukum Creek, and anadromous steelhead and chinook salmon utilize the creek up to the falls. Specimens collected are interior redband rainbow trout. Meristic characters are typical for redband rainbow (scales in the lateral series: 158-165, 160.5; scales above the lateral line: 29-33, 30.8; pyloric caeca numbers: 38-45, 42; gill rakers: 18-21, 19.5). All specimens lack basibranchial teeth. There is some variability in the spotting pattern (spots on the head), and all specimens have primitive parr marks. It is likely that anadromous steelhead have contributed to the genetic structure of the population. While there is no indication of cutthroat influence from the sample, it was not possible to determine unambiguously the degree, if any, coastal rainbow alleles have affected the trout at this location. The specimens are judged to be "good" interior redband rainbow trout. Knowledge of the stocking history of this stream would be valuable to further clarify the status of this population.

Chiwawa River Watershed

Chiwawa River, mainstem (Sites 401 & 407, N=7, 163-246 mm)

The Chiwawa River was sampled at river kilometer 30.6 (site 407), just above 19 Mile Campground, and near its headwaters (site 401) at river kilometer 56.0. Specimens from the lower site are interior redband rainbow with influence from non-native coastal rainbow trout (undoubtedly the result of stocking). Scale counts are in the range of redband rainbow trout (lateral series: 142-153, 147.5; above the lateral line: 24-28, 26.0) but are too high for a population derived solely from coastal rainbow trout. Pyloric caeca numbers are high (58-63, 60.5) and can only be the result of genetic input from coastal (hatchery) rainbow trout. None of the specimens have basibranchial teeth, and spotting patterns are very different from any "pure" interior redband rainbow population collected from the Mid-Columbia basin (size and distribution of spots, particularly number of spots on the head). Parr marks were not visible in the specimens and are assumed to be derived (coastal). Most interior redband rainbow trout retain primitive parr marks as adults.

Specimens collected at river kilometer 56.0 (site 401) are westslope cutthroat trout. While these fish are not entirely

"pure", they are "good" representatives of native westslope cutthroat trout. All specimens have basibranchial teeth (5-12, 7) as is expected of a population of pure cutthroat trout. Scale counts are high (lateral series: 187-214, 197.0; above the lateral line: 38-42, 40.4), and pyloric caeca numbers are low (27-39, 33), all within the range of westslope cutthroat. Gill raker numbers are somewhat high (19-20, 19.6), and spotting patterns are not completely consistent within the population. One specimen has 49 spots on the head, which is not found in "pure" populations of westslope cutthroat. Size and shape of the spots are typical of *O. lewisi* (small, irregular in outline). Stocking information indicates a planting of Tokul Creek hatchery fish (coastal cutthroat trout) in 1962. No exact location is given, but if coastal cutthroat trout were planted above Buck Creek in 1962, it would explain the spotting variability observed in this sample. The same effect could be derived from small amounts of gene flow with rainbow trout. While there is some indication of a small amount of historic gene flow with non-native cutthroat or rainbow trout in this location, this population remains a "good" example of native cutthroat from the Mid-Columbia basin and should be managed accordingly.

Chikamin Creek (Sites 410, 411 & 412, N=32, 113-154 mm)

Chikamin Creek which enters the Chiwawa River at river kilometer 22.2 was sampled above the confluence at river kilometer 2.3 (site 410), river kilometer 5.5 (site 411), and at an upstream location at river kilometer 8.9 (site 412). Bull trout were collected at all sites, but brook trout were also present at site 410. Despite the sympatric occurrence of bull trout and brook trout at site 410, no hybridization was detected in any of the specimens. All individuals identified as brook trout in the field have morphological characteristics (tri-colored ventral fins, and rows of spots in the dorsal fin), and mtDNA haplotypes that are diagnostic for brook trout (RFLP patterns of the cytochrome b and ND-1 genes (Table 4)). All specimens identified as bull trout had mtDNA haplotypes specific to bull trout.

Rainbow trout collected at site 410 are mostly interior redband rainbow, but show evidence of genetic input from various other sources. The extensive stocking of Chikamin Creek is apparent primarily in the spotting characteristics of this sample. Scale counts are in the range of redband rainbow trout (lateral series: 133-153, 144.5; above the lateral line: 24-28, 26.4). Pyloric caeca numbers are high (39-56, 48.6) and are likely the result of genetic input from coastal (hatchery) rainbow trout.

None of the specimens have basibranchial teeth, and spotting patterns are different from "pure" interior redband rainbow trout (size and distribution of spots, particularly number of spots on the head). Parr are marks rounded (coastal rainbow) in 2 specimens, and intermediate in the rest.

At site 411 (river kilometer 5.5), cutthroat trout were collected in addition to bull trout. Specimens from this location are judged to be mostly cutthroat in outward appearance, but are clearly hybridized with rainbow trout. Scale counts are much lower than "pure" or "essentially pure" westslope cutthroat sampled in the Chiwawa River drainage (lateral series: 139-194, 160.7; above the lateral line: 31-43, 36.2). Pyloric caeca numbers are not outside the range for westslope cutthroat, but are higher than nearby "pure" populations (32-49, 39.0). Only one of six specimens collected has basibranchial teeth- a clear indication of extensive hybridization with rainbow trout. Presence of primitive parr marks in all of the specimens suggests that hybridization was with interior redband, as opposed to coastal rainbow trout. Chikamin Creek was stocked on several occasions in the 1930's and 1940's with cutthroat trout from Chikamin hatchery and rainbow trout originally from the nearby Leavenworth Hatchery. This stocking history is reflected in the hybrid population now present in Chikamin Creek.

Four of the five bull trout collected at site 411 show no indication of hybridization with brook trout (specimens- 041101-041104 lacked spots in the dorsal fin, and no tri-colored pigmentation of the ventral fins was detected from photographs). Specimen 041105 is a bull trout X brook trout hybrid. Photographs show light spots in the dorsal fin, and tri-colored ventral fins. Mitochondrial DNA haplotypes from RFLP patterns ND-1 and cytochrome b genes are specific to bull trout of the Columbia basin in all specimens except the putative hybrid, which has diagnostic brook trout haplotypes in the cytochrome b and ND-1 genes (Table 4). Based on earlier studies (Leary et al. 1983, 1991) it is likely this fish is a male F-1 hybrid.

Only bull trout were collected at the upstream sampling location (site 412). No indication of hybridization with brook trout was detected at this site (all specimens 041201-041205 lacked spots in the dorsal fin, and no tri-colored pigmentation of the ventral fins was detected from photographs). Digestion of amplified ND-1 and cytochrome b genes of the mitochondrial genome revealed RFLP patterns specific to bull trout of the Columbia basin (Table 4).

The presence of brook trout in the lower portion of Chickamin Creek is worthy of concern. Though hybridization was detected in only one specimen collected, brook trout are likely to have an increasingly negative impact on the bull trout of this stream. Further hybridization and/or replacement of bull trout is likely when these two species come into contact (Leary et al. 1983, 1985). It is also possible that maternally inherited mitochondrial DNA did not detect more hybridization in the specimens collected.

Rock Creek (Sites 408 & 409, N=9, 98-239 mm)

Rock Creek was sampled near its confluence with the Chiwawa River at river kilometer 0.8 (site 408) and at an upstream location at river kilometer 9.6 (site 409). Bull trout were sampled at both locations. Photographs were taken of specimens only from site 409. No indication of hybridization with brook trout was detected from either location. Photographs of bull trout specimens (040801, 040802) taken at site 409 show no spots on the dorsal fin and lack tri-colored ventral fins. From both sites, mtDNA haplotypes from RFLP patterns of the ND-1 and cytochrome b genes are typical of bull trout of the Columbia basin (Table 4).

Rainbow trout were also collected at site 408. All of the specimens have primitive parr marks, pyloric caeca numbers (44-52, 46.4), and scale counts (lateral series: 141-162, 150.4; above the lateral line: 26-31, 28.0) typical of interior redband. There is some variability within the sample in spotting patterns, and the overall phenotype is different from other "pure" interior redband rainbow in the Mid-Columbia basin. It is likely there has been some gene flow with nearby non-native rainbow trout in the Chiwawa River (e.g. site 407). Stocking records indicate extensive stocking in Rock Creek which is evident in the observed variability of the specimens collected. The sample is judged to be "good", but not "pure" interior redband rainbow trout.

Cutthroat trout collected at site 409 are judged to be "good", but not "pure" westslope cutthroat trout. All specimens have basibranchial teeth (3-11, 6.8), low pyloric caeca numbers (26-36, 31.3), and very high scale counts (lateral series: 202-219, 210.3; above the lateral line: 44-5, 46.5); but the spotting pattern variability within the sample is different than typical "pure" westslope cutthroat populations (spots are distributed in front of the dorsal fin, and too many spots on the heads of 3 specimens). Size and shape of the spots is typical of westslope cutthroat. Available information concerning the stocking history of Rock Creek indicates the stream has been planted with both rainbow and

cutthroat (presumably Twin Lakes progeny) on several occasions. With such high scale counts, low pyloric caeca numbers, and consistent basibranchial dentition, it is evident that the population in the upper portion of Rock Creek has had very limited influence from rainbow trout, and should be managed as a "good" population of westslope cutthroat pending further investigation. It is also possible that this phenotypic pattern (high scale counts, and atypical spotting with many spots on the head) is an indigenous form of westslope cutthroat trout in the MCRB that differs from westslope cutthroat throughout its range.

Phelps Creek (Sites 405 & 406, N=10, 149-208 mm)

Phelps Creek is a 3rd order tributary to upper Chiwawa River entering the latter stream at river kilometer 48.6. It was sampled at river kilometer 5.2 (site 405) and river kilometer 10 (site 406). Specimens from both locations are judged to be "good" and "essentially pure" westslope cutthroat trout, respectively. Fish from both sampling sites are similar in meristic characteristics (respectively: scales in the lateral series: 186-213, 201.6; 199-224, 211.0; scales above the lateral line: 43-45, 43.8; 34-39, 35.6; pyloric caeca: 32-47, 36.4; 32-39 35.6; gill rakers: 19-22, 20.4; 17-20, 18.8), but the upper site (406) tends to be more indicative of "pure" westslope cutthroat. All of the specimens from both locations have well developed basibranchial teeth (4-12, 8.6; 5-11, 7.6). There is more variability in spotting characteristics within the sample at the lower location, with individuals having more spots on the head and more spots in front of the dorsal fin. It appears that there has been limited gene flow with interior redband rainbow trout in the lower portions of Phelps Creek (spotting characteristics), but not in the headwater reaches.

This stream was stocked with both cutthroat and rainbow trout from the Chiwaukum hatchery in the late 1930's and 1940, which could explain the observed variability. Mitochondrial DNA analysis of cytochrome b and ND-1 genes found all specimens from site 406 to have diagnostic haplotypes associated with westslope cutthroat trout. Phelps Creek should be protected from further stocking of non-native trout, as it appears to have a strong population of westslope cutthroat trout.

Buck Creek (Site 402, N=5, 154-179 mm)

Buck Creek was sampled above its confluence with the upper Chiwawa River at river kilometer 0.8. Specimens collected are

"essentially pure" westslope cutthroat trout. All individuals have basibranchial teeth (1-6, 3.8), but the number of teeth in each specimen is somewhat low. Scale counts are very high (lateral series: 198-212, 206.4; above the lateral line: 42-44, 43.0) and pyloric caeca numbers low (25-32, 29.2), both indicative of a "pure" population of cutthroat trout. No development of the posterior side of the first gill raker was observed. The range of variation in these characters is quite low, which is typical of an indigenous, or single source population. Gill raker numbers are high for westslope cutthroat (18-22, 20.2), but other populations of westslope cutthroat in the Mid-Columbia basin collected in this study tend to have relatively high gill raker counts. The spotting patterns are very typical of "pure" westslope cutthroat. All specimens have spots well concentrated toward the caudal region, with no spots below the lateral line anterior to the dorsal fin. One specimen has slightly larger spots than the rest of the sample. Mitochondrial DNA haplotypes from RFLP products of the cytochrome b and ND-1 genes are diagnostic for westslope cutthroat, corroborating the morphological findings.

Nason Creek Watershed

Nason Creek, mainstem (Sites 704 & 705, N=13, 133-231 mm)

Nason Creek, a 3rd order tributary to the Wenatchee River, enters at river kilometer 86.3 and contributes 18 % of the flow of the Wenatchee River. Steelhead and Chinook dominate the creek up to river kilometer 27, where Gaynor falls provides a barrier to anadromous fish. Collections were made below the falls from several locations (sites 704, & 705) at river kilometers 8.0, 25.3, and 26.6. An additional collection was made at a headwater location at river kilometer 41.2 (site 701), near the source of Nason Creek.

Specimens collected from below the barrier falls at river kilometers 8.0, 25.3 and 26.6 are very similar, and are considered as one population. Meristic characters are indicative of a major influence from interior redband rainbow in the population, but spotting characteristics, particularly the presence of derived (coastal rainbow) rounded parr marks in the specimens, demonstrates the presence of genetic input from hatchery rainbows (scales in the lateral series: 142-156, 150.1; scales above the lateral line: 22-34, 28.8; pyloric caeca numbers: 28-59, 39.3; total number of gill rakers on the first gill arch: 16-20, 18.4.

None of the specimens have basibranchial teeth, indicating cutthroat trout alleles are probably not present in the population. About half of the specimens have rounded parr marks which are the result of genetic input from coastal rainbow in the population. This is undoubtedly the result of historic stocking of hatchery rainbow trout in Nason Creek.

Roaring Creek (Sites 711 & 712, N=15, 105-201 mm)

Roaring Creek is a 2nd order tributary to the Nason Creek entering at river kilometer 86.3. It was sampled at two locations: site 712 near the mouth (river kilometer 1.3) and site 711 at river kilometer 3.9. Brook trout were collected at both sites and show no evidence of hybridization with bull trout (all specimens have distinct rows of spots in the dorsal fin and tri-colored ventral fins; mtDNA haplotypes from restriction digests of the cytochrome b, ND-1 and ND-2 genes are diagnostic for brook trout (Table 4)).

Rainbow trout were also collected at site 712. Specimens are primarily interior redband rainbow that are hybridized with cutthroat trout. Meristic analysis found characters more typical of westslope cutthroat: (scales in the lateral series: 172-188, 177.2; scales above the lateral line: 30-36, 34.6 pyloric caeca numbers: 37-43, 39.8; total number of gill rakers on the first gill arch: 18-21, 19.2; one specimen has basibranchial teeth). Spotting patterns are intermediate with few spots on the heads but randomly distributed along the anterior portion of the body. All specimens have primitive parr marks. The samples from site 712 are indicative of a rather extensive stocking history of the Wenatchee River and are clearly hybrids.

Gill Creek (Sites 709 & 710, N=18, 99-187 mm)

Gill Creek is a very small (1st order), high gradient (18%) tributary to Nason Creek entering at river kilometer 16.3. Collections were made just above the confluence at river kilometer 0.5 (site 710) and at an upstream location (site 709) at river kilometer 4.2. Specimens from site 709 are "good" westslope cutthroat, and the collection from the lower (site 710) location are rainbow X cutthroat hybrids.

Meristic analysis of specimens from site 709 shows characters within the expected ranges for westslope cutthroat, but scale counts are lower than "pure" populations in other tributaries to Nason Creek (scales in the lateral series: 162-197, 176.3; scales above the lateral line: 31-36, 32.8; pyloric caeca numbers:

29-46, 35.1; total number of gill rakers on the first gill arch: 18-21, 19.4). Two of the 8 specimens lacked basibranchial teeth, which is evidence of hybridization with rainbow trout. Spotting patterns are fairly uniform within the sample, but enough variability exists to challenge the absolute purity of this population. It is possible that this collection was made near the end of a hybrid zone, as most of the individuals have excellent westslope cutthroat phenotypes, and only slight indications of gene flow from rainbow is detectable in the sample. Additional sampling to the source at Lake Ethel would be helpful to determine if a barrier or "pure" westslope cutthroat are present higher in the drainage.

Samples taken from near the confluence with Nason Creek represent a rainbow X cutthroat hybrid swarm. Meristic characters (high scale counts, and relatively low pyloric caeca numbers) indicate interior redband rainbow to be a major contributor to the genetic structure of the population (scales in the lateral series: 161-208, 184.7; scales above the lateral line: 30-47, 39.9; pyloric caeca numbers: 29-47, 38.5; total number of gill rakers on the first gill arch: 17-20, 18.3). Over half of the specimens (6 of 10) lack basibranchial teeth. The four specimens that have basibranchial teeth, also have very high scale counts and low pyloric caeca numbers, as well as excellent cutthroat spotting patterns, suggesting some degree of reproductive isolation or movement from an upstream cutthroat population may be occurring in the stream. The remaining individuals have spotting patterns that are intermediate between rainbow and cutthroat and are clearly hybrids.

Whitepine Creek (Sites 707 & 708, N=12, 133-189 mm)

Whitepine Creek is a 3rd order stream that enters Nason Creek below the barrier at Gaynor Falls at river kilometer 24.8. Collections were made at river kilometer 2.1 (site 708) and at river kilometer 4.8 (site 707). Though Mullan et al. (1992) report the limits of anadromous fishes at kilometer 2.4 of Whitepine Creek, suggesting the presence of a barrier, collections from above and below this point are very similar. Samples from these two locations are considered as one population here. Meristic analysis shows a dominance of interior redband rainbow alleles in the population, (sites 707 & 708 respectively: scales in the lateral series: 159-164, 162.2; 147-160, 152.8; scales above the lateral line: 26-35, 31.2; 32-34, 33.2; pyloric caeca numbers: 47 (one specimen only); 26-55, 36.7; total number of gill rakers on the first gill arch: 18-21, 19.4; 17-20, 18.6; and

all specimens lack basibranchial teeth). Spotting patterns suggest a limited amount of gene flow with cutthroat trout (several specimens with spots strongly concentrated toward the caudal region, and no spots on the head). Overall spotting variability within the sample, and the presence of rounded parr marks in roughly 1/3 of the specimens suggest the influence of coastal (hatchery) rainbows in the population. The trout from Whitepine Creek are judged to be redband rainbow with limited hybridization with both cutthroat and coastal rainbow trout.

Wildhorse Creek (Site 706, N=4, 133-213 mm).

Wildhorse Creek is a tributary to Whitepine Creek. Specimens collected at river kilometer 1.3 (site 706) are mostly interior redband rainbow, but spotting patterns suggest some limited hybridization with cutthroat trout has occurred in this stream. Meristic characters are indicative of redband rainbow (scales in the lateral series: 148-164, 158.5; scales above the lateral line: 32-35, 33.3; total number of gill rakers on the first gill arch: 18-21, 19.3); pyloric caeca numbers are not available as internal organs were not present in the specimens). None of the specimens have basibranchial teeth, indicating genetic contribution from cutthroat is limited. Spotting patterns are variable, with one individual having "good" westslope cutthroat spotting characteristics. Overall, the sample is similar to collections from Whitepine Creek.

Smith Brook (Sites 702 & 703, N=10, 105-178 mm)

Smith Brook is a small headwater tributary to Nason Creek (entering at river kilometer 36.2). Samples were collected near the confluence, at river kilometer 0.5 (site 703) and about half way to the source (river kilometer 1.9) at site 702. Specimens from the upstream location are judged to be "essentially pure" westslope cutthroat trout. Meristic characters are all within the expected ranges of westslope cutthroat (scales in the lateral series: 162-192, 178.0; scales above the lateral line: 31-41, 36.2; pyloric caeca numbers: 29-37, 34.6; total number of gill rakers on the first gill arch: 18-20, 18.8). All of the specimens have well developed basibranchial teeth (3-11, 6.4). Spotting patterns represent excellent cutthroat phenotypes with spots strongly concentrated toward the caudal region and no spots in an arc extending from about the pelvic fins to just above the lateral line. No spots are present in front of the dorsal fin and below the lateral line, except a few on the head which seems to be typical of westslope in the Mid-Columbia basin. One specimen has

distinctly larger spots than the rest of the sample. This population is similar to those known to have been stocked from Twin Lakes and could be the result of historic stocking. Restriction digests of the cytochrome b gene produced RFLP patterns diagnostic for westslope cutthroat.

Specimens collected at site 703 above the mouth of Smith Brook (river kilometer 0.5) are rated as "good" westslope cutthroat trout, but slight variability in the spotting patterns suggests a very limited input from another source in the population. Specimens have meristic characters similar to the fish collected about 1.6 kilometers upstream (scales in the lateral series: 182-195, 187.4; scales above the lateral line: 32-37, 33.8; pyloric caeca numbers: 34-41, 37.0; total number of gill rakers on the first gill arch: 18-20, 19.6; and all specimens have basibranchial teeth (3-7, 4.2). There is slightly more variability within the sample in spotting patterns, with 2 individuals having spots in the lower anterior portion of the body where westslope cutthroat trout generally lack spots, and one specimen has larger spots. Available information on historic stocking shows that coastal cutthroat trout from the Tokul River Hatchery were planted in Smith Brook in the 1960's. Historic gene flow with coastal cutthroat would explain the spotting variability in the sample from site 703. Based on the subtle variability in spotting, the population in the lower section of Smith Brook is not "pure" westslope cutthroat trout. Specimens from the upper reaches of the stream of Smith Brook, however, do not show signs of introgression with coastal cutthroat or rainbow trout, and should be managed as a native westslope cutthroat trout population.

Nason Creek Headwaters (Site 701, N=6, 133-175 mm)

Specimens collected at site 701 are "essentially pure" westslope cutthroat trout. Scale counts in the lateral series were 197-216, 206; and above the lateral line: 44-48, 46.3. Specimens also have relatively low pyloric caeca numbers (30-41, 34.2), and higher than typical gill raker numbers (20-22, 21.5). All of the specimens have well developed basibranchial teeth (4-10, 7.2). Spotting patterns are consistent within the sample, and spots are typical of westslope (small, irregular in outline). The presence of this population of native cutthroat trout in the headwaters of Nason Creek is interesting, as samples from the lower portions of the creek indicate historic stocking has occurred in the system. There are records of stocking Twin Lakes westslope cutthroat in Nason Creek, but specific locations were not

determined. It is not known if the uppermost reaches of the stream were stocked with a "pure" cutthroat or if this represents a relict native population. Mitochondrial DNA analysis of cytochrome b and ND-1 genes found all specimens from site 701 to have diagnostic haplotypes associated with westslope cutthroat trout.

Little Wenatchee River Watershed

Little Wenatchee River, mainstem (Sites 601 & 611, N=10, 104-232 mm)

The Little Wenatchee River has a barrier to upstream fish migration at river kilometer 12.6. Specimens were collected above the falls at river kilometers 14 (site 601) and below the falls at river kilometer 13.5 (site 611). Above the falls, "essentially pure" westslope cutthroat were collected. Specimens have very high scale counts (lateral series: 206-217, 211.6; above the lateral line: 41-47, 44.0), which are among the highest reported for any taxa of trout (Behnke 1992). Pyloric caeca numbers are low (29-41, 33.4) as expected for a pure population of cutthroat trout. All of the specimens collected have well developed basibranchial teeth (3-10, 5.4). Gill raker numbers are higher than most populations of westslope cutthroat (19-22, 20.2), but not unlike other populations of westslope cutthroat in the Mid-Columbia basin collected in the present study. Spotting patterns are very consistent in the collection, and all of the specimens have typical *O. c. lewisi* spots (small, irregular in outline). The presence of this "essentially pure" westslope cutthroat population above an apparent barrier falls is likely due to stocking. Available information regarding the stocking history of the river note plants of cutthroat trout from Chiwaukum Hatchery and several plantings of rainbow trout and steelhead, but exact locations are not recorded. It is possible that these fish are indigenous cutthroat (based on high scale and gill raker counts) that were either native above the falls or carried above the falls from a source that contained indigenous cutthroat.

Specimens collected from site 611 below the falls are "good" interior redband rainbow, but probably not "pure". Scale counts (lateral series: 139-152, 145.8; above the lateral line: 26-28, 26.5) and pyloric caeca numbers (36-45, 40.5) are typical of interior redband rainbow. None of the specimens have basibranchial teeth, and all have primitive parr marks. There is variability in the spotting patterns within the sample and there are subtle differences between this population and others

determined to be "pure" interior redband rainbow in this study (prominence of secondary rows of parr marks, and distribution of spots on the front of the body and head). Records of extensive planting of rainbow and steelhead tend to support this conclusion.

Lost Creek (Site 612, N=5, 156-195 mm)

Lost Creek is a very steep (29% gradient), small (2nd order) stream that enters the Little Wenatchee River 6.4 kilometers above Lake Wenatchee. The stream originates in Lost Lake 3.2 kilometers above the confluence. Lost Creek was sampled at river kilometer 0.3. Specimens collected are "essentially pure" westslope cutthroat trout. This is a bit of a quandary considering the close proximity of the site to the lower portion of the Little Wenatchee River (found to have rainbow trout below barrier falls at river kilometer 14 (site 611)) and Lake Wenatchee. Meristic characters are all within the expected ranges of the local form of westslope cutthroat, (scales in the lateral series: 188-219, 200.2; scales above the lateral line: 46-48, 46.6; pyloric caeca numbers: 29-36, 32.8; total number of gill rakers on the first gill arch: 19-22, 20.6). All specimens have basibranchial teeth (2-4, 3.0), though the number of teeth in each specimen is rather low. Spotting patterns within the sample are very uniform, and spots are well concentrated toward the caudal region with no spots below the lateral line in the front half of the fish. As is the case with several other populations of westslope cutthroat trout in this study, the spots are somewhat larger than most westslope cutthroat throughout its range. The shape of the spots is typically *lewisi* (irregular in outline, "X-shaped"). Given the extensive stocking in and around Lake Wenatchee it is possible that this population was stocked from some source of "pure" westslope cutthroat.

Rainy Creek (Site 608 & 609, N=19, 85-201 mm)

Rainy Creek is a 3rd order tributary of the Little Wenatchee River, entering at river kilometer 12.7. It was sampled near the confluence (river kilometer 0.6) at site 609, and at an upstream location at river kilometer 9 (site 608). The lower sampling location was found to have rainbow trout that show slight signs of genetic input from cutthroat trout. Meristic characters are within the expected range of redband rainbow (scales in the lateral series: 139-178, 156.4; scales above the lateral line: 28-40, 32.3; pyloric caeca numbers: 29-44, 37.5; total number of gill rakers on the first gill arch: 18-22. 19.5). One specimen has 4 basibranchial teeth, which is most probably the result of gene flow with cutthroat trout, although occasionally pure redband

populations will have individuals with a few basibranchial teeth. Spotting patterns are quite variable within the population, ranging from "good" cutthroat-like spotting patterns to "good" redband rainbow patterns. Several specimens are intermediate between both. There is also a wide range in the number of spots on the heads of individuals (0-51, 18), providing further evidence of gene flow with cutthroat trout. Stocking records indicate the planting of cutthroat in 1934 from the Chiwaukam Hatchery, and rainbow trout on several occasions from both the Leavenworth and Chiwaukum hatcheries.

Samples collected from river kilometer 9 (site 608) are judged to be "pure" westslope cutthroat trout. Meristic elements strongly support this conclusion. Specimens have very high scale counts that are quite unlikely in a population that has been genetically influenced by rainbow trout (scales in the lateral series: 214-221, 216.8; scales above the lateral line: 45-47, 46.2). Pyloric caeca numbers are within the expected range of westslope cutthroat (36-41, 37.2), as are total number of gill rakers on the first gill arch (17-20, 18.6). All specimens have basibranchial teeth (1-9, 4), although the numbers of teeth are a bit low in several individuals. Spotting characteristics are consistent within the sample, and all of the specimens have no spots below the lateral line and in front of the dorsal fin. Size and shape of spots are typical of *O. c. lewisi* (irregular in outline, "X-shaped") although somewhat larger than westslope cutthroat throughout most of its range. As previously stated, records confirm a planting of cutthroat trout in Rainy Creek in 1934. Whether these are native cutthroat persisting above the stocking location or the result of that 1934 planting (or other undocumented events) is speculative. The recurrence of the pattern of high scale counts throughout the Mid-Columbia basin supports the likelihood that these are native cutthroat. Restriction digests of the cytochrome b and ND-1 mitochondrial gene produced RFLP patterns diagnostic for westslope cutthroat, corroborating the morphological conclusions.

Snowy Creek (Site 610, N= 5, 174-198 mm). Snowy Creek is a small tributary to Rainy Creek entering at river kilometer 6.4. It was sampled at river kilometer 1.5 (site 610) above the confluence with Rainy Creek. The population of "pure" westslope cutthroat in Snowy Creek is one of the most unique sampled in this extensive survey of the Mid-Columbia River subbasin. Both meristic characters and spotting patterns are different from other populations of westslope cutthroat in this

study, suggesting this population has been isolated from gene flow, and/or was founded by a few number of individuals. There is no recorded evidence of stocking in Snowy Creek.

Scale counts are not as high as those of "pure" cutthroat in nearby tributaries of the Little Wenatchee River, and are closer to "typical" westslope cutthroat throughout its range (scales in the lateral series: 181-198, 186.6; scales above the lateral line: 31-35, 33.8). Pyloric caeca numbers are low and very indicative of a "pure" westslope population (27-37, 30.8). Total number of gill rakers on the first gill arch are somewhat high compared to other reported westslope populations (20-22, 21.0), and all specimens have basibranchial teeth (2-4, 3.4). Basibranchial teeth in most individuals are completely imbedded and rather small, quite different from most of the other populations of westslope cutthroat examined in this study. The spotting pattern clearly distinguishes this collection from other westslope cutthroat populations. Spots are very concentrated at the caudal region with no spots whatsoever in front of the anal fin, but all individuals have several spots on top of the head in front of a line extending up from the anterior margin of the opercles. Spots are typical *lewisi* in shape and size (small, irregular in outline and "X-shaped"). General taxonomic characteristics of this population indicate localized differentiation in a small isolated population, probably the result of genetic bottleneck and founder effects. Mitochondrial DNA analysis corroborates the morphological conclusions. Restriction digests of the cytochrome b and ND-1 genes produced RFLP patterns typical of westslope cutthroat.

Lake Creek (Sites 602 & 603, N=11, 126-196 mm)

Lake Creek is a 2nd order, 7% gradient stream that originates from Heather Lake. Lake Creek was sampled near its confluence with the Little Wenatchee River at river kilometer 0.6 (site 603) and at river kilometer 6.4 (site 602). Specimens collected at the lower sampling site are "good" interior redband rainbow trout, while individuals from the upstream location show influence from non-native coastal rainbow trout. Meristic analysis of the sample from the lower site (603) found characters within the expected ranges of interior redband rainbow (scales in the lateral series: 144-158, 149.4; scales above the lateral line: 28-36, 30.4; pyloric caeca numbers: 38-44, 40.8; total number of gill rakers on the first arch: 19-21, 20.0). None of the specimens have basibranchial teeth. All of the fish collected had primitive

(oval) parr marks with clear secondary rows visible, and spotting patterns were consistent within the sample.

Specimens collected from river kilometer 6.4 (site 602) are mostly redband rainbow, but have meristic elements that show signs of genetic input from coastal rainbow trout (scales in the lateral series: 140-147, 143.3; scales above the lateral line: 25-27, 25.2; pyloric caeca numbers: 39-57, 47.7; total number of gill rakers on the first gill arch: 19-21, 19.5). Specifically, individual specimens with caeca numbers outside the range for interior redband rainbow and low scale counts above the lateral line are the result of gene flow with coastal rainbow. None of the specimens have basibranchial teeth. Spotting patterns are variable within the sample, and half of the specimens do not have primitive parr marks with secondary rows as expected from "pure" redband rainbow. While individual specimens from site 602 appear to be "pure" redband rainbow, the presence of obvious non-native (hatchery) rainbow trout in the same stream suggests that these fish have been influenced by historic stocking.

Unnamed Creek (Site 604, N=5, 153-203 mm). "Unnamed Creek" refers to a small tributary to Lake Creek that enters from the south at river kilometer 0.6 of Lake Creek. It is a small, high gradient stream only about 1.6 kilometers in length. Specimens collected at river kilometer 1.3 are "pure" westslope cutthroat. This is interesting because of the close proximity of rainbow trout in Lake Creek. Meristic characters are indicative of a population of "pure" westslope (scales in the lateral series: 192-219, 205.6; scales above the lateral line: 45-52, 48.0; pyloric caeca numbers: 33-37, 35.4; total number of gill rakers on the first gill arch: 18-20, 19.0). Although scale counts are very high for westslope cutthroat (Behnke 1992), they are similar to other populations of "pure" westslope cutthroat assessed in this study. All specimens have basibranchial teeth (1-6, 3), and spotting patterns are also indicative of a "pure" cutthroat population (spots are all well concentrated toward the caudal region, with few spots anterior to the dorsal fin and below the lateral line). Specimens have somewhat larger spots than typical westslope cutthroat, but the irregular shape and outline is typical *O. c. lewisi*. Larger spots in westslope cutthroat has been documented in the John Day River and appears to be common in the Mid-Columbia as well. Restriction digests of the cytochrome b mitochondrial gene produced RFLP patterns diagnostic for westslope cutthroat.

Fish Creek (Site 606, N=5, 158-212 mm)

Fish Creek is a 2nd order tributary of the Little Wenatchee River entering at river kilometer 25.4. It was sampled at river kilometer 1.3 (site 606). Specimens collected are similar to those from nearby Caddy Creek. The population is "essentially pure" westslope cutthroat having meristic characters indicative of *lewisi*, but slight variability in spotting patterns. Specimens have high scale counts (scales in the lateral series: 187-226, 199.2; scales above the lateral line: 35-38, 36.4) and low pyloric caeca numbers (24-40, 33.6). Total number of gill rakers on the first gill arch (19-20, 19.8) are similar to fish from Caddy Creek. All specimens have basibranchial teeth which is a "good" indicator for cutthroat trout. There is some variability in the spotting patterns (individuals with spots in front of the dorsal fin and below the lateral line), and several specimens have more than 15 spots on the head. This spotting pattern, along with high scale and gill raker numbers, is not likely explained by hybridization because the pattern is too consistent. Rather, it may be the native westslope cutthroat phenotype in the Mid-Columbia area.

Caddy Creek (Site 605, N=5, 173-212 mm)

Caddy Creek is in the headwaters of the Little Wenatchee River, with its confluence at river kilometer 26.6 of the latter. Samples were collected at river kilometer 2.6 (site 605). Specimens are "good", but not "pure", westslope cutthroat. Scale counts are within the reported range of westslope cutthroat, but somewhat lower than nearby "pure" populations (scales in the lateral series: 184-201, 191.6; scales above the lateral line: 38-42, 39.4). Pyloric caeca numbers (29-36, 32.0) are indicative of westslope cutthroat, and total number of gill rakers on the first gill arch (18-22, 20.4) are typical of *lewisi* collected in the Mid-Columbia, but somewhat higher than most *lewisi* throughout its range. One of five specimens lacks basibranchial teeth, which indicates some influence of rainbow trout in the population. Spotting patterns are also indicative of genetic input from rainbow trout. Specimens have spots below the lateral line and in front of the dorsal fin, as well as too many spots on the head. Stocking in the Wenatchee River subbasin that occurred in the mid-1930's and 1940's included Caddy Creek, and the effects of plantings are observable in this stream. Hybridization with rainbow trout, however, has been minimal in this population, and the fish are phenotypically "good" westslope cutthroat trout.

White River Watershed

White River, mainstem (Sites 501 & 503, N=12, 177-239 mm)

The White River was sampled at river kilometer 20.9 (site 503) and river kilometer 28.6 (site 501). A barrier to anadromous fish is present at river kilometer 23.0. Specimens from both locations are mostly interior redband with likely genetic input from cutthroat trout in the upstream site, and show definite influence from non-native rainbow trout (likely the result of historic stocking). Scale counts for both populations are fairly typical of interior redband rainbow (site 503 & 501 respectively-lateral series: 145-154, 150.2; 154-212, 171.0; above the lateral line: 26-42, 29.2; 26-44, 34.6). Very high scale counts in the upstream site most probably is the result of gene flow with cutthroat trout, as no population of rainbow trout is known to have individuals with 212 scales in the lateral series. One specimen has extremely high pyloric caeca numbers at the lower location (site 503 (36-68, 47.2)) which must be the result of genetic input from coastal (hatchery) rainbow trout. Pyloric caeca numbers are typical for redband rainbow at the upper site 501 (32-47, 38.4). None of the specimens from either location have basibranchial teeth, which indicates that cutthroat genetic input has been minimal. Spotting patterns at both locations are variable within the samples. Most parr mark, where visible, are rounded in outline with lack of secondary rows, another indication of non-native coastal rainbow influence in the population. The sample from site 501 was also evaluated by DNA analysis. Multiple haplotypes, diagnostic for both rainbow and cutthroat, were observed from restriction digests of the cytochrome b and ND-1 genes, which is also a clear indication of hybridization. In summary, specimens from the White River samples are a mixture of native and non-native rainbow, with a slight genetic input from cutthroat the higher location.

Panther Creek (Sites 504 & 505, N=8, 155-214 mm)

Panther Creek, a 3rd order stream, enters the White River at river kilometer 21.1. It was sampled near its mouth (site 504 at river kilometer 1.6) and at river kilometer 3.2 (site 505). The samples from Panther Creek are unusual in that "good" westslope cutthroat were collected at the lower location, and only rainbow trout from the upper site. Specimens from site 504 are "good", but probably not "pure" cutthroat. They have high scale counts (lateral series: 184-203, 195; above the lateral line: 34-40,

37.3) typical of westslope cutthroat, but pyloric caeca numbers are somewhat high for a "pure" population (34-46, 41.7). All specimens have basibranchial teeth, with one individual having 2 weakly developed and deeply imbedded teeth. Spotting characteristics show slight influence from rainbow trout (many spots on the head and in front of the dorsal fin below the lateral line). Gill raker numbers (19-21, 20.3) are also high for westslope cutthroat in general, but we have observed several populations with higher gill raker numbers in this survey, and Twin Lakes cutthroat have somewhat higher gill raker numbers as well.

Specimens from site 505 (river kilometer 3.2) are a mixture of coastal and interior redband cutthroat trout, with no visible signs of cutthroat influence. Specimens have scale counts that are within the range of interior redband rainbow (lateral series: 137-154, 143.2; above the lateral line: 27-34, 31.4). Pyloric caeca numbers (36-58, 46.4) are clearly indicative of influence from non-native coastal rainbow in the population, with individual specimens having caeca numbers outside the range of interior redband rainbow trout. None of the specimens have basibranchial teeth. Spotting characteristics are variable in the sample. Both primitive (oval) and derived (rounded) parr marks are present in individual specimens, and a wide range of number of spots on the head was noted. The population appears to have been influenced by historic stocking of non-native rainbow trout. Although we did not sample bull trout in Panther Creek, it is an important spawning area for adfluvial bull trout from Lake Wenatchee. It is unknown if juvenile bull trout were in the stream.

Indian Creek (Site 502, N=6, 186-261 mm)

Indian Creek, a 2nd order stream that enters the White River at river kilometer 28.6, was sampled above the confluence at river kilometer 2.3. Specimens collected are rainbow X cutthroat hybrids, but mostly resemble interior redband rainbow trout. Scale counts in the lateral series (155-209, 174.0), and above the lateral line (34-45, 37.3), as well as numbers of pyloric caeca (29-43, 34.5) are indicative of redband X cutthroat hybrids. This is supported by the rather wide ranges of meristic counts. One specimen (50201) is phenotypically a "good" westslope cutthroat, and is the only individual in the collection that has basibranchial teeth (1).

DISCUSSION

The primary conclusion from the baseline information collected in this study is that populations of native westslope cutthroat, interior redband rainbow trout, and bull trout do remain in the Mid-Columbia basin (Appendix G, Table 2). Westslope cutthroat and bull trout were generally found in small, isolated populations in headwater reaches above some kind of physical barrier. While populations of interior redband rainbow were also found in the MCRB, extensive stocking of both coastal derived rainbow trout and steelhead confounds identification with absolute certainty.

The impacts of historic stocking of salmonids in the MCRB are extensive. Mainstem rivers and larger tributaries are dominated by trout that were stocked or show effects of hybridization with hatchery fish. The general pattern of altitudinal distribution observed in several streams in the MCRB was rainbow trout in the lower elevation, low gradient reaches, followed by a hybrid zone and finally cutthroat and bull trout in the steeper and colder headwater reaches. In our sampling, however, the hybrid zone tended to be generally longer than described by Mullan et al. (1992), and often extended to the uppermost sampling locations. It is assumed that natural barriers to reproductive interaction, such as those that are known to occur between rainbow and cutthroat trout (e.g. John Day River), tend to break down when non-indigenous trout are planted. Finally, in many tributaries of the MCRB, non-native brook trout dominate or have completely replaced native *Salvelinus* species. Locations where brook trout were the dominant or only *Salvelinus* species collected in this survey were: Beaver Creek, War Creek, Cub Creek, and Boulder Creek in the Methow subbasin; mainstem Entiat River in the Entiat subbasin; and East Fork Mission Creek and Roaring Creek in the Wenatchee subbasin.

There was strong concordance between classical taxonomic methods and mtDNA analysis. While mtDNA is an effective tool for questions of taxonomic concern, it is important to recognize some constraints (Hillis and Moritz 1990). Mitochondrial DNA is located within the cytoplasm of the cell, and is maternally inherited. It is haploid and does not undergo recombination every generation, as is the case with nuclear DNA. Populations in which hybridization has occurred will generally have multiple mtDNA haplotypes, while "pure" populations have only one haplotype. Due to the maternal inheritance of mtDNA, results must be interpreted with proper caution. It is assumed that there is no assortative mating (e.g. females of cutthroat mating with only rainbow trout males), in which case hybridization would not be

detected with mtDNA alone (Dowling and Childs 1992). Based on our experience, however, hybridization is usually detected by the presence of multiple mtDNA haplotypes, even with small sample sizes, indicating females from both taxa are usually involved in hybridization events. Low frequency introgression, however, is more readily detected at the molecular level by nuclear DNA markers or protein electrophoresis, which evaluate multiple loci expressed from the nuclear genome. These may be the preferred method when sacrifice of specimens is not an issue.

Populations identified as "pure" westslope cutthroat by classical methods all had only one mtDNA haplotype indicative of *lewisi* for the restriction enzymes used in the diagnosis. Several populations identified as introgressed by meristic analysis had two mtDNA haplotypes within the population, containing both cutthroat and rainbow diagnostic RFLP patterns. Introgression of this type is usually more recent (within the last 50 years), as closed populations tend to have a common mitochondrial haplotype.

The molecular methods employed in this study were designed to address taxonomic concerns at the species and subspecies level. We found specific diagnostic markers to be very useful for this purpose, but not sufficient to provide insight to population level questions. For population genetic considerations, more restriction enzymes would be required to show variation among and between populations of interest. Further investigations into the genetic structure of populations surveyed in this study could also incorporate allozyme information which is very effective in identifying subtle genetic differences between and among populations.

While it is difficult to ascertain the source of some "pure" populations, their persistence in the MCRB is important. All three species of interest in this study are rare throughout their ranges. Considering the paucity of large populations of westslope cutthroat, interior redband rainbow trout, and bull trout in the MCRB, special attention should be given those identified as "pure" in this baseline study.

Westslope Cutthroat

From what is known about the evolutionary histories of cutthroat trout, we assume that westslope cutthroat should have been in the Mid-Columbia basin prior to the inter-glacial period of the mid-Pleistocene (< 70,000 ybp). The presence of inland cutthroat above major barriers on the Snake, Columbia, Spokane,

and Kootenay rivers, known to have formed about that time, attest to this fact (Behnke 1988, 1992). Divergence into two distinct evolutionary groups (Yellowstone and westslope cutthroat) must have also occurred prior to the formation of these barriers. Based on this probable time of separation, we would expect to see significant divergence within the westslope cutthroat clade, but such is not the case. In general, *lewisi* tend to be uniform. The greatest divergence from the "ideal" *lewisi* occurs in the upper and mid Columbia basins, where larger spots (John Day drainage) and higher scale counts and gill raker numbers (upper and mid Columbia basins) have been documented. A major effect of the Lake Missoula floods, referred to in the Introduction, may have been to bring a lacustrine adapted population into these regions which possibly mixed with the westslope cutthroat that were already present in some systems.

We assume that the "typical *lewisi*" observed in the MCRB is the result of stocking cutthroat trout from Twin Lakes (or Kings Lake) which have the typical *lewisi* characters described above. The populations of westslope cutthroat with higher scale counts, more erratic spotting, and higher gill raker numbers are assumed to be the "local native" Mid-Columbia cutthroat: remnants of pre-Missoula floods, and/or typical of what came downstream with the floods. It is also likely that there has been interaction between indigenous westslope cutthroat and stocked cutthroat in some locations. Despite the fact that many of the "pure" cutthroat populations were founded from, or affected by, historic stocking, we were able to observe phenotypic diversity within the MCRB. Further homogenization of westslope cutthroat within the MCRB should be avoided to conserve the genetic diversity that remains.

Interior Redband Rainbow Trout

Identification of native redband rainbow trout was confounded by the extensive stocking history on the MCRB and the lack of distinct mitochondrial DNA markers available at this time. The most reliable characters for the specimens collected in this study were a combination of meristic elements (scales, pyloric caeca), spotting patterns, and presence of primitive parr marks. Mitochondrial DNA restriction patterns were able to clearly identify rainbow trout, but confident separation of coastal and redband rainbow, based on DNA alone, awaits further research. The most promising protocol to corroborate classical taxonomic methods is a combination of mitochondrial and nuclear markers including Random Amplified Polymorphic DNA (RAPD) (Williams et al.

1990), and traditional protein electrophoresis (e.g. Allendorf 1975, Currens et al. 1990; Chapman et al. 1994).

Bull Trout

When this study was initiated, there was some question, particularly in the State of Washington, as to the taxonomic status of bull trout. In particular, distinction between bull trout and Dolly Varden (*Salvelinus malma*) was assumed to be problematic. Washington's policy toward bull trout at that time are reflected in recommendations by Brown (1992) to regard Dolly Varden and bull trout as the same ecological unit, and manage these two distinct species as though there were no essential differences.

Based on earlier studies of morphology, chromosomes, and DNA, there is sound evidence as to the validity of species level differences between bull trout and Dolly Varden (Cavender 1978, 1980; Hartley 1987; Bond 1992; Phillips et al. 1989, 1992, 1995). Dolly Varden appear to be phylogenetically related to Arctic charr, whereas bull trout appear to be aligned to Japanese charr (kondzha in Russia) (*Salvelinus leucomanis*) and stone charr (*Salvelinus pluvius*) or (*S. albus*) (Cavender 1980; Behnke 1980; Phillips et al. 1994). The Haas and McPhail (1991) linear discriminant function, based on measurements of 3 taxonomic characters, also supports this conclusion. However, it should be noted that this LDF developed by Haas and McPhail is only one of many tools that may be applied to distinguish these two species, and difficulty in its application should not be interpreted as taxonomic uncertainty.

There should be no doubt as to the taxonomic identity of bull trout in the MCRB. In this study, three independent lines of taxonomic information (osteological analysis, morphological and meristic evaluation, as well as molecular genetic data generated from mitochondrial DNA) all point to the expected conclusion that the MCRB bull trout are clearly distinct from Dolly Varden. If there is sympatric occurrence of Dolly Varden and bull trout in Washington (Haas and Mcphail 1991, Brown 1992), or areas in which hybridization is occurring (Brown 1992), it is not in the MCRB.

We strongly question if bull trout and Dolly Varden should be managed as a single unit. There are certain shortcomings to such an approach, which involves the concept of "typological species" (see Mayr 1963; 1982; Mayr and Ashlock 1992; and Behnke 1992 for extensive discussion of the pitfalls of a typological

species concepts). In addition to taxonomic differences, there are significant ecological difference between these two species. Dolly Varden are generally considered to be anadromous fishes that have, in some cases, colonized inland to a limited extent. The southern form of Dolly Varden (McPhail 1961), which is native to the state of Washington, has not been reported from the eastern Cascade drainages of the Columbia basin. Dolly Varden life history revolves around anadromy, and they seem to have developed some unique migratory habits (Armstrong and Morrow 1980; Armstrong 1984). Thermal regimes and constraints may also be potentially different between the two species. Bull trout are known almost exclusively from freshwater habitats. They have adapted particular life-history strategies that are not predominant in Dolly Varden; among which are the resident, fluvial, and adfluvial strategies for energy acquisition and reproduction. Pratt (1992), Goetz (1991) Brown (1992) and Rieman and McIntyre (1993) provide excellent reviews of the life history and biology of bull trout. From an evolutionary perspective, and in light of their current listed status of bull trout under the Endangered Species Act, it would indeed be prudent to manage Dolly Varden and bull trout as the distinct species that they are. Current thinking with regard to salmonid fishes and their classification as Distinct Population Segments or Evolutionary Significant Units for the purposes of the Endangered Species Act would also support this contention (Waples 1991; Monroe and Nielsen 1994).

Recommendations for Further Evaluation

The present survey included specimens from 142 sites in the Methow, Entiat, and Wenatchee river drainages. Information on interior redband rainbow trout from several locations in the Okanagan subbasin is included in Appendix B. Taxonomic diagnoses in 1998 for westslope cutthroat trout and interior redband rainbow trout in some tributaries to the Wenatchee and Yakima River subbasins are also included in Appendix D. The Lake Chelan subbasin was not sampled, and may contain populations of bull trout and westslope cutthroat trout (interior redband rainbow were not native above Chelan Falls). This project was intended to provide a broad overview of the distribution of native trout in the MCRB, and should serve as a starting point for continued monitoring and investigation.

Specific populations determined to be important to regional managers could be examined in finer detail by molecular methods

including protein electrophoresis, chromosome number, and additional DNA analysis. In particular, the genetic structure of populations on interior redband rainbow trout in the MCRB remains unclear. We assume redband rainbow trout of the MCRB possess the LDH B2 76 allele (Currens et al. 1990), which would provide a distinctive marker between redband and non-native coastal rainbow trout in the MCRB. Another interesting question concerns the differences between "local native" westslope cutthroat identified by high scale counts and more gill rakers and the "typical" *lewisi*.

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TABLES

Table 1. Comparison of Mitochondrial DNA haplotypes of Mid-Columbia basin rainbow and cutthroat trout. In samples where RFLP products could not be unambiguously scored, haplotype designation was not included (/). Populations determined to have rainbow X westslope cutthroat hybrids (HYB), presumed interior redband rainbow, (IRRB), and "pure" westslope cutthroat (WCTP) are listed in diagnosis.

Site	Location	Cyt HinfI	Cyt MboI	Cyt RsaI	ND1 MboI	ND1 DdeI	ND1 MspI	Diagnosis
9	Crater Cr	A/B	A/C	A/B	A/B	A/ B	/	HYB
15	Little Bridge Cr	B	B	C	B	B	B	IRRB
17	W Fk Buttermilk Cr	B	C	B	B	B	B	IRRB
23	Eagle Cr	A	A	A	A	A	A	WCTP
24.2	W Oval Lk	A	A	A	A	A	A	WCTP
25	War Cr	A/B	A/C	A/B	A/B	A/ B	/	HYB
30	North Cr	A	A	A	A	A	A	WCTP
42	Cutthroat Cr	A	A	A	A	A	A	WCTP
45	Robinson Cr	A	A	A	A	A	A	WCTP
36	Methow R	B	C	B	B	/	B	IRRB
66	Tungston Lk	A	A	A	A	A	A	WCTP

Site	Location	Cyt HinfI	Cyt MboI	Cyt RsaI	ND1 MboI	ND1 DdeI	ND1 MspI	Diagnosis
77	Chewuch R	A	A	/	A	A	A	WCTP
108	Tommy Cr	A	A	A	A	A	A	WCTP
114	Cougar Cr	A	A	A	A	A	A	WCTP
206	Negro Cr	B	B	/	B	B	B	IRRB
403	Buck Cr	A	A	A	/	/	/	WCTP
406	Phelps Cr	A	A	A	A	A	A	WCTP
604	Unnamed Cr	A	A	A	/	/	/	WCTP
608	Rainy Cr	A	A	/	A	A	A	WCTP
610	Snowy Cr	A	A	A	/	A	A	WCTP
701	Nason Cr	A	A	A	A	A	A	WCTP
702	Smith Brook	A	A	A	/	/	/	WCTP

Table 2. Location of collection by river kilometer and number of specimens collected of bull trout (*S. confluentus*) captured in the Methow River (I), the Entiat River (II) and the Wenatchee River (III) subbasins.

Location	River Kilometer	N	Collection No.
I. Twisp River	43.5	5	JMR 305-309
I. W.Fk Buttermilk Cr.	5.5	10	JMR 26-27, MSR 84-91
I. South Cr	0.2	1	MSR 172
I. Reynolds Cr.	0.5	5	JMR 300-304
I. Goat Cr.	10.5, 14.5, 17.7	1,6,5	MSR 276-286, MSR 255
I. Early Winters Cr.	20.9	6	MSR 304-310
I. Lost River	6.3	1	JMR 86
I. Cougar Lake	-	5	JMR 81-85
I. Methow River	130.8	5	JMR 9103
I. Monument Cr.	0.2	5	JMR 219, MSR 456-459
I. Lake Cr.	12.4	1	MSR 403
II. Cougar Cr.	0.3	5	011501-05
II. Mad River	16.4, 29.5	10	11601-05, 11701-05
III. Rock Cr.	0.8, 9.7	12	40801-05, 40901-07
III. Chikamin Cr.	2.3, 5.5, 8.9	16	41001-5, 41101-5, 41201-07

Table 3. Comparison of Mitochondrial DNA haplotypes from select bull trout populations in the MCRB with other bull trout populations in the Columbia and Klamath rivers, and with Dolly Varden from Slippery Lake Alaska. Restriction enzyme fragment patterns are denoted by letters, and represent patterns observed by digestion by the following enzymes: for cytochrome b: AluI, DdeI, HaeIII, HinfI, MspI, RsaI, and Sau3AI; for ND-1: AluI, MspI, and RsaI; for ND-2: HaeIII, HinfI, MboI, MspI, RsaI, and Sau3AI

LOCATION	CYT-B	ND-1	ND-2
W.Fk. Buttermilk	CBC/BBB	BDD	CCCBBC
Twisp River	DBCBBBB	BDD	CCCBBC
Goat Creek	DBCBBBB	BDD	CCCBBC
Cougar Lake	CBCBBBB	BDD	CCCBBC
Methow River	CBCBBBB	BDD	-
Monument Creek	CBCBBBB	BDD	CCCBBC
Other Columbia River	CBCBBBB DBCBBBB	BDD, BED BDB, BEB	CCCBBC
Klamath River	CBCBBBB	BED	CCDBBD
Brook Trout	AAAAAAA	AAA	AAAAAA
Dolly Varden	CCBBBCB	DCB, CAB, CDE	CGBGGB

Table 4. Comparison of Mitochondrial DNA haplotypes of Mid-Columbia basin bull trout and brook trout. In samples where RFLP products could not be unambiguously scored, or PCR products were not strongly amplified haplotype designation was not included (/).

Site	Location	Cyt Alu I	Cyt Dde I	Cyt Sau 3AI	ND1 AluI	ND1 Msp I	ND1 RsaI	Diag-no sis
18	W.Fk. Buttermilk Cr	C	B	B	B	D	D	BULL
25	War Cr	A	A	A	A	A	/	BROOK
26	War Cr	A	A	A	A	A	/	BROOK
27	Reynolds Cr	C	B	B	B	D	D	BULL
28	South Cr	C	B	B	B	D	D	BULL
31	Twisp R	C	B	B	B	D	D	BULL
38, 39	Goat Cr	C	B	B	B	D	D	BULL
41	Early Winters	C	B	B	B	D	D	BULL
43	Lost R	C	B	B	B	D	D	BULL
44	Cougar Lk	C	B	B	B	D	D	BULL
47	Methow R.	C	B	B	/	/	/	BULL
51	Eightmile Cr	A	A	A	A	A	A	BROOK
50	Falls Cr	A	A	A	A	A	A	BROOK
53	Lake Cr	C	B	B	B	D	D	BULL
67	Cub Cr	A	A	A	A	A	A	BROOK
71	Twentymile Cr	A	A	A	A	A	A	BROOK
78, 79	S Fk Beaver Cr	A	A	A	A	A	A	BROOK

Site	Location	Cyt Alu I	Cyt Dde I	Cyt Sau 3AI	ND1 AluI	ND1 Msp I	ND1 RsaI	Diag-no sis
199	S Fk Beaver Cr	A	A	A	A	A	A	HYB BROOK
83	Monument Cr	C	B	B	B	D	D	BULL
101	Entiat R	A	A	A	A	A	/	BROOK
105	N Fk Entiat	A	A	A	A	A	A	BROOK
115	Cougar Cr	C	B	B	B	D	D	BULL
116	Mad R	C	B	B	B	D	D	BULL
117	Mad R	C	B	B	B	D	D	BULL
307	Mission Cr	A	A	A	A	A	A	BROOK
308	E. Fk Mission Cr	A	A	A	A	A	A	BROOK
408	Rock Cr	B	B	B	B	D	D	BULL
409	Rock Cr	B	B	B	B	D	D	BULL
410	Chikamin Cr	A	A	A	A	A	A	BROOK
411	Chikamin Cr	B	B	B	B	D	D	BULL
411	Chikamin Cr	B/A	B/A	B/A	B/A	B/A	B/A	BULL/BRO OK
412	Chikamin Cr	B	B	B	/	/	/	BULL
711, 712	Roaring Cr	A	A	A	A	A	A	BROOK

Table 5. Means, standard deviations and ranges of diagnostic meristic characters from bull and brook trout in the MCRB. All bull trout are from locations in the Methow subbasin, and brook trout are from locations in the Methow, Entiat, and Wenatchee subbasins. Data from this study (*) is compared to published data for bull trout, Dolly Varden, and brook trout from Cavender (1980).

Character	Species	Mean	Range	St. Dev.
<u>Branchiostegal Rays</u>	Bull Trout *	26.9	24-30	1.3
	Brook Trout*	22.1	19-25	1.8
Cavender (1980)	Bull Trout	27.4	24-31	/
Cavender (1980)	Dolly Varden	22.6	19-25	/
Cavender (1980)	Brook Trout	22.8	20-25	/
<u>Mandibular Pores</u>	Bull Trout *	14.6	12-16	0.9
	Brook Trout*	13.9	12-16	1.1
Cavender (1980)	Bull Trout	15.7	12-19	/
Cavender (1980)	Dolly Varden	11.9	10-14	/
Cavender (1980)	Brook Trout	14.9	12-17	/
<u>Gill Rakers</u>	Bull Trout *	16.8	14-20	1.4
	Brook Trout*	17.9	13-20	1.4
Cavender (1980)	Bull Trout	16.6	14-20	/
Cavender (1980)	Dolly Varden	20.9	19-22	/
Cavender (1980)	Brook Trout	17.7	16-22	/
<u>Pyloric Caeca</u>	Bull Trout *	26.7	22-33	2.9
	Brook Trout*	38.0	24-54	7.0
Cavender (1980)	Dolly Varden	23.1	13-24	/
Cavender (1980)	Brook Trout	38.4	23-46	/

Table 6. Site number, location, and specimen number of individual bull trout for which osteological preparations were made.

Site	Location	Collection No.
016	Buttermilk Cr	MSR 084
016	Buttermilk Cr	MSR 087
018	W Fk Buttermilk Cr	MSR 090
027	Reynolds Cr	JMR 301
027	Reynolds Cr	JMR 303
031	Twisp River	JMR 305
031	Twisp River	JMR307b
038	Goat Cr	JMR 277
039	Goat Cr	JMR 284
044	Cougar Cr	JMR 083
044	Cougar Cr	JMR 085
047	Methow River	JMR 097
047	Methow River	JMR 100
053	Lake Cr	MSR 403
083	Monument Cr	MSR 456
083	Monument Cr	JMR 219

FIGURES

Figure 1. Characteristic premaxillae from bull trout (*Salvelinus confluentus*) (right column) and Dolly Varden (*Salvelinus malma*) (left column). Bull trout specimens are from this collection. Dolly Varden are after Cavender (1980). (Line drawings by Lynn Bjork)

Figure 2. Characteristic supraethmoids from bull trout (*Salvelinus confluentus*) (right column) and Dolly Varden (*Salvelinus malma*) (left column). Bull trout specimens are from this collection. Dolly Varden are after Cavender (1980). (Line drawing by Lynn Bjork)

Figure 3. Characteristic hyomandibulae from bull trout (*Salvelinus confluentus*) (right column) and Dolly Varden (*Salvelinus malma*) (left column). Bull trout specimens are from this collection. Dolly Varden are after Cavender (1980). (Line drawings by Lynn Bjork)

APPENDICES

Appendix A. Summary of egg shipments of Yellowstone cutthroat trout eggs from Yellowstone National Park to Washington (from Varley 1979).

Appendix B. Taxonomic report on collections of interior redband rainbow trout from the Okanagan River subbasin.

Appendix C. Maps depicting the collection sites and purity ratings for salmonids captured during this study in the Methow, Entiat, and Wenatchee river subbasins.

Collection sites of bull trout (*Salvelinus confluentus*) in the Mid-Columbia basin. Collection locations are given in circles. Boxes indicate collection locations where bull trout X brook trout hybrids (*S. confluentus* X *S. fontinalis*) were collected. Collection locations are described in the text and Appendix G. Stars indicate other locations where bull trout are known to occur.

Collection locations of "pure" and "essentially pure" populations of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) in the Methow River subbasin. Collection locations are given in circles and are described in Appendix G.

Collection locations of "pure" and "essentially pure" westlope cutthroat trout (*Oncorhynchus clarki lewisi*) in the Entiat and Wenatchee river subbasins. Collection locations are given in circles and are described in the text and Appendix G.

Collection locations of "pure", "essentially pure", and "good" redband rainbow trout (*Oncorhynchus mykiss gairdneri*) in the Methow River subbasin. Collection locations in circles indicate "pure" and "essentially pure" populations, those given in squares are "good" populations. Populations are described in the text and Appendix G.

Collection sites of redband rainbow trout (*Oncorhynchus mykiss gairdneri*) in the Entiat and Wenatchee river subbasins. Collection locations given in boxes indicate "pure" and "essentially pure" populations. Collection locations are described in the text and Appendix G.

Collection sites in the Mid-Columbia River basin where brook trout (*Salvelinus fontinalis*) were captured.

Appendix D. Taxonomic diagnosis of westslope cutthroat trout and interior redband rainbow trout from the Wenatchee River and Yakima River, Washington.

TAXONOMIC DIAGNOSIS OF WESTSLOPE CUTTHROAT TROUT AND INTERIOR REDBAND RAINBOW TROUT FROM THE WENATCHEE RIVER AND YAKIMA RIVER, WASHINGTON

Report to the U.S. Fish and Wildlife Service and U.S Forest Service

by

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BACKGROUND

This report presents a taxonomic diagnosis of specimens of cutthroat and rainbow trout collected from four streams in the Wenatchee River subbasin and nine streams from the Yakima River subbasin. Five of the streams are divided into upper and lower sampling locations and are designated separate collection sites (Table 1). Specimens collected from each collection site are analyzed as separate populations. In three of these streams, there is a significant difference in specimens from the upper and lower collection sites. A total of 18 collection locations are assessed in this report (five from the Wenatchee River subbasin and 13 from the Yakima River subbasin). Diagnosis is based on three distinct lines of taxonomic information: morphological or classical taxonomic evaluation, mitochondrial DNA analysis, and analysis of a nuclear DNA gene that codes the internal transcribed spacer region (ITS) of ribosomal DNA coding regions. Morphological analysis (M) includes 212 specimens from 16 locations, and mitochondrial DNA (mtDNA) and nuclear DNA (N) analysis includes 97 specimens from nine locations. Taxonomic conclusions are summarized in Table 1. The following background information on westslope cutthroat trout (*Oncorhynchus clarki lewisi*) (WCT) and interior redband rainbow trout (*Oncorhynchus mykiss gairdneri*) (IRRB) is after Proebstel et al. (1998).

Westslope Cutthroat Trout

The westslope cutthroat trout is native to South Saskatchewan and the upper and middle Columbia and upper Missouri basins. It is assumed from karyological evidence (Thorgaard 1983) that westslope cutthroat (2N=66) are intermediate between the primitive coastal cutthroat trout (*O. c. clarki*) (2N=68) and the more derived interior subspecies: Lahontan (*O. c. henshawi*) and Yellowstone cutthroat trout (*O. c. bouvieri*) (2N=64).

Very little has been published regarding westslope cutthroat trout in the MCRB. For example, the symposium held by the American Fisheries Society (Gresswell 1988) concerning the status of interior stocks of cutthroat trout produced several papers that focus on westslope cutthroat trout, but none mention the presence of this subspecies in the Methow, Entiat, Wenatchee or Yakima river drainages. Behnke (1988) documented the occurrence of westslope cutthroat trout in the Chelan drainage, and noted the occurrence of isolated populations throughout the Mid-Columbia region (Behnke 1992). Surveys conducted in the late 1940s and early 1950s have brief references to the occurrence of cutthroat trout in the Mid-Columbia basin (Bryant and Parkhurst 1950). Surveys by Mullan et al. (1992) documented the occurrence of "cutthroat trout" throughout the Mid Columbia headwater tributaries. We assume that westslope cutthroat were present in the upper and mid Columbia by mid-Pleistocene (Behnke 1992), but it appears that sporadic distribution of *O. lewisi* in this portion of the Columbia basin is the result of late glacial or postglacial dispersion. Dymond (1931) described the mountain cutthroat (*O. c. alpestris*) from disjunct populations of *O. lewisi* in the upper Columbia basin in British Columbia (Behnke 1992).

The presence of westslope cutthroat trout in the Mid-Columbia basin was clearly affected by one of the most catastrophic geological events in the history of western North America. Glacial Lake Missoula was a massive body of water (about the size of Lake Erie and Lake Ontario combined, some 500 cubic miles of water) created by glacial ice dams where the Clark Fork River enters Lake Pend Orielle in Idaho. It is now known that these ice dams broke about 100 times during the Pleistocene (Parfit 1995), initiating a series of mammoth flood events that shaped the topography of the Columbia plateau. These floods presumably carried Lake Missoula westslope cutthroat trout into the upper and middle Columbia basins. Staggering volumes of water (more than 600 million cfs- equaling ten times the volume of all the present rivers in the world) raged through the scablands of Washington (Parfit 1995), creating massive backwater pools that presumably allowed westslope cutthroat to colonize the drainages of the east slope Cascades. These massive floods occurred repeatedly, and it is likely that trout coming in the various flood events were able to find some refugia. How these "naturally introduced" cutthroat interacted with the westslope we assume were already present is unclear.

Later invasions by redband rainbow trout replaced cutthroat in most drainages, but "pure" westslope cutthroat populations remained above barriers (e.g. Lake Chelan) and in some headwaters where temperature regimes favored cutthroat.

Based on collections described by Mullan et al. (1992) and collections in the summers of 1992 and 1993 (Proebstel et al. 1998), it is obvious that both redband rainbow trout and westslope cutthroat trout often occur in the same system. They may be allopatric or sympatric, but rainbow trout generally occupy the lower reaches. There is a transition zone where both species and/or intermediates occur. Cutthroat trout (along with bull trout) dominate the upper, higher gradient sections where annual temperature units are considerably less (Mullan et al. 1992). Confident identification is often difficult, particularly where low levels of introgression have occurred. Natural zones of sympatry between redband rainbow and westslope cutthroat trout are known to occur in the John Day River drainage in northern Oregon and southern Washington (Behnke 1992) and in the Yakima River subbasin (Yakima Fisheries Project

1995). Given the thermal regimes of the region, it is quite possible that similar zones of sympatry existed in the study area prior to settlement and subsequent translocation of trout. The extensive planting of both native and non-native rainbow and cutthroat subspecies that has occurred in the last century has undoubtedly affected natural mechanisms of reproductive isolation, adding another layer of complexity to correct identification.

Westslope cutthroat in the MCRB are either indigenous relict populations, or introduced *lewisi* from two Washington broodstock lakes (Twin Lakes- introduced population from Lake Chelan or local indigenous stocks in the Wenatchee subbasin; Kings Lake- introduced population from Priest Lake, Idaho (Behnke 1992)). The Washington Department of Fish and Wildlife (WDFW) also lists Nelson Lake, Ford Hatchery, and Washoe Park Trout Hatchery, Montana, as sources for westslope cutthroat trout in Washington (unpublished WDFW data). In most cases, Twin Lake progeny were used for cutthroat stocking in the Methow, Entiat and Wenatchee drainages (Ken Williams, WDFW, personal communication). Records of stocking from 1952-1993 in the MCRB list only Twin Lakes as a source, with the exception of one stocking in 1984 of Lake Lenore (Lahontan) cutthroat in Big Twin Lake. Lahontan cutthroat from Lake Lenore were not stocked in streams of interest here. Non-native cutthroat trout in the MCRB would most likely be from Yellowstone Lake cutthroat that were introduced many years ago, or coastal cutthroat (*O. c. clarki*) that were transported within the state of Washington from the western slope of the Cascades to the MCRB. The State of Washington received many millions of eggs from Yellowstone Lake but very few of the shipments were made directly to locations in the Wenatchee or Yakima river subbasins (Varley 1979). Two shipments of 100,000 eggs were received in "good" condition at Pateros by the Methow Hatchery in 1930, and the Okanagon County Game Commissioner in 1932. Chelan State Fish Hatchery received more than 700,000 eggs between 1930 and 1941, Colville received 550,000 eggs in six years between 1917 and 1938, Leavenworth Hatchery received more than 500,000 eggs in 1951, and the Wenatchee Hatchery received one shipment of 100,000 eggs in 1932 (Varley 1979). Shipments to the Yakima County Hatchery or the Yakima State Fish Hatchery were made in 1930, 1932, and 1938 of 100,000 eggs each year, and 500,000 eggs were received in 1950 at the Yakima State Fish Hatchery.

Interior Redband Rainbow Trout

There has been considerable confusion in regards to natural variability and historic distribution of rainbow trout in the Columbia basin. Based on many years examination of museum collections and field collections, Behnke (1992) summarized the taxonomy of interior redband trout of North America. A review of this subject is presented in Appendix B, Proebstel et al. (1998).

Long standing perceptions that resident rainbow trout and anadromous steelhead are always genetically distinct and reproductively isolated may be suspect (e.g. Currens et al. 1988; Mullan et al. 1992; Chapman et al. 1994). It is likely that in some situations, steelhead contribute to the genetic structure of "resident" populations. Conversely, based on circumstantial evidence in the Methow River, "resident" rainbow trout may contribute to steelhead runs (Mullan et al. 1992). Gene flow occurs to prevent complete separation, and gene flow is likely stimulated by introduction of both non-native hatchery rainbows and steelhead. Genetic mixing of steelhead from stocking in the study area may have begun as early as 1918 and has been extensive. Non-native coastal steelhead contributed to the gene pools of various hatchery stocks in the Mid-Columbia basin (Mullan et al. 1992, Chapman et al. 1994), and have potentially impacted populations of native resident interior redband rainbow trout. In the 1930's and 1940's, "rainbow trout" were reared in the Leavenworth, Chiwaukum and Methow Hatcheries for stocking of waters in the MCRB. In more recent years, between 1961 and 1973, a "common" Mid-Columbia broodstock of steelhead was developed and used in all Mid-Columbia River hatcheries. This broodstock was derived from commingled stocks gathered below Priest Rapids Dam (Mullan et al. 1992). In 1974, another broodstock was developed for the Wells Dam Hatchery and "residual" fish from this broodstock have been stocked in the rivers and streams of the Mid-Columbia basin (Ken Williams, WDFW, personal communication). In addition, the Winthrop National Fish Hatchery, and its historic efforts toward propagation of the "Winthrop-strain rainbow trout" has certainly impacted the rivers and streams in at least the Methow River system. Chapman et al. (1994) summarized the available information concerning the origin and number of rainbow trout stocked in the MCRB from 1949-1994: a total of 12,626,274 rainbow trout from at least 15 different brood sources. Recent records of the WDFW indicate stocking rainbow trout in the MCRB relied heavily on coastal hatchery rainbows from the Glendale, South Tacoma, Spokane, and Tokul Creek Hatcheries. Furthermore, intentional hybrid crosses were made between rainbow and cutthroat trout very early in Washington (Cranford 1912) and were widely stocked.

The accurate identification of relict populations of interior redband rainbow trout in the Mid-Columbia basin, is clearly confounded by likely genetic interaction with non-native steelhead, rainbow trout, and cutthroat trout. In light of these factors, there is little doubt that very few uncontaminated indigenous native rainbow trout populations remain.

METHODS

Collection of Specimens and Preservation of Tissue

Preservation of tissue samples for various forms of DNA analysis is normally accomplished by freezing tissue, or whole specimens on dry ice or in liquid nitrogen (Dessauer and Hafner 1984). As an alternative to freezing, tissue preservation buffers (e.g. Proebstel et al. 1993) or 100% ethyl alcohol may be used. For the purposes of the present study, specimens were collected by

electroshocking and frozen on dry ice. A small fin clip was preserved in 100% ethanol from all specimens. Morphological examination was performed on all specimens prior to DNA analysis.

Classical Taxonomic data- basis of diagnosis

Cutthroat Trout

All populations were evaluated on the basis of morphological and meristic characters. Characters useful in identification for cutthroat trout are summarized in Behnke (1992). These include: number of scales in the lateral series and above the lateral line, number of pyloric caeca, number of basibranchial teeth, number of gill rakers on the first gill arch, number and degree of development of gill rakers on the posterior side of the first gill arch, spotting pattern, coloration, size and shape of parr marks, pelvic fin ray numbers, and number of vertebrae.

For westslope cutthroat trout (*O. clarki lewisi*) coloration and spotting pattern, number and degree of development on basibranchial teeth, number of scales in the lateral series and above the lateral line, and number of gill rakers are very diagnostic. Pure populations of westslope cutthroat trout (WCT) typically have lateral series scale means between 150-200 while coastal rainbow trout have fewer scales (most specimens have between 120-140). Westslope cutthroat trout from populations in the upper and Mid Columbia basin have the highest lateral series scale counts, averaging more than 200 in some collections. It may be assumed that all native populations of westslope cutthroat trout have pyloric caeca numbers between 25-50. Numbers of gill rakers on the first arch are typically 17-21 (means of 18-19). Genetically pure cutthroat trout typically have basibranchial teeth. With the exception of a few populations of interior redband rainbow trout (*O. mykiss gairdneri*), and some populations on the western side of the Kamchatkan peninsula in Russia, rainbow trout do not have basibranchial teeth. The occurrence of more than 10% of individuals without these small teeth in a population of cutthroat trout is a good indicator of historic gene flow with rainbow trout. Conversely, in a population with predominately rainbow phenotypes, an incidence of more than 10% of basibranchial teeth is indicative of cutthroat trout genetic influence. In addition, pelvic fin ray counts in most cutthroat trout populations have mean values near 9.

Based on the examination of thousands of specimens of cutthroat and rainbow trout, spotting patterns have proven to be very useful in taxonomic evaluation (Behnke 1992). This character is not amenable to quantification, and requires the judgement of an experienced observer. For evaluation of spotting patterns in this study, a series of spotting indices was used to evaluate the observed variability in spotting patterns of each specimen, and the results were interpreted for the sample as a whole. Individual specimens were rated for their overall spotting phenotype (**SI**) from 1 to 5, where 1 represents a typical "pure" westslope cutthroat spotting pattern, and 5 represents a typical "pure" rainbow spotting pattern. The size of spots (**Ssz**) was rated from 1 to 3, with 1 being relatively small and 3 greater than or equal to the diameter of the pupil of the eye. The total number of spots on the head (**SH**) was counted for each specimen, and the sample was rated for uniformity among the specimens, or sample spotting index (**SSI**).

Identification of rainbow trout influence in a population of cutthroat trout is important because of the widespread stocking of hatchery propagated fish in the last century. With few exceptions, hatchery rainbow trout are derived from coastal rainbow trout and steelhead (*O. mykiss irideus*). Coastal rainbow typically have 120-140 scales in the lateral series, with means around 130, and much higher pyloric caeca numbers than most subspecies of interior cutthroat trout (40-70 with averages about 55). Rainbow trout have mean pelvic fin ray counts of 10. Coastal rainbow trout also lack basibranchial teeth. Spotting patterns of rainbow trout are dramatically different from cutthroat, with an even distribution of spots across the body and numerous spots on top of the head. Parr marks of coastal rainbow trout are rounded as opposed to elliptical parr marks observed in cutthroat and interior redband rainbow trout. In addition, dorsal and ventral supplemental rows of parr marks present in cutthroat and interior redband rainbow are absent or reduced in coastal (hatchery) rainbow trout. Parr marks are rated with an index where 1=primitive elliptical parr marks, 2= intermediate and 3= rounded derived parr marks. Morphological diagnosis of rainbow trout is based on scales in the lateral series and above the lateral line, pyloric caeca numbers, pelvic fin ray counts, gill raker numbers, basibranchial teeth counts, types of parr marks, and spotting patterns (Behnke 1992).

Populations are rated as "pure" (P) if all diagnostic criteria are in the expected range for the subspecies. A rating of "essentially pure" (EP) indicates the population is considered to be representative of the subspecies, but one or more of the diagnostic criteria is slightly outside the expected range. It is difficult to assess, in some cases, whether this is natural variability or due to multiple sources of the population. A population may be rated "essentially pure" if all characteristics are within the expected range, but other taxa are present in the system that are capable of hybridizing. A rating of "good" (G) describes a population where obvious gene flow with another subspecies of cutthroat or rainbow trout is detectable, but the overall phenotype is characteristic of the native taxa. In some basins, "good" populations are all that remain of the native taxa, and these should be given special attention (e.g., Dowling and Childs 1992). A "hybrid" (H) or introgressed population is one where obvious genetic input from two or more taxa of trout is present and the overall phenotype is predominately intermediate.

Molecular Genetic Data

DNA isolation and analysis

Methods described in Current Protocols in Molecular Biology (Ausubel et al. 1991), abridged by P. Evans, Brigham Young University, and D. Proebstel, Colorado State University were used for isolation of total DNA. Mitochondrial DNA methods are similar to those of Cronin et al. (1993) and Shiozawa and Evans (1994).

Total DNA was extracted from ground muscle tissues by digestion in a 400 µl solution of 10 mM Tris-HCl, (pH 8.0), 25 mM EDTA, 100 mM NaCl, 0.5% SDS, proteinase K (0.5-0.1 µg/ml) for 8-10 hours at 55 °C. The DNA was purified by one or two extractions with phenol/chloroform/isoamyl alcohol (25:24:1; vol/vol/vol) and precipitated in one to two volumes of 100% ethanol. An alternative purification procedure involved precipitation of proteins and desalting with 200 µl of 5 M potassium acetate, followed by one extraction with phenol/chloroform/isoamyl alcohol (25:24:1; vol/vol/vol) and a second extraction with chloroform/isoamyl alcohol (24:1; vol/vol). Precipitation of DNA was in one to two volumes of 100% ethanol. The DNA was then resuspended in 400 µl 10 mM Tris and 1.0 mM EDTA (pH 7.2, TE buffer) or sterile distilled water. DNA concentration was determined by absorbance at 260 nm and by estimations from ethidium bromide-stained gels. A negative control with no tissue was taken through the entire procedure.

Polymerase Chain Reaction

Mitochondrial DNA.

Amplification was performed in a 25-50 µl volume reaction containing 67 mM Tris (pH 8.8), 6.7 mM MgSO₄, 16.6 mM (NH₄)₂SO₄, 10 mM 2-mercaptoethanol, four dNTP'S (dATP, dTTP, dGTP, and dCTP) at 1 mM, two primers at .2-1 µM, total DNA (10-500 ng), and 0.2-0.5 units of Taq polymerase (Perkin Elmer). Negative controls with no added DNA and the supernatant from the tissue extraction control were included in each reaction. Primers 765 and 766 (LGL Ecological Genetics Inc.) were used in the amplification of a 1.3 kb region of the mitochondrial genome encompassing the cytochrome B gene sequence. In this analysis, the cytochrome B gene was amplified in 97 individuals and digested with 7 restriction enzymes to detect polymorphism in maternal mtDNA haplotypes. The PCR cycle consisted of 32 cycles of denaturation for 45 seconds at 95 °C, annealing for 40 seconds at 50 °C, and extension for 2.5 minutes at 70 °C.

Nuclear DNA.

Amplification was performed in a 25-50 µl volume reaction containing 67 mM Tris (pH 8.8), 6.7 mM MgSO₄, 16.6 mM (NH₄)₂SO₄, 10 mM 2-mercaptoethanol, four dNTP'S (dATP, dTTP, dGTP, and dCTP) at 1 mM, two primers at .2-1 µM, total DNA (10-500 ng), and 0.2-0.5 units of Taq polymerase (Perkin Elmer). Negative controls with no added DNA and the supernatant from the tissue extraction control were included in each reaction. Primers (BYU-LSU and BYU-SSU) were used in the amplification of a 1.6 kb region of the nuclear genome that spans three conserved genes including the ITS 1 and ITS 2 ribosomal DNA subunits and flanking region. This region was amplified in 97 individuals and cut with the restriction enzyme AluI which detects polymorphisms between rainbow and cutthroat trout. The PCR cycle consisted of 32 cycles of denaturation for 45 seconds at 95 °C, annealing for 40 seconds at 50 °C, and extension for 2.5 minutes at 70 °C.

Restriction Enzyme Digestion

The PCR amplification products of mtDNA were screened by digestion with seven restriction enzymes. These enzymes were AluI, CfoI, HaeIII, HinfI, MboI, MseI, MspI, RsaI, and Sau3AI, all of which recognize and cut four base-pair sequences of the PCR product (MboI and Sau3AI are isoschizomers- or recognize the same 4 base-pair sequence). The nuclear DNA gene coding the ITS ribosomal DNA was digested with AluI. Digestion reactions were performed in 10 µl volumes in 10X TAE buffer (1:10 volume), 1-2 units of digestion enzyme, 5 µl PCR product and a TE buffer. Reaction mixture was incubated for 1-3 hours at 37 °C, and reconstituted with 1-5 µl SDW and 1 µl dye. Digested fragments were then separated according to size on 2% agarose gels containing ethidium bromide. Gels were illuminated under ultraviolet light and photographed to identify restriction fragment patterns with a computerized imaging apparatus (Eagle-eye). Different patterns were assigned a letter to designate different mitochondrial haplotypes and nuclear genotypes.

Mitochondrial DNA

Mitochondrial DNA is maternally inherited and therefore represents maternal lineages within a population, much the same way a pedigree exhibits relatedness. The major advantage of mtDNA is to determine the relationship among populations of the same or closely related taxa. It can give insight as to the genetic make-up of populations by comparing differences in these lineages, and it can also detect the presence of unrelated lineages within a population. Due to the nature of inheritance of the mitochondria, mtDNA cannot be used to definitively identify hybrids (e.g., Dowling and Childs 1992). The direction of hybridization is apparent when mtDNA is used to support morphological, nuclear DNA or allozyme information, and the relative degree of introgression can be inferred by mtDNA frequencies within a population. The presence of several mtDNA haplotypes within a small sample is also clear indication of more than one genetic source for the population.

RESULTS AND DISCUSSION

Wenatchee River Subbasin

Site 1. Peshastin River, lower N=12 IRRB-G

Twelve specimens collected from the lower location on the Peshastin River between Ruby and Negro creeks are phenotypically redband rainbow trout, but show morphological signs of limited gene flow with coastal rainbow trout. Given the stocking history of this river, it is likely that multiple sources of rainbow trout, including hatchery coastal rainbow, and residual redband rainbow or steelhead have been introduced onto the native redband rainbow population. Twelve specimens were examined by classical methods. Based on this sample, the population is judged to be "good" (B) redband rainbow trout.

Ranges and means for diagnostic characters are: scales in the lateral series: (136-168, 151.75); scales above the lateral line: (29-36, 32.42); pyloric caeca numbers: (31-41, 35.50); total gill rakers on the first gill arch: (16-20, 18.33); pelvic fin ray counts: (9-10, 9.83); no specimens have basibranchial teeth; and spotting patterns are quite variable, with spots concentrated toward the caudal region in some individuals and evenly distributed across the body in others within the sample. Sample Spot Index is 2, indicating there is some uniformity of spotting characteristics within the sample, but variants are observable. Of particular significance is the variability in primary parr marks, both in size and shape. Six of 12 specimens have very elliptical parr marks, with well developed secondary parr marks both above and below the primary parr marks. These individuals are very typical of redband rainbow trout. Three of 12 specimens have rounded parr marks and absence of secondary parr marks, which is the expected condition of coastal rainbow trout, and 3 individuals are intermediate. Posterior gill raker development is consistent within the sample, with little or no posterior gill rakers. None of the specimens have posterior gill rakers on the first gill arch. The predominant phenotype is interior redband rainbow trout, but morphological characters suggest limited gene flow with coastal rainbow trout.

Site 2. Peshastin River, upper N=13 IRRB-EP

Thirteen specimens from site Number 2 on the upper Peshastin River were examined by classical methods. Based on this sample, the population is judged to be "essentially pure" interior redband rainbow trout. There is some variability in spotting patterns and parr mark characteristics that infer the population has multiple sources. While this collection indicates that this population is phenotypically representative of IRRB, stocking history of this section of Peshastin River should be considered.

Ranges and means for diagnostic characters are: scales in the lateral series: (150 -176, 157.82); scales above the lateral line: (30-35, 32.82); pyloric caeca numbers: (31-38, 33.80); total gill rakers on the first gill arch: (14-19, 16.50); pelvic fin ray counts: (9-10, 9.73); no specimens have basibranchial teeth or cutthroat marks; and spotting patterns are variable within the sample, with spots concentrated toward the caudal region in 3 of 12 individuals, and randomly distributed in the remaining 9 specimens. Sample Spot Index is: 1-, indicating there is good uniformity of spotting characteristics within the sample, but some variability is noticeable. The high scale counts, low pyloric caeca numbers and mean pelvic fin ray counts of 9.73 are very indicative of IRRB. No obvious characteristics of coastal rainbow trout are depicted in meristic characters. Three of the specimens have atypical parr marks and secondary rows are weak or absent. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers.

Site 3. Sand Creek N=12 IRRB-EP

Twelve specimens from site Number 3 from Sand Creek, tributary to Mission Creek, were examined by classical methods. Specimens were collected near the trailhead for Red Hill trail#1223. Based on this sample, the population is judged to be "essentially pure" interior redband rainbow trout. There is some variability in spotting patterns and parr mark characteristics that infer the population has multiple sources. Most individuals have elliptical parr marks, but 3 specimens have round parr marks. All of the specimens have well developed secondary parr marks, and orange coloration. While this collection indicates that this population is phenotypically representative of IRRB stocking history of Sand Creek should be considered.

Ranges and means for diagnostic characters are: scales in the lateral series: (148-161, 151.67); scales above the lateral line: (29-35, 32.17). These are consistently high and do not indicate coastal rainbow trout. Pyloric caeca numbers are relatively low: (31-39, 34.92) and are below the range of coastal rainbow trout. Total gill rakers on the first gill arch are: (17-20, 18.55). Pelvic fin ray counts are typical for IRRB: (9-10, 9.75) and no specimens have basibranchial teeth. Spotting patterns are very typical of pure IRRB, with the exception of 3 individuals that have rounded parr marks. All specimens have well developed secondary parr marks that are characteristic of IRRB. Sample Spot Index is: 1-, indicating there is uniformity of spotting characteristics within the sample, with slight variability. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Coloration of specimens was consistent within the sample. Individuals have orange coloration, with pronounced white on the ventral region below secondary parr marks.

Site 4. Icicle River N=12 IRRB-EP

Twelve specimens from Icicle River, collected between Frosty and Doughgod junctions where trail first comes back to the river above Frosty Creek, were examined by classical taxonomic methods. Based on this sample, the population is judged to be "essentially pure" (A-) interior redband rainbow trout.

Ranges and means for diagnostic characters are: scales in the lateral series: (144-182, 158.33); scales above the lateral line: (36-45, 37.50); pyloric caeca numbers: (36-50, 41.83); total gill rakers on the first gill arch: (17-20, 18.36); pelvic fin ray counts: (9-10, 9.58); no specimens have basibranchial teeth. The scale counts are very high for rainbow trout, and do not indicate influence of coastal rainbow trout. Pelvic fin ray counts are somewhat low for IRRB with a mean for the sample of 9.58. Typical mean pelvic fin ray counts for "pure" IRRB are between 9.6 and 9.9. Spotting patterns vary somewhat variable within the sample. Two specimens have characteristics more typical of pure WCT, with spots concentrated toward the caudal region. Sample Spot Index is: 1-, indicating there is good uniformity of spotting characteristics within the sample, but subtle variability is observable. Posterior gill raker development is consistent within the sample, with little or no posterior gill rakers. This collection depicts very good phenotypic representatives of interior redband rainbow trout, but high scale counts and low pelvic fin ray mean for the collection, along with subtle spotting variability indicate that limited gene flow with cutthroat trout cannot be ruled out. Icicle Creek has a rather extensive stocking history (Proebstel et al. 1998) and location of historic stocking relative to the collection location should be considered. Nuclear DNA and mtDNA information would be helpful to clarify the taxonomic status of this population.

Site 5. Negro Creek N=12 WCT-EP

Twelve specimens from Negro Creek, collected above the falls along road #400, were examined by classical methods and by RFLP analysis of the cytochrome B mitochondrial DNA gene and RFLP analysis of the nuclear Ribosomal RNA coding region. Based on this sample, the population is judged to be "essentially pure" westslope cutthroat trout.

Ranges and means for diagnostic characters are: scales in the lateral series: (188-216, 204.17); scales above the lateral line: (42-52, 48.17); pyloric caeca numbers: (27-36, 30.10); total gill rakers on the first gill arch: (17-20, 18.42); pelvic fin ray counts are consistent within the sample, with all specimens having 9 pelvic fin rays: (9, 9); all specimens have basibranchial teeth (1-9, 3.25). Basibranchial tooth development is feeble in 2 of 12 individuals, with only vestigial tooth development under the skin and 3 other specimens have only one basibranchial tooth. This is somewhat less than other "pure" populations of westslope cutthroat trout in the Mid-Columbia basin (Proebstel et al. 1998). Spotting patterns are somewhat atypical of WCT and subtle differences between specimens is apparent. Some individuals have spots concentrated toward the caudal region with a size and shape very typical of most WCT. The predominant spotting phenotype in the sample is with larger more rounded spots. Sample Spot Index is: 1-, indicating there is relative uniformity of spotting characteristics within the sample, but differences in distribution and size are apparent. Two of 12 specimens have spots on the top of the head. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Some specimens had one or two vestigial or very weakly developed posterior gill rakers on the first gill arch.

There are no strong indications from morphological analysis of non-native cutthroat trout. While larger rounded spots are present (which are characteristic of Yellowstone cutthroat trout), no other YCT characteristics, such as posterior gill raker development and high basibranchial teeth counts are present in the sample. The unique spotting characteristics are considered to be localized variability, or possibly the result of historical introduction of pure WCT from another source onto an indigenous population in Negro Creek. Molecular data from both mtDNA and nuclear DNA only produced haplotypes and genotypes that are typical of westslope cutthroat trout (Table 3). For the sample of 12 individuals, molecular data corroborates the conclusions based on morphological characters.

Yakima River Subbasin

Site 6. Stafford Creek N=21 IRRB-EP-H, WCT-EP-H

Twenty-one specimens from Stafford Creek were assessed by molecular methods only. Stafford Creek is a tributary to the North Fork Teanaway River. Fish were collected off of road #9703 at an elevation of approximately 2,880 ft. From the mtDNA haplotypes and nuclear DNA genotypes present in the collection, it is apparent that there are IRRB, WCT and hybrids in this population. The predominant mtDNA haplotypes are from rainbow trout, with 13 of 21 individuals having haplotypes associated with rainbow trout in RFLP digests with *HinfI*, *RsaI*, and *HaeIII*. The remaining 8 specimens have haplotypes expected from westslope cutthroat trout (Table 3). Nuclear DNA information corroborates the mtDNA data, but also shows 5 of 21 individuals with both cutthroat and rainbow genotypes. The most probable explanation of mtDNA haplotype frequency distribution and nuclear DNA data is that the population is not completely reproductively isolated. As with other locations in the Yakima River subbasin, this may be a "natural" hybrid zone. Specimens from the headwaters, and lower sections of Stafford Creek would be informative as to whether this population is essentially a hybrid swarm, or if taxa are partitioned along an altitudinal gradient.

Site 7. Silver Creek N=13 WCT-EP

Twelve specimens from Silver Creek, tributary to the Yakima River, were collected above a steep gradient area with some small falls. A hiking trail was taken to this area and the elevation was approximately 1,100 ft. Fish were examined by classical methods and by RFLP analysis of the cytochrome B mitochondrial DNA gene and RFLP analysis of the ITS nuclear ribosomal DNA coding region. Based on this sample, the population is judged to be "essentially pure" westslope cutthroat trout.

Ranges and means for diagnostic characters are: scales in the lateral series: (199-221, 209.58); scales above the lateral line: (47-51, 48.75); pyloric caeca numbers: (28-34, 30.22); total gill rakers on the first gill arch: (18-21, 18.83); pelvic fin ray counts: (9);

all specimens have basibranchial teeth (2-9, 6.50). The scale counts are very high and strongly indicate a "pure" cutthroat population. Low pyloric caeca numbers and well-developed basibranchial teeth are also typical of "pure" cutthroat. Spotting patterns are within the range of pure WCT, but variability in size of spots, and distribution on the body are slightly more than expected from a pure relict population. The source of spotting variability could be either historic gene flow with a larger population, or the result of historic stocking of trout in Silver Creek. Sample Spot Index is: 1-, indicating there is good uniformity of spotting characteristics, but some noticeable variability within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Some specimens had one or two vestigial or very weakly developed posterior gill rakers on the first gill arch. Coloration is consistent within the sample, with a predominance of red-orange shades. There is no indication of historic gene flow with Yellowstone cutthroat trout from the morphological characters.

Molecular genetic data is consistent with morphological analysis. All individuals have only mtDNA haplotypes that are associated with westslope cutthroat trout (Table 3). One individual (SIL-8) had a rare *Hinf*I haplotype C that was also observed in both WCT and IRRB from Wildcat Creek (Sites 15 and 16). Nuclear DNA analysis also produced genotypes associated with cutthroat trout. No rainbow genotypes were observed in the sample of 12 individuals (Table 3).

Site 8. Big Creek N=12 WCT-EP

Twelve specimens from Big Creek, tributary of the Yakima River were collected off of road #4110-115 at an elevation of 3,280 ft. Fish were collected in an area where there was a beaver dam and pond in part of the creek. Several brook trout were also present. Fish were analyzed by molecular methods only. From the mtDNA data it may be assumed that only cutthroat trout haplotypes are present in the collection, but digests with *Alu*I, *Msp*I and *Hae*III all produced two restriction digest patterns (Table 3). The most plausible explanation for multiple haplotypes in several restriction enzymes is that there is more than one source for the population. Relict populations tend to have common haplotypes in a small sample, or show limited haplotype variability in one or two restriction enzymes. Nuclear DNA information corroborates the mtDNA data. All 12 individuals have *Alu*I restriction digest patterns of the ITS gene that are expected from cutthroat trout (Table 3). Based on the molecular information from 12 individuals, this population is considered to be "essentially pure" westslope cutthroat trout. Stocking history of Big Creek should be considered to address the possibility of multiple sources for this population.

Site 9. Cold Creek N=1 WCT

One specimen from Cold Creek, tributary of Keechelus Creek, is a westslope cutthroat trout. This specimen has mtDNA and nuclear DNA that is characteristic of cutthroat trout. Morphological diagnostic characters are: scales in the lateral series: (191); scales above the lateral line: (41); pyloric caeca numbers: (33); total gill rakers on the first gill arch: (19); pelvic fin ray count: (9); the specimen has 2 basibranchial teeth (2); and spotting patterns are very typical of pure WCT, with spots concentrated toward the caudal region. It is not possible to make any dramatic conclusions as to the nature of the population from Cold Creek based on one individual, but it is obvious that westslope cutthroat trout are present in the stream.

Site 10. Oak Creek N=12 WCT-EP

Twelve specimens from Oak Creek were collected off of road #1400 at a beaver ponds area, elevation 3,900 ft. Fish were examined by classical methods and by RFLP analysis of the cytochrome B mitochondrial DNA gene and RFLP analysis of the nuclear Ribosomal RNA coding region. Based on this sample, the population is judged to be "essentially pure." The overall phenotype is excellent for westslope cutthroat trout, but there are mtDNA haplotypes in 2 individuals that are associated with rainbow trout (OAK-4 and OAK-7), and nuclear DNA data depicts these as having both cutthroat and rainbow trout genotypes. This population may be considered to be worthy of protection as native westslope cutthroat trout, but would not be a good candidate for transplants or recovery efforts, based on this analysis.

Ranges and means for diagnostic characters are: scales in the lateral series: (182-204, 190.08); scales above the lateral line: (44-50, 46.82); pyloric caeca numbers: (28-38, 31.45); total gill rakers on the first gill arch: (18-20, 18.92); pelvic fin ray counts: (9); all specimens have basibranchial teeth (2-8, 3.91); and spotting patterns are very typical of pure WCT, with spots concentrated toward the caudal region and very consistent within the sample. Sample Spot Index is: 1, indicating there is uniformity of spotting characteristics within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. The scale counts are not quite as high as other populations of WCT in the Mid-Columbia region, but are more typical of WCT from the John Day River (Behnke 1992).

The molecular genetic information from RFLP analysis of the cytochrome B mitochondrial DNA gene, and nuclear ribosomal DNA coding regions do not depict a genetically "pure" westslope cutthroat trout population. Diagnostic mtDNA haplotypes indicative of rainbow trout are present in 2 of 12 individuals. Nuclear DNA data depicts these 2 individuals as hybrids, with genotypes of both rainbow and cutthroat trout. While this collection shows signs of gene flow with rainbow trout, the overall phenotype is excellent WCT, and no observable characteristics of past hybridization with rainbow trout is apparent in the phenotype. The sample is very

consistent in WCT morphological characters. In the Yakima drainage, where both interior redband and westslope cutthroat trout are indigenous, it is not completely unexpected to find a certain amount of "natural" gene flow occurring between these two taxa. The important question concerning Oak Creek is whether or not this limited gene flow is the result of historic stocking, or a natural phenomenon. Phenotypically, there are no outward signs of rainbow trout in the sample. I would recommend treating this as an indigenous population, pending further information concerning the stocking history of the stream.

Site 11. Rattlesnake Creek, mainstem N=7 IRRB- "good" (G)

Seven specimens from the mainstem of Rattlesnake Creek (collection No. LRAT-1 to LRAT- 7) were examined by classical taxonomic methods. Based on this sample, the population is judged to be "good" interior redband rainbow trout (G). It is apparent from this analysis that multiple sources of rainbow trout have influenced this population, and slight coastal rainbow trout influence is inferred from meristic counts and spotting phenotypes.

Ranges and means for diagnostic characters are: scales in the lateral series: (135-167, 147.80); scales above the lateral line: (28-37, 31.14); pyloric caeca numbers: (34-48, 42.0); total gill rakers on the first gill arch: (17-20, 18.71); pelvic fin ray counts: (10-11, 10.29); no specimens have basibranchial teeth. There is a greater range in scale counts and pyloric caeca numbers than is generally observed in populations of "pure" redband rainbow. Two specimens have 11 pelvic fin rays, which is assumed to be derived from gene flow with coastal rainbow trout. Spotting patterns are variable within the sample, and parr marks in 2 of 7 individuals are indicative of coastal rainbow influence in the population. Sample Spot Index is: 2, indicating there is variability in spotting characteristics within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers.

Site 12. Rattlesnake Creek, middle N=11 IRRB X WCT HYB; upper N=1 WCT-EP

Eleven specimens from middle Rattlesnake Creek and one specimen from upper Rattlesnake Creek were examined by classical taxonomic methods. Based on this sample, it is apparent that the population is comprised primarily of interior redband rainbow trout and there is limited gene flow with westslope cutthroat trout in the middle sections. Two specimens are determined to be WCT X IRRB hybrids. This section of Rattlesnake Creek should be considered to be a hybrid zone with a predominance of redband rainbow influence. It is quite possible this is a "natural" hybrid zone. The one specimen collected from the upper section (where bull trout were also collected) is judged to be "essentially pure" westslope cutthroat trout (A-).

Ranges and means for diagnostic characters of specimens from middle Rattlesnake site (MRAT-1 to MRAT- 11) are: scales in the lateral series: (147-173, 157.55); scales above the lateral line: (28-38, 31.09); pyloric caeca numbers: (33-40, 36.25); total gill rakers on the first gill arch: (19-21, 19.82); pelvic fin ray counts: (9-10, 9.64); two specimens have basibranchial teeth; and spotting patterns are very variable, with most individuals having redband rainbow spotting characteristics. Two individuals have spotting patterns typical of WCT, with spots concentrated toward the caudal region. Sample Spot Index is: 2, indicating there is variability in spotting characteristics within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers.

Morphological characters for the specimen collected in the upper section (UR-1) are: scales in the lateral series: (189); scales above the lateral line: (39); pyloric caeca numbers: (30); total gill rakers on the first gill arch: (17); pelvic fin ray count: (9); the specimen has 6 well-developed basibranchial teeth (6); and a spotting pattern very typical of pure WCT. This individual is determined to be "pure" westslope cutthroat trout, but dramatic conclusions about the population in the upper reaches of Rattlesnake Creek would require more individuals.

Site 13. Little Rattlesnake Creek, lower (Site # 1) N=12 IRRB-EP

Twelve specimens from lower Little Rattlesnake Creek were examined by classical methods and by RFLP analysis of the cytochrome B mitochondrial DNA gene and RFLP analysis of the nuclear Ribosomal RNA coding region. Based on this sample, the population is judged to be "essentially pure" interior redband rainbow trout.

Ranges and means for diagnostic characters are: scales in the lateral series: (141-156, 148.92); scales above the lateral line: (31-36, 32.42); pyloric caeca numbers: (32-37, 34.70); total gill rakers on the first gill arch: (16-20, 17.75); pelvic fin ray counts: (9-10, 9.75); no specimens have basibranchial teeth; and spotting patterns are very typical of pure IRRB, with smaller spots as seen in other collection locations in the Mid-Columbia River subbasin. Sample Spot Index is: 1, indicating there is uniformity of spotting characteristics within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Secondary parr marks are present in all individuals, and very consistent within the sample. None of the specimens have a cutthroat mark.

Molecular information corroborates the morphological findings, but does give an indication of very limited historic gene flow with cutthroat trout. Diagnostic restriction enzymes that distinguish rainbow and cutthroat trout for digests of the cytochrome B

Mitochondrial DNA gene (Hinf I, HaeIII, RsaI, and Sau3AI) are all typical of rainbow trout. Digests with CfoI and MspI produced a polymorphisms that were observed in westslope cutthroat specimens from the upper collection location (Site 2- see below). I do not consider CfoI or MspI digests to be representative of fixed differences between rainbow and cutthroat trout, but the presence of haplotypes dominate in the cutthroat trout from the upper sampling location does suggest limited historic gene flow with cutthroat trout. Digests of the nuclear DNA ITS-1 gene also show all individuals with IRRB genotypes. My interpretation of the combined data is that this population is interior redband rainbow trout, but does potentially contain limited genetic input from westslope cutthroat trout. It is interesting that there is no morphological indication of cutthroat trout in the sample. It is likely this is a "natural" occurrence, and should not affect managing this population as IRRB.

Site 14. Little Rattlesnake Creek, upper (Site #2) N=12 WCT-EP

Twelve specimens from upper Little Rattlesnake Creek (Site 2) were examined by classical methods and by RFLP analysis of the cytochrome B mitochondrial DNA gene and RFLP analysis of the nuclear Ribosomal RNA coding region. Based on this sample, the population is judged to be "essentially pure" westslope cutthroat trout.

Ranges and means for diagnostic characters are: scales in the lateral series: (186-202, 193.00); scales above the lateral line: (39-51, 47.08); pyloric caeca numbers: (26-32, 29.09); total gill rakers on the first gill arch: (18-21, 19.33); pelvic fin ray counts: (9); all specimens have basibranchial teeth (3-13, 6.08); and spotting patterns are very typical of pure WCT, with smaller spots concentrated toward the caudal region. There is very slight variation in the size of spots within the sample, but all individuals have very characteristic westslope cutthroat spotting patterns. Sample Spot Index is: 1-, indicating there is uniformity of spotting characteristics within the sample, but slight variability in spot size. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Some specimens had one or two vestigial or very weakly developed posterior gill rakers on the first gill arch. All of the specimens have a well-defined cutthroat mark. Coloration is very consistent, with orange coloration in all specimens.

Molecular information corroborates the morphological findings, but does give an indication of very limited historic gene flow with interior redband rainbow trout. Diagnostic restriction enzymes that distinguish rainbow and cutthroat trout for digests of the cytochrome b Mitochondrial DNA gene (Hinf I, HaeIII, RsaI, and Sau3AI) are all typical of cutthroat trout. Digests with CfoI produced a polymorphism in 6 individuals that was observed in 4 of 12 IRRB specimens from the lower collection location (Site 1- see above). MspI produced a haplotype in 2 of 12 specimens observed in half of the individuals from the lower collection site. As in the sample from above, I do not consider CfoI or MspI digests to be representative of fixed differences between rainbow and cutthroat trout, but the presence of these different haplotypes that are found in the IRRB from Site 1 on Little Rattlesnake Creek suggests very limited historic gene flow with redband rainbow trout. Digests of the nuclear DNA ITS-1 gene show all individuals with a pattern that is characteristic of cutthroat trout. My interpretation of the combined data from the upper location is that this population is "essentially pure" westslope cutthroat trout. There is no morphological indication of IRRB in the sample, and the population should be managed as an essentially pure, indigenous WCT population. The presence of IRRB in the same system makes this stream very interesting from an evolutionary and ecological perspective.

Site 15. Wildcat Creek, lower (Site # 2) N=12 IRRB-EP

Twelve specimens from Wildcat Creek, tributary to the Tieton River, were collected up road #1306 at an elevation of 2,900 ft. Fish were examined by classical methods and by RFLP analysis of the cytochrome B mitochondrial DNA gene and RFLP analysis of the nuclear Ribosomal RNA coding region. Based on this sample, the population is judged to be "essentially pure" interior redband rainbow trout (A-).

Ranges and means for diagnostic characters are: scales in the lateral series: (135-159, 146.3); scales above the lateral line: (28-35, 31.42); pyloric caeca numbers: (36-42, 38.55); total gill rakers on the first gill arch: (17-19, 18.0); pelvic fin ray counts: (9-10, 9.80); no specimens have basibranchial teeth; and spotting patterns are very typical of pure IRRB, with small to medium size spots. Sample Spot Index is: 1-, indicating there is uniformity of spotting characteristics, but slight variability is observable within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Secondary parr marks are present in all individuals, and relatively consistent within the sample. None of the specimens have a cutthroat mark. Secondary parr marks are weak or absent in smaller individuals, but prominent in all larger specimens. Overall, parr marks are primitive and consistent within the collection. Red-orange coloration is consistent, and well defined in the area of the lateral band. One specimen with 135 scales in the lateral series, and very slight differences in parr marks suggest the possibility of very limited gene flow with coastal rainbow trout. Stocking history of Wildcat Creek would be helpful to determine the possibility of coastal rainbow genetic influence on the population. If any, this would be very minimal.

Molecular genetic information suggests that there are multiple sources to the population and points to the likelihood of small amounts of gene flow with cutthroat trout in Wildcat Creek. Diagnostic mtDNA enzymes have patterns associated with rainbow trout, but more than one haplotype is present in digests with HinfI, Sau3AI and HaeIII. Multiple haplotypes suggest more than one genetic source to the population. There is a rare HinfI haplotype that is present in 7 of 12 individuals, and is also present in 6 of 12 individuals from the upper collection location on Wildcat Creek which has westslope cutthroat trout (see below-site # 1). This seems to point to current or historic gene flow with cutthroat trout in the system, but it should be noted that phenotypic integrity of redband rainbow is

maintained in the lower collection location. Nuclear DNA RFLP patterns show all 12 specimens with rainbow trout polymorphisms. The presence of common mtDNA digest patterns opens the possibility of gene flow with WCT, but it is possible in this system this is part of a "natural" process. It is important to consider the phenotypic dominance of redband rainbow trout in this collection location.

Site 16. Wildcat Creek, upper (Site #1) N=12 WCT-EP

Twelve specimens examined from the upper collection location on Wildcat Creek, elevation 3,400 ft, are determined to be "essentially pure" westslope cutthroat trout. One of 12 specimens is identified both by morphological and molecular data as a hybrid WCT X IRRB (with a predominantly cutthroat phenotype). Ranges and means for diagnostic characters are: scales in the lateral series: (158-206, 187.0); scales above the lateral line: (34-51, 45.64); pyloric caeca numbers: (27-37, 31.27); total gill rakers on the first gill arch: (18-20, 18.89); pelvic fin ray counts: (9); all specimens have basibranchial teeth, with the exception of UWCA-12 which is lacking basibranchial teeth and is identified as a hybrid based on this and other meristic character values (0-12, 5.18). Spotting patterns are very typical of pure WCT, with spots concentrated toward the caudal region and very consistent within the sample. Sample Spot Index is: 1- indicating there is uniformity of spotting characteristics within the sample. The individual identified as a hybrid is also variable in spotting characteristics. No posterior gill rakers are present in the sample. Coloration is consistent and orange as has been observed in other collections described in this report.

Molecular genetic analysis reveals several interesting points. First, the individual identified morphologically as a hybrid (UWCA-12) has rainbow trout mtDNA digestion patterns for diagnostic enzymes and both rainbow and cutthroat trout nuclear DNA digestion patterns. This confirms it is a hybrid, and that the maternal genetic material is rainbow trout. Second, as stated above, there is a rare *HinfI* haplotype that is present in 6 of 12 specimens that is also found in 7 of 12 specimens from the lower sampling location where the predominant phenotype is IRRB (Table 3). This points to the conclusion that gene flow has occurred between the two taxa present in the stream, but is very significant that the overall phenotypes are being maintained along an altitudinal gradient. A small amount of hybridization (around 10%) is likely a "natural" occurrence here. This is similar to inferences from historic collections from the John Day drainage where westslope cutthroat and redband rainbow trout are sympatric (Behnke 1992).

Pending further information concerning the stocking history of Wildcat Creek, this stream should be considered to have a sympatric occurrence of "essentially pure" westslope cutthroat in the headwaters, and interior redband rainbow in the lower reaches, with an expected "hybrid zone" in the middle. As such, this is an important and unique location for native trout.

Site 17.1. Crow Creek, lower N=4; IRRB-EP

Site 17.2. Crow Creek, middle N=11 IRRB-EP, H WCT-EP, H

Specimens collected from Crow Creek (N=21) depict a distribution of redband rainbow and westslope cutthroat trout along an altitudinal gradient with rainbow trout in the lower portion, a hybrid zone in the middle, and westslope cutthroat trout in the headwaters. The specimens collected at the lowest location (Site Crow-1), elevation 2,800 ft, are judged to be "essentially pure" redband rainbow trout. There is little doubt, based on the specimens collected at the middle collection location (Site Crow-2), elevation 2,900 ft, that there is some degree of gene flow occurring between the two taxa in the middle portions of the stream. Specimens from Site 17.2 have a full range of phenotypes including excellent westslope cutthroat, interior redband rainbow and two intermediates. There are fewer individuals with intermediate phenotypes than would be expected from a hybrid swarm where there is a complete lack of reproductive isolation. Based on the specimens examined from the three collection sites, it is apparent that westslope cutthroat and redband rainbow have managed to maintain phenotypic integrity in at least portions of the stream. This is the expected condition of a naturally occurring sympatric population. The extent or length of the "hybrid zone" cannot be determined from this relatively small sample and it would be interesting to examine specimens from the stream section between sites 2 and 3. The headwaters (Site CROW-3: see below), elevation 3,200 ft, have excellent westslope cutthroat trout.

Four specimens from the lower collection location of Crow Creek (Site CROW- 1) were examined by classical methods. Based on this sample, the population is judged to be "essentially pure" interior redband rainbow trout (A-). Ranges and means for diagnostic characters are: scales in the lateral series: (148-168, 156.25); scales above the lateral line: (28-31, 29.5); pyloric caeca numbers: (33-38, 2.36); total gill rakers on the first gill arch: (19-20, 19.5); pelvic fin ray counts: (10); no specimens have basibranchial teeth; Sample Spot Index is: 1-, indicating there is uniformity of spotting characteristics, but slight variability is observable within the sample. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Secondary parr marks are present in all individuals, and relatively consistent within the sample. None of the specimens have a cutthroat mark. The overall phenotype is typical of interior redband rainbow trout.

Specimens collected from the middle location (Site Crow-2) have a complete range of phenotypes Sample Spot Index= 3. Four of 11 specimens (CRO-6, CRO-7, CRO-8 & CRO-16) are phenotypically "pure" westslope cutthroat trout. All of these individuals have morphological characters that are strongly indicative of WCT: ranges of diagnostic characters in these individuals are: scales in the lateral series: (212-226); scales above the lateral line: (43-48); pyloric caeca numbers: (29-36); total gill rakers on the first gill arch: (18-20); pelvic fin ray counts: (9); all specimens have basibranchial teeth (6-8); and spotting patterns are very typical of pure

WCT, with smaller spots concentrated toward the caudal region. There is very slight variation in the size of spots among these four specimens, but all individuals have very characteristic westslope cutthroat spotting patterns.

Of the remaining seven specimens, five have typical interior redband rainbow trout phenotypic characteristics, and two specimens are hybrids. Ranges of diagnostic characters in these individuals are: scales in the lateral series: (153-212); scales above the lateral line: (28-46); pyloric caeca numbers: (29-40); total gill rakers on the first gill arch: (18-22); pelvic fin ray counts: (9-10); none of these specimens have basibranchial teeth; and secondary parr marks and overall spotting pattern is typical of redband rainbow trout. Two remaining specimens (CRO- 17 and CRO- 19) have intermediate meristic characters and basibranchial teeth, and are hybrids based on morphological characters. This group of specimens clearly show gene flow is occurring between the two taxa of trout in Crow Creek. Ranges and means for all of the specimens collected from Site 17.2 are given in Table 2. As with other locations in this survey, this could be a natural hybrid zone. Stocking history of Crow Creek should be considered in this regard.

Site 17.3 Crow Creek, upper N=6 WCT-EP

The collection from upper Crow Creek (Site CROW-3) is interesting in that 5 of 6 specimens are "essentially pure" westslope cutthroat trout and the remaining one individual has most phenotypic characteristics of interior redband rainbow trout, but has some degree of cutthroat genetic input. It appears that there is substantial (but not complete) reproductive isolation occurring in the headwaters of Crow Creek. Specimens from Crow Creek were examined by classical methods. Ranges and means are given for the five "essentially pure" WCT, and the IRRB slightly hybrid specimen separately. Ranges and means for diagnostic characters (N=5 WCT) are: scales in the lateral series: (189-213, 205.80); scales above the lateral line: (42-46, 44.80); pyloric caeca numbers: (28-31, 29.40); total gill rakers on the first gill arch: (19-22, 20.0); pelvic fin ray counts: (8-9, 8.80); all specimens have basibranchial teeth (2-11, 7.60); and spotting patterns are very typical of pure WCT, with spots concentrated toward the caudal region and very consistent within the sample. Sample Spot Index is: 1- , indicating there is slight variability in spotting characteristics within the sample. Four of Five specimens have spots on the top of the head. Posterior gill raker development was consistent within the sample, with little or no posterior gill rakers. Some specimens had one or two vestigial or very weakly developed posterior gill rakers on the first gill arch. Lower scale counts, basibranchial teeth numbers in one specimen, along with spotting variability suggest a very limited amount of gene flow with sympatric IRRB, but the integrity of the overall WCT phenotype is maintained in these five specimens. The specimen diagnosed as IRRB with slight WCT genetic input has the following morphological characters: scales in the lateral series: (169); scales above the lateral line: (33); pyloric caeca number: (36); total gill rakers on the first gill arch: (18); pelvic fin ray count: (10); and no basibranchial teeth.

In conclusion, based on the specimens examined, Crow Creek has "essentially pure" WCT in the headwaters, a hybrid zone where there appears to be only limited gene flow, and "essentially pure" IRRB in the lower section. Reproductive isolation is not complete, but the phenotypes are maintained for both WCT and IRRB in this stream.

Table 1. Summary of diagnosis of 1996 collections from the Wenatchee and Yakima River subbasins. Interior redband rainbow trout (IRRB) and westslope cutthroat trout (WCT) are rated as "pure" (P) if all characters are within expected range for the taxa and "essentially pure" (EP) if the overall phenotype is typical of the taxa, but one or more characters may be slightly outside the expected range. Hybrids (H) are determined by morphological and nuclear DNA information. Coastal rainbow trout (CRBT) characters were obvious in the collections from lower Peshastin Cr (site 1) and Rattlesnake Creek. Morphological analysis (M) includes 212 specimens from 16 collection locations. Mitochondrial DNA (mtDNA) analysis includes 97 specimens from 9 locations. Nuclear DNA (N-DNA) analysis includes 97 specimens from 9 locations.

Site	Subbasin	#	M	mt DNA	N-DNA	Diagnosis
1. Peshastin lower	Wenatchee	12	IRRB/CRBT	/	/	IRRB/Mixed CRBT
2. Peshastin upper	Wenatchee	13	IRRB EP	/	/	IRRB EP
3. Sand Creek	Wenatchee	12	IRRB-EP	/	/	IRRB EP
4. Icicle Creek	Wenatchee	12	IRRB EP	/	/	IRRB EP
5. Negro Creek	Wenatchee	12	WCT P	WCT-12	WCT-12	WCT P
6. Stafford Creek	Yakima	21	/	IRRB -13 WCT-8	WCT-6 IRRB-10 H-5	MIXED IRRB WCT some hybrids
7. Silver Creek	Yakima	13	WCT EP	WCT-12	WCT-12	WCT EP/P
8. Big Creek	Yakima	12	/	WCT-12	WCT-12	WCT-EP
9. Cold Creek	Yakima	1	WCT (N=1)	WCT (N=1)	WCT-1	WCT ?
10. Oak Creek	Yakima	12	WCT EP	WCT-10 IRRB-2	WCT-10 H-2	WCT EP
11. Rattlesnake Cr mainstem	Yakima	12	IRRB G (CRBT)	/	/	IRRB G (CRBT)
12. Middle Rattlesnake Cr	Yakima	11	IRRB EP IRRB X WCT WCT	/		IRRB EP IRRB X WCT WCT
13. Little Rattlesnake lower	Yakima	12	IRRB EP	IRRB-12	IRRB-12	IRRB EP
14. Little Rattlesnake upper	Yakima	12	WCT EP	WCT-12	WCT-12	WCT EP
15. Wildcat Cr lower	Yakima	12	IRRB EP IRRB X WCT(1)	IRRB* HYB 12	IRRB-12	IRRB EP
16. Wildcat Cr upper	Yakima	12	WCT EP	WCT* 12	WCT-11 H-1	WCT EP
17.1 17.2 Crow Cr lower, middle	Yakima	15	IRRB EP	/	/	IRRB EP
17.3 Crow Cr upper	Yakima	6	WCT EP	/	/	WCT EP

Table 2. Ranges means and standard deviation (StDev) for diagnostic morphological characters used to evaluate specimens of trout from the Wenatchee and Yakima river subbasins. Sample Spot Uniformity Index (SSUI) refers to the uniformity of spotting characteristics within the sample. Collection locations are listed by Site reference number and stream name.

LOCATION		LATERAL LINE SCALES	SCALES ABOVE LATERAL LINE	PYLORIC CAECA	BASI-BANCHIAL TEETH	TOTAL GILL RAKERS	PELVIC FIN RAYS	SSUI
Site 1	RANGE	136-168	29-36	31-41	absent	16-20	9-10	2
Peshastin R	MEAN	151.75	32.42	35.5		18.33	9.83	
lower N=12	StDev	9.44	2.54	3.26		1.15	0.39	
Site 2	RANGE	150-176	30-35	31-38	absent	14-19	9-10	1-
Peshastin R	MEAN	157.82	32.82	33.8		16.5	9.73	
upper N=13	StDev	7.11	2.09	2.39		1.9	0.47	
Site 3	RANGE	148-161	29-35	31-39	absent	17-20	9-10	1-
Sand Cr	MEAN	151.67	32.17	34.92		18.55	9.75	
N=12	StDev	4.05	2.08	2.71		0.82	0.45	
Site 4	RANGE	144-182	36-45	36-50	absent	17-20	9-10	1-
Icicle Cr	MEAN	158.33	37.5	41.83		18.36	9.58	
N=12	StDev	10.59	3.83	4.34		1.03	0.51	
Site 5	RANGE	188-216	42-52	27-36	1-9	17-20	9	1-
Negro Cr	MEAN	204.17	48.17	30.1	3.25	18.42	9	
N=12	StDev	8.22	2.59	2.69	2.38	1.16	0	
Site 7	RANGE	199-221	47-51	28-34	2-9	18-21	9	1-
Silver Cr	MEAN	209.58	48.75	30.22	6.5	18.83	9	
N=12	StDev	7.74	1.42	1.86	2.02	1.11	0	
Site 9 Cold Cr	N=1	191	41	33	2	19	9	1
Site 10	RANGE	182-204	44-50	28-38	2-8	18-20	9	1
Oak Cr	MEAN	190.08	46.82	31.45	3.91	18.92	9	
N=12	StDev	6.58	2.36	2.84	2.17	0.67	0	
Site 11	RANGE	135-167	28-37	34-48	absent	17-20	10-11	1-
Rattlesnake Cr	MEAN	147.8	31.14	42		18.71	10.29	
mainstem N=7	StDev	11.5	3.89	5.42		1.11	0.5	
Site 12	RANGE	147-173	28-38	33-40	present	19-21	9-10	2
Rattlesnake Cr	MEAN	157.55	31.09	36.25	in N=2	19.82	9.64	

middle N=11	StDev	8.55	2.66	2.55		0.6	0.5	
upper N=1		189	39	30	6	17	9	/
Site 13	RANGE	141-156	31-36	32-37	absent	16-20	9-10	1-
Little Rattlesnake	MEAN	148.92	32.42	34.7		17.75	9.75	
lower N=12	StDev	4.9-3	2.07	1.89		1.06	0.45	
Site 14	RANGE	186-202	39-51	26-32	3-13	18-21	9	1-
Little Rattlesnake	MEAN	193	47.08	29.09	6.08	19.33	9	
upper N=12	StDev	5.64	3.12	2.21	3.06	0.78	0	
Site 15	RANGE	135-159	28-35	36-42	absent	17-19	9-10	1-
Wildcat Cr	MEAN	146.3	31.42	38.55		18	9.8	
Lower N=12	StDev	7.4	2.75	2.02		0.95	0.42	
Site 16	RANGE	158-206	34-51	27-37	0-12	18-20	9	1-
Wildcat Cr	MEAN	187	45.64	31.27	5.18	18.89	9	
upper N=12	StDev	12.17	4.48	3.61	3.12	0.72	0	
Site 17.1	RANGE	148-168	28-31	33-38	absent	19-20	10	1-
Crow Cr .	MEAN	156.25	29.5	36.25		19.5	10	
lower N=4	StDev	8.66	1.29	2.36		0.58	0	
Site 17.2	RANGE	153-223	28-48	29-40	0-8	18-20		3
Crow Cr	MEAN	181.5	36.7	35	absent in 5	18.45		
middleN=11	StDev	30.99	8.03	3.37		0.82		
Site 17.3	RANGE	189-213	42-46	28-31	2-11	19-22	8-9	1-
Crow Cr	MEAN	205.8	44.8	29.4	7.6	20	8.8	
upper N=5	StDev	10.03	1.64	1.52	3.65	1.41	0.45	
IRRB N=1		169	33	36	absent	18	10	/

Table 3. Summary of mtDNA haplotypes from RFLP analysis of Cytochrome B gene, and genotypes from RFLP analysis of nuclear ITS ribosomal DNA gene. Haplotypes and genotypes are assigned letter designations. Haplotypes and genotypes considered diagnostic for westslope cutthroat trout are designated "A" and those considered diagnostic for interior redband rainbow trout are designated "B" for nuclear DNA AluI and mtDNA RFLP enzymes: HinfI, Sau3AI, HaeIII, and RsaI only. Letter designations for remaining mtDNA enzymes (CfoI, AluI, and MspI) are not diagnostic for species level differences. Additional haplotypes (C, D and E) are not considered diagnostic for either taxa, but represent diversity in maternal mtDNA lineages. Frequencies of haplotypes or genotypes are given as numerical value

Site	Location	N	HinfI	CfoI	Sau3AI	AluI	MspI	HaeIII	RsaI	NUCLEAR AluI
5	Negro Cr	12	A-12	A-12	A-12	A-12	A-12	A-12	A-12	A-12
6	Stafford Cr	12	A-8 B-13	A-21	A-7 B-8 C-6	A-8 B-13	A-7 B-14	A-8 B-13	A-8 B-13	A-6 B-10 A/B-5
7	Silver Cr	12	A-11 C-1	A-12	A-12	A-12	A-12	A-12	A-12	A-12
8	Big Cr	12	A-12	A-9 C-3	A-12	A-7 B-5	A-6 B-6	A-9 C-3	A-12	A-12
9	Cold Cr	1	A	A	A	A	A	A	A	A-1
10	Oak Cr	12	A-10 B-2	A-1 C-11	A-10 B-2	A-7 B-5	A-7 B-5	A-5 C-7	A-10 B-2	A-10 B-2
13	Lower Little Rattlesnake Cr	12	B-12	A-8 C-4	B-12	A-12	A-6 B-6	B-12	B-12	B-12
14	Upper Little Rattlesnake Cr	12	A-12	A-6 C-6	A-12	A-12	A-10 B-2	A-12	A-12	A-12
15	Lower Wildcat Cr	12	B-5 C-7	A-12	B-7 C-5	A-12	A-10 B-2	B-5 E-5 D-2	B-12	B-12
16	Upper Wildcat Cr	12	A-6 C-6	A-12	A-11 C-1	A-12	A-12	A-11	C-1	A-11 B-1

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Appendix E. Summaries of meristic and morphological data for specimens of cutthroat and rainbow trout from sampling locations in the Mid-Columbia River basin.

Appendix F. Meristic and morphological data from specimens of brook and bull trout from sampling locations in the Mid-Columbia River basin.

Appendix G. Summary of diagnosis of specimens of cutthroat, rainbow, brook and bull trout in the Mid-Columbia River basin.

APPENDIX G. Summary of diagnosis of cutthroat, rainbow, bull and brook trout from sampling locations in the Mid-Columbia basin. Locations are ordered according to major drainages. Drainage code is: 1= Methow River, 2= Twisp River, 3= Lost River, 4= Chewuch River, 5= Entiat River, 6= Lower Wenatchee River, 7= Nason Creek, 8= Little Wenatchee River, 9= White River. Taxonomic code is: RBT= coastal or presumed hatchery rainbow trout, IRRB= interior redband rainbow trout, WCT= westslope cutthroat trout, BULL= bull trout, BROOK= brook trout. Purity Rating is coded as follows: P= "pure" -considered genetically pure based on this analysis; EP= "essentially pure" -one or more of the diagnostic criteria was outside the expected range for the species or subspecies (this may be due to natural variability, or multiple sources of the population; essentially pure populations should be managed as pure populations unless additional evidence -e.g stocking history or allozyme data is considered); G= "good" -the specimens are phenotypically representative of the subspecies, but are not considered to be genetically pure; HYB-R/C -the sample is considered to be introgressed with a predominately rainbow phenotype in the collection; HYB-C/R -the sample is considered to be introgressed, with a predominately cutthroat phenotype in the collection; HYB-BROOK/BULL -hybrids between brook trout and bull trout.

Location - Drainage	Site Number	River-kilometer	Diagnosis- Purity Rating
Black Canyon Creek -1-	1, 2	0.3, 4	RBT
Methow River Mainstem -1-	3	6	RBT
Gold Creek -1-	6	8.7	IRRB-G
South Fork Gold Creek -1-	5	11.6	RBT
Foggy Dew Creek -1-	7	5.6	HYB-R/C
Foggy Dew Creek -1-	8	8.7	WCT-EP
Lower Crater Creek -1-	9	1.9	HYB-R/C
Crater Creek -1-	11	7.7	RBT
Libby Creek -1-	12	5.6	HYB-R/C
North Fork Libby Creek -1-	13	13.7	HYB-C/R
Beaver Creek -1-	78	12.4	IRRB-G, BROOK
South Fork Beaver Creek -1-	79	7.7	IRRB-G, BROOK
Beaver Creek -1-	199	head-water	HYB-BROOK/BULL
Twisp River -2-	14	26	RBT
Upper Twisp River -2-	31	43.5	BULL
Upper Twisp River -2-	32	45.1	WCT-G
Little Bridge Creek -2-	15	5.5	IRRB-P
Buttermilk Creek -2-	16	5.3	IRRB-G
W. Fork Buttermilk Creek -2-	17	2.7	IRRB-P
W. Fork Buttermilk Creek -2-	18	5.5	BULL
E. Fork Buttermilk Creek -2-	19	2	HYB-R/C

Location - Drainage	Site Number	River-kilometer	Diagnosis- Purity Rating
E. Fork Buttermilk Creek -2-	20	5.2	HYB-C/R
Lower Eagle Creek -2-	21	0.3	HYB-R/C
Middle Eagle Creek -2-	22	3.2	HYB-C/R
Upper Eagle Creek -2-	23	5.2	WCT-EP
Middle Oval Lake -2-	24.1	N A	HYB-R/C
West Oval Lake -2-	24.2	N A	WCT-EP
War Creek -2-	25	3.1	HYB-R/C; BROOK
Lower War Creek -2-	26	2.9	HYB-R/C; BROOK
Reynolds Creek -2-	27	0.5	BULL
Lower South Creek -2-	28	0.2	HYB-R/C, BULL
Upper South Creek -2-	29	4	WCT-G
North Creek -2-	30	1	WCT-EP
Wolf Creek -1-	33	2.7	HYB-R/C
Wolf Creek -1-	34	9.5	HYB-C/R
Wolf Creek -1-	35	16.1	WCT-G
Upper Methow River -1-	36	98.2	RBT
Goat Creek -1-	37	2.1	IRRB-EP
Goat Creek -1-	38, 39	10.5, 12.9	HYB-R/C; BULL
Goat Creek -1-	70	14.7	WCT-G
Early Winters Creek -1-	40	6	HYB-R/C
Early Winters Creek -1-	41	20.9	WCT-P; BULL
Cedar Creek -1-	8??	3.9	HYB-R/C
Cedar Creek -1-	81	8.9	WCT-G
Cedar Creek -1-	82	10.6	WCT-G
Cutthroat Creek -1-	42	1.6	WCT-P
Lost River -3-	43.1	1.3	RBT
Lost River -3-	43.2	6.3	BULL
Cougar Lake -3-	44	N A	HYB-C/R; BULL
Monument Creek -3-	83	0.3	BULL
Robinson Creek -3-	45	1.9	WCT-P

Location - Drainage	Site Number	River-kilometer	Diagnosis- Purity Rating
Trout Creek -1-	46	0.8	RBT
Upper Methow River -1-	47	125.5, 130.8	HYB-C/R; BULL
Upper Methow River -1-	48	133.9	WCT-G
Cub Creek -4-	68	10.6	BROOK
Boulder Creek -4-	73-75	/	IRRB-G; BROOK
Eight Mile Creek -4-	49	1.6	RBT
Eight Mile Creek -4-	51	12.9	BROOK
Falls Creek -4-	50	7.1	WCT-G
Twentymile Creek -4-	71	5.8	BROOK
Lake Creek -4-	52	8	HYB-R/C
Lake Creek -4-	53	12.4	BULL
Lake Creek -4-	54	13.5	HYB-C/R
Lake Creek -4-	55	15.3	WCT-G
Andrews Creek -4-	56	0.5	IRRB-G
Andrews Creek -4-	57	1.8	IRRB-G
Andrews Creek -4-	58	4	HYB-R/C
Andrews Creek -4-	59	5.3	HYB-R/C
Chewuch River -4-	60	48.8	HYB-R/C
Chewuch River -4-	63	49.9,53.6	WCT-G
Chewuch River -4-	64	67.9	RBT
Chewuch River -4-	76	73.5	WCT-G
Chewuch River -4-	77	74.3	WCT-EP
Tungston Lake -4-	66	N A	WCT-EP
Entiat River -5-	110	46.7	RBT; HYB-R/C
Entiat River -5-	101	69.2	BROOK, HYB-R/C
Roaring Creek -5-	122	3.2	IRRB-EP
N. Fork Entiat River -5-	105	0.2	RBT; BROOK
N. Fork Entiat River -5-	103	5.2	HYB-R/C
N. Fork Entiat River -5-	102	11.6	WCT-G
Mad River -5-	117	16.4	IRRB-G; BULL

Location - Drainage	Site Number	River-kilometer	Diagnosis- Purity Rating
Mad River -5-	116	29.5	HYB-C/R; BULL
Mad River -5-	112	38.5	WCT-G, or WCT-EP
Cougar Creek -5-	115	0.3	HYB-R/C; BULL
Cougar Creek -5-	114	3.2	WCT-EP
Hornet Creek -5-	119	0.2	RBT
Tillicum Creek -5-	121	4.8	RBT
South Pyramid Creek -5-	104	1.9	IRRB-G
Lake Creek -5-	106	6	IRRB-G
Tommy Creek -5-	107, 108	6.0, 1.9	WCT-P
Mission Creek -6-	16.1	10	IRRB-G; BROOK
E. Fork Mission Creek -6-	308	2.1	BROOK
Ingalls Creek -6-	210	1.2	IRRB-G
Negro Creek -6-	206	1.2	IRRB-G
North Shaser Creek -6-	203	1.9	IRRB-G
Chiwakum Creek -6-	301	4.8	IRRB-G
Chiwawa River -6-	407	30.6	RBT
Chiwawa River -6-	401	56	WCT-G
Chikamin Creek -6-	410	2.3	RBT; BROOK; BULL
Chikamin Creek -6-	411	5.5	HYB-R/C; BULL; HYB-BROOK/BULL
Chikamin Creek -6-	412	8.9	BULL
Rock Creek -6-	408	0.8	RBT; BULL
Rock Creek -6-	409	9.6	WCT-G; BULL
Buck Creek -6-	402	0.8	WCT-EP
Phelps Creek -6-	405	5.2	WCT-G
Phelps Creek -6-	406	10	WCT-EP
Nason Creek Mainstem -7-	704, 705	5.0,16.0	RBT
Nason Creek Headwaters -7-	701	41.2	WCT-EP
Roaring Creek -7-	711	1.3	BROOK
Roaring Creek -7-	712	3.9	HYB-R/C; BROOK
Gill Creek -7-	710	0.50	HYB-R/C

Location - Drainage	Site Number	River-kilometer	Diagnosis- Purity Rating
Gill Creek -7-	709	4.2	WCT-G
Whitepine Creek -7-	708	2.1	IRRB-G
Whitepine Creek -7-	707	4.8	IRRB-G
Wildhorse Creek -7-	706	1.3	IRRB-G
Smith Brook -7-	703	0.5	WCT-G
Smith Brook -7-	702	1.9	WCT-EP
Little Wenatchee River -8-	611	13.5	IRRB-G
Little Wenatchee River -8-	601	14.0	WCT-EP
Lost Creek -8-	612	0.3	WCT-EP
Rainy Creek -8-	609	0.6	HYB-R/C
Rainy Creek -8-	608	9.0	WCT-P
Snowy Creek -8-	610	1.5	WCT-P
Fish Creek -8-	606	1.3	WCT-EP
Caddy Creek -8-	605	2.5	WCT-G
Lake Creek -8-	603	0.6	RBT
Lake Creek -8-	602	6.4	IRRB-G
Unnamed Creek -8-	604	1.3	WCT-P
White River -9-	503	20.9	RBT
Panther Creek -9-	504	1.6	WCT-G
Panther Creek -9-	505	3.2	RBT
Indian Creek -9-	502	2.3	HYB-R/C
White River -9-	501	28.6	HYB-R/C