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GENETIC AND PHENOTYPE CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

POPULATIONS OF THE PEND OREILLE, KETTLE, AND SANPOIL RIVER BASINS OF COLVILLE NATIONAL FOREST

Fiscal Year 1999 Report

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GENETIC AND PHENOTYPE CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

FY-99 REPORT: POPULATIONS OF THE PEND OREILLE, KETTLE, AND SANPOIL RIVER BASINS OF COLVILLE NATIONAL FOREST

Report Prepared For

NORTHWEST POWER PLANNING COUNCIL
UPPER COLUMBIA BASIN FISH AND WILDLIFE PROGRAM

BPA Project Number 1998-026-00

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By

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EXECUTIVE SUMMARY

In fiscal year 1999, we collected nonlethal fin tissues for genetic analysis from nineteen stream trout populations (fifteen initially expected to be cutthroat populations and four initially expected to be interior rainbow populations) residing in headwater tributaries of the Pend Oreille River, Kettle River, Sanpoil River, and Sherman Creek basins, all on federal lands of the Colville National Forest in northeastern Washington. Using a portable aquarium, we also photographed representative specimens of each population for a color catalog of appearance phenotypes. Analysis of paired interspersed nuclear DNA elements (PINEs) was used to characterize each population as to subspecies and level of hybridization, and a genetic purity rating was assigned to each using a modification of the Binns system originally developed in Wyoming to gauge the genetic purity of interior cutthroat trout populations.

Nine of our collection sites were free of hybrids and contained only genetically pure westslope cutthroat trout *Oncorhynchus clarki lewisi*. Four of these (South Fork Sanpoil, Rocky Creek, Silver Creek, and East Fork Smalle Creek) were given A-ratings for genetic purity and are considered native populations owing to the absence of any record of stocking with cutthroat trout. The other five populations were rated B because these streams had been stocked in the past with cutthroat trout. However, based on results of another recent project conducted by the Washington Department of Fish and Wildlife, we now believe that two of these populations (Upper Sullivan and North Fork Sullivan creeks) should be elevated to A-rank because they bear no genetic resemblance to the commonly used hatchery cutthroat stock.

All other cutthroat trout populations contained hybrids with rainbow trout ranging from 5 percent to 63 percent of the individuals collected. Owing to a limitation of the PINE technique, we are unable to state whether the rainbow trout contribution to these hybrids was from the Columbia River redband subspecies *O. mykiss gairdneri*, which is the indigenous form, or from the coastal rainbow subspecies *O. mykiss irideus* which has been widely stocked in the basin.

Three of our collection sites contained only rainbow trout and were free of evidence of hybridization with cutthroat trout. Unfortunately, we were not able to clearly distinguish between interior and coastal rainbow trout or hybrids of the two in this study. Even so, based on the absence of any record of past stocking of rainbow trout, two populations, those of Lone Ranch Creek and Canyon Creek, were given A-ratings and are taken to be pure native *O. mykiss gairdneri*.

Brook trout *Salvelinus fontinalis* were found at nine of our collection sites and comprised a significant proportion (range from slightly less than 5 percent to 75 percent) of the salmonids present at these sites. We never found a site where brook trout had completely displaced either cutthroat or rainbow trout, but in one site brook trout did outnumber the cohabiting species (rainbow trout in that particular case) by 3 to 1. On the other hand, we found only cutthroat trout now present in four streams where brook trout had predominated in earlier U. S. forest Service surveys, and only redband rainbow trout in one stream where brook trout had been previously stocked.

The precise locations of our collection sites as well as site descriptions, site photographs, and habitat conditions as we found them are given in the report. Maps showing the distribution of genetically pure and hybridized populations are also included. Although not a complete inventory by any means, this information should be of great value to managers in coming years for stewardship of the native resident trout populations, especially in the face of the potential listing of the westslope cutthroat trout under the U. S. Endangered Species Act.

GENETIC AND PHENOTYPE CATALOG OF NATIVE RESIDENT TROUT OF THE INTERIOR COLUMBIA RIVER BASIN

FY-99 REPORT: POPULATIONS OF THE PEND OREILLE, KETTLE, AND SANPOIL RIVER BASINS OF COLVILLE NATIONAL FOREST

INTRODUCTION

The 1994 Fish and Wildlife Program of the Northwest Power Planning Council specifies the recovery and preservation of population health of native resident fishes of the Columbia River Basin. Among the native resident species of concern are interior rainbow trout of the Columbia River redband subspecies *Oncorhynchus mykiss gairdneri*[1] and westslope cutthroat trout *O. clarki lewisi*. The westslope cutthroat trout has been petitioned for listing under the U. S. Endangered Species Act (American Wildlands et al. 1997).

Before at-risk populations can be protected, their presence and status must be established. Where introgression from introduced species is a concern, as in the case of both westslope cutthroat trout and redband rainbow trout, genetic issues must be addressed as well. As is true with native trout elsewhere in the western United States (Behnke 1992), most of the remaining pure populations of these species in the Columbia River Basin are in relatively remote headwater reaches.

The objective of this project is to photo-document upper Columbia Basin native resident trout populations in Washington, and to ascertain their species or subspecies identity and relative genetic purity using a nonlethal DNA technique. FY-99 was year two of a five-year project in which we conducted field visits to remote locations to seek out and catalog these populations. In FY-99 we worked in collaboration with the Colville National Forest and Kalispel Indian Tribe to catalog populations in the northeastern corner of Washington State.

[1] The common and scientific names used here are those of Behnke (1992).

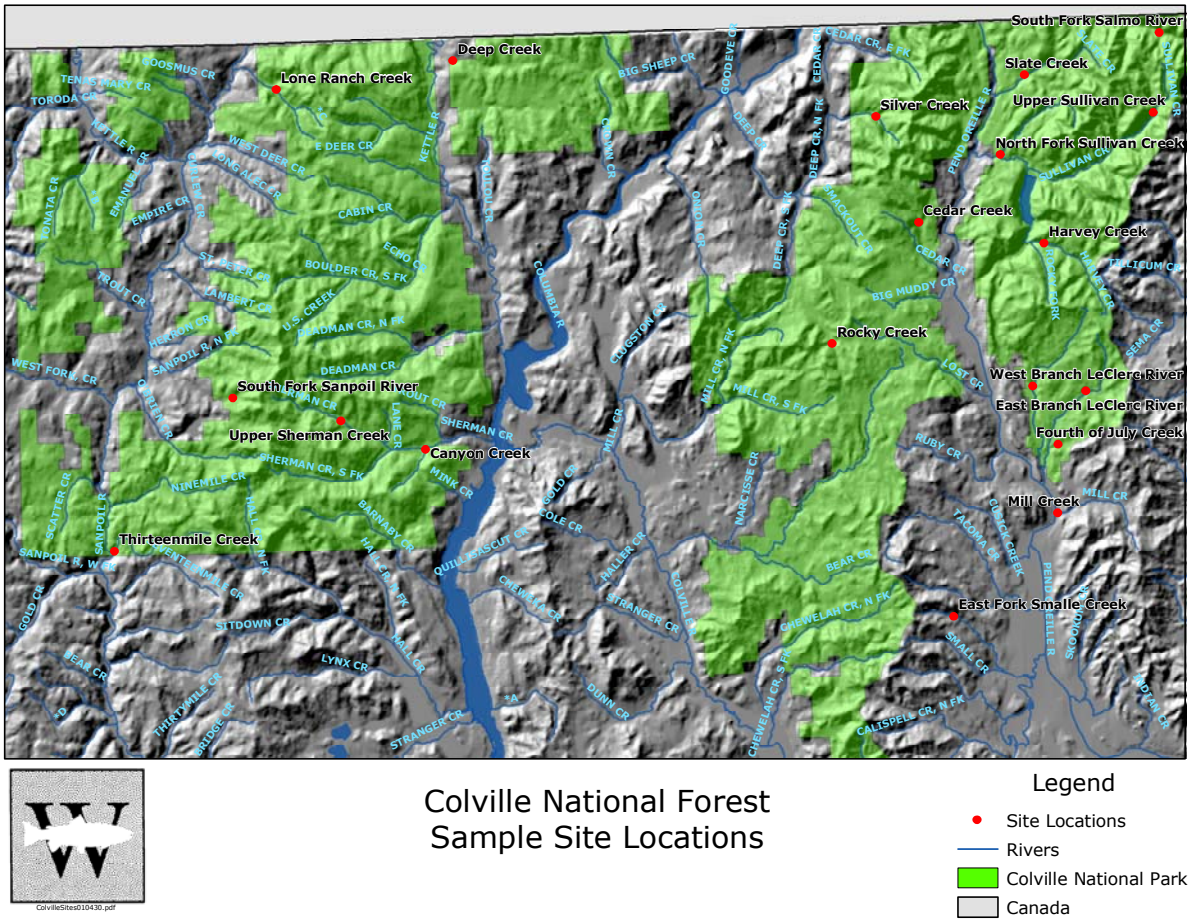
THE STUDY AREA

All of our FY-99 collection sites were located on federal lands in the Colville National Forest (Figure 1). The Colville National Forest occupies a land area of about 770,000 ha (1.9 million acres) and contains approximately 796 km (495 miles) of stream. There are approximately 1,423 ha (3,515 surface acres) of lakes and reservoirs located on the Forest as well. Our collection sites were located on small streams in the Pend Oreille, Kettle River, Sanpoil River, and Sherman Creek basins, which are tributaries of the Columbia River above Grand Coulee Dam.

The entire study area lies within the Northern Rockies Ecoregion defined by Omernik and Gallant (1986). Franklin and Dyrness (1973, reprinted 1988) earlier referred to this same general area as the Okanogan Highlands Province. Both Provinces and Ecoregions are terms used to delineate unique combinations of landscape features having distinctive terrestrial vegetation and climate. Rugged, high mountains are the dominant feature of this particular ecoregion (Omernik and Gallant 1986) with elevations varying from 396 to 2,477 m (1,300 to 8,000 feet) and local relief commonly around 914 m (3,000 feet). Mountains have sharply crested ridges and steep slopes that are cut by steep-walled, narrow stream valleys. Average annual precipitation varies from 508 mm (20 inches) to greater than 1,524 mm (60 inches), depending on elevation and exposure. Stream density varies accordingly, ranging from 0.006 km of stream per ha of land area (1 mile per sq. mi.) to 0.018 km of stream per ha (3 miles per sq. mi.). Perennial flows can occur in channels draining as little as 518 ha (1280 acres or 2 sq. mi.) in this ecoregion.

The study area is largely forested with stands of western white pine, lodgepole pine, western red-cedar, western hemlock, western larch, Douglas-fir, subalpine fir, and Engleman spruce (Franklin and Dyrness 1973 reprinted 1988; Omernik and Gallant 1986). Ponderosa pine is found in some locations as well. Principal land uses are forestry and recreation, with mining and wildlife habitat also being important. Livestock grazing occurs in the ecoregion in open forests at lower elevations, and land in the river valleys is planted for forage, grain, and peas.

FIGURE 1



During the Pleistocene, most of the study area was covered by Cordilleran ice (Richmond et al. 1965; Heusser 1983; Booth 1987). During the last major advance of the ice between about 30,000 and 11,000 years ago (referred to as the Pinedale glaciation in this region), lobes of the Cordilleran ice sheet extended southward, with the Little Spokane Lobe covering the Pend Oreille River drainage and the Colville, Columbia River, and Sanpoil lobes covering the remainder of the study area. Glacial Lake Spokane and Glacial Lake Columbia also existed at times during this period, and flood waters from ice dam breaks at Glacial Lake Missoula coursed across the landscape just to the south of the study area (Bretz 1969; Waitt 1980). What all this means is that our study sites would have had to be recolonized by fishes after retreat of the ice, with the Pend Oreille River drainage most probably being recolonized from the refugium associated with Glacial Lake Missoula and the

Kettle River and Sanpoil River sites from either the Lake Missoula refugium or the unaffected downstream portions of the Columbia River.

METHODS

Selection of Collection Sites

Collection sites for FY-99 were selected by the fisheries staff of the Colville National Forest in consultation with the Kalispel Indian Tribe. We added only one criterion, that being that there be no lakes in the headwaters of the drainage. The no-lakes criterion was included to avoid at least one possible source of hatchery origin fish that could confound our genetic analysis. The Washington Department of Fish and Wildlife propagates the Kings Lake strain of westslope cutthroat trout and stocks these fish widely in northeastern Washington high lakes (Crawford 1979, 1998). Even though great pains are taken not to stock lakes where the fish might escape to reproduce downstream, such escapes have been known to occur nevertheless, so we try to avoid streams with lakes in their headwaters.

A list of twenty-five sites was compiled initially, from which collections were actually made from nineteen sites. The other six sites were dropped, either because they turned out to be too small to yield to our collection method, or because they were deemed too small to hold trout at all. Fifteen of the nineteen collection sites were thought to be populated predominately by westslope cutthroat trout, and the other four sites by interior rainbow trout. The locations of these sites are mapped in Figure 1. We also obtained a collection of specimens of the Kings Lake strain of westslope cutthroat trout from the Washington Department of Fish and Wildlife's Colville Hatchery.

Stocking History of Collection Streams

Since 1932, when the Washington legislature vested all responsibility for fish and wildlife management in what is now the Washington Department of Fish and Wildlife, the stocking of hatchery-reared trout in State waters has been a bread-and-butter practice of that agency. We examined three separate compilations of Washington Department of Fish and Wildlife stocking

records for study area streams dating from 1933 to the present. One of these data sets was compiled by the Department itself (see Crawford 1998); one by Pend Oreille County Public Utility District No. 1 (see Pend Oreille County PUD 1999); and one by the Colville National Forest (Tom Shuhda, Forest Fish Biologist, personal communication 1999). Prior to 1932, many other agencies and entities, including predecessor State agencies, the old U. S. Bureau of Fisheries, county fish and game agencies, and even individuals, also stocked trout in state waters. Unfortunately, no neat institutional history exists for any of these activities. We canvassed the archives of the Washington Department of Fish and Wildlife, all of the old State Fish Commissioner's reports that we could locate (Washington State Fish Commissioner 1905, 1907…through 1919), and any other sources that came to our attention (e.g., Varley 1979) for whatever records might exist of trout-stocking activities in our collection streams and nearby waters. Although we cannot vouch for the completeness of these archives—and thus, can never be completely certain that the absence of a record for a given stream means no stocking ever occurred there—we nevertheless took the absence of a record as evidence that the population we found was native and untainted by stocking unless our genetic results indicated otherwise.

Based on available records and reports, we determined that hatchery origin or non-native fish most likely to be encountered in the course of our work would include coastal rainbow trout *Oncorhynchus mykiss irideus*, coastal cutthroat trout *O. clarki clarki*, Yellowstone cutthroat trout *O. clarki bouvieri* (imported into the state in the past under the name "Montana black-spotted trout") and the Kings Lake strain of westslope cutthroat trout mentioned above (a brief history of the Kings Lake westslope cutthroat broodstock is given later in this report). Past shipments of "Montana black-spotted trout" into the state may have also included westslope cutthroat trout. According to Crawford (1979), all of Washington's hatchery rainbow broodstocks are derived from *irideus*, the coastal rainbow subspecies. However, we learned that the Washington Department of Fish and Wildlife has recently developed a broodstock from local populations of *Oncorhynchus mykiss gairdneri*, the inland rainbow subspecies. This broodstock is held at Phelan Lake north of Colville in Stevens County, where eggs are taken, and the fish are reared for distribution at the Department's Colville Hatchery.

Other Recent Genetic Studies on the Colville National Forest

Leary (1997) and Kanda and Leary (1998, 1999) have carried out recent genetic studies of resident trout populations of Colville National Forest streams. We reviewed the results of these studies and incorporated peritnent findings in this report. Washington Department of Fish and Wildlife also recently reported on genetic studies of Pend Oreille basin cutthroat trout populations (Shaklee and Young 2000), which should add to the collected genetic knowledge of the region's fish populations.

Fish Collection and Work-Up

In the field, upon arrival at a collection stream, we first prospected for a convenient work-up site where we could set up our aquarium and other gear. We recorded the township, range, and section coordinates of this site from the appropriate USGS 7-1/2' quadrangle map, and also its GPS coordinates read from a Garmin II-Plus unit. We also photographed the site and recorded its altitude and stream order (Strahler 1957) as determined from the map. When our equipment was set up, we deployed upstream and down from the work-up site to collect fish. We seldom had to cover more than 1.2 km (3/4 mile) of stream to collect all the fish we needed.

We collected all fish specimens by hook and line angling using artificial flies with barbless hooks. Non-target fishes were released straight away, although we kept track of the number captured. When a fish of a target species (cutthroat trout, rainbow trout and hybrids of the two) was brought to hand, we removed the fly and quickly placed the fish in a bucket of clean stream water, which itself was kept in the shade. We exchanged the holding water frequently to keep the fish cool and well-aerated. After 30-45 minutes of angling, we brought the fish to the work-up site, regardless of how many had been captured. If more fish were needed to complete our collection after the initial batch had been processed and released, additional 30-45 minute angling periods were employed. We recorded the total time spent angling to capture the requisite number of fish of the target species from each site, and from that calculated a catch per unit of effort (CPUE) value which we used as a surrogate for abundance of target species at the site. In the field, we recorded fish as either cutthroat trout, rainbow trout, or hybrid on the basis of visual inspection. We corrected these field calls later, when necessary, after the results of the genetic tests were in hand.

Fish were anesthesized in groups of two or three, using the procedure described below. Each fish was then measured (fork length to the nearest mm) on a measuring board, weighed (wet weight to the nearest gram) using calibrated Pesola precision spring scales, and the adipose fin (or, on fish smaller than about 76 mm, a small snippet from the lower tip of the caudal fin) was removed with sharp, clean, stainless-steel scissors. These fin-tissue snippets were carefully placed in individual pre-labeled vials of preservative and saved for later use in the genetic analysis. The fish were then placed either in a bucket of clean stream water, or in a still but not stagnant part of the stream itself to recover from the anesthetic prior to release.

Anesthetic Protocol

We used clove oil (Anderson et al. 1997; Preiser et al. 1997; Keene et al. 1998) at 50 mg/L as our anesthetic in this work. During our FY-98 field work (see Trotter et al. 1999), we found that clove oil produces the same levels of anesthesia on about the same timetable, and recovery times are also about the same, as MS-222 at equal concentrations. Plus, clove oil carries a GRAS (Generally Recognized As Safe) rating from the U. S. Food and Drug Administration whereas MS-222 must be used with a 21-day withdrawl period before the fish can become fodder for human consumption. This can be an important consideration when collecting from streams open to recreational angling, as most of our sites were.

Clove oil is not completely soluble in water, and must first be dissolved in ethanol. We prepared stock solutions consisting of 3 mL of clove oil (density approximately 1 g/mL) made up to 30 mL with denatured 95-percent ethanol. Three-mL quantities of this stock solution were measured out into individual ethanol-proof capped vials which were kept in the dark in a refrigerator until taken into the field. The contents of one vial dispersed in 6 L of stream water in a 18.9 L (5 gal.) bucket gave us our 50 mg/L field concentration.

Fish Photography

Five to eight fish (most often six) from each collection site were not anesthesized immediately, but were placed individually in a small portable aquarium through which stream water was flowing, allowed time to acclimatize, and then photographed. Following its photo session, each fish was removed from the aquarium, anesthesized, and worked up as described above while the next fish was becoming acclimatized to the aquarium.

The aquarium used in this work is a portable "photarium" unit of the type described by Rinne and Jakle (1981) and built according to their specifications. The unit is made of Plexiglas and measures 356 mm (14 inches) wide by 203 mm (8 inches) high by 51 mm (2 inches) deep. A small submersible pump (Teel model 1P811A) powered by a 12-volt gel-cell battery circulates stream water through the unit, thus maintaining an environment similar in temperature and oxygen content to the fish's natural habitat. The current in the tank induces the fish to assume a natural position without undue stress, enabling high quality color photographs to be obtained that are useful for documentation, taxonomic studies, and publication.

We photographed under direct natural lighting, with the light impinging on the front of the aquarium to minimize glare and reflections (midday lighting in bright sunlight, say between 10 AM and 2 PM, works best but we could not always control the timing of our photo-shoots, nor the quality of the light). The aquarium itself was shifted and reoriented when necessary to eliminate shadows. We always placed a layer of clean gravel in the bottom of the aquarium (after first filling the tank with water to prevent scratching the Plexiglas) to avoid having the bottom of the unit show in our photographs. We always shot against a plain background which consisted of a colored backdrop cloth stretched over a board. We experimented with various background colors, but settled on forest green for photographing in strong direct light, and light blue for photographing under cloudy or mixed-light conditions. Figure 2 shows the assembled unit in operation at streamside.

To mimimize the potential for camera malfunction, unforseen loss of film, or accidents in processing, we shot two sets of photographs, a primary set and a backup set, of each fish. For the primary set, we used a Minolta Maxxum 600si camera equipped with autofocus and autoexposure

features. We spot-metered exposures directly off the side of the fish, then used the camera's bracketing program to bracket the metered readings by ± 0.5 ev unit. All images in the primary photo set were made on 35-mm Kodachrome 200 transparency film, and all film was processed by the Kodak laboratory in Tukwila, Washington. The backup photo set was taken with a Nikon FE camera equipped with a 55 mm F 3.5 Micro-Nikkor lens. Backup images were exposed on several film types that included Ectachrome EV100VS at 100 ASA or Fujichrome Sensia at 400 ASA.

FIGURE 2

Because of the tradition for taxonomic measurements to be made on the left side of the fish (Behnke 1992), we photographed all fish facing left.

A photo catalog of our collections was prepared by first selecting the best images (one each) of each fish. These images were sent to the Kodak laboratory for scanning onto a master compact disk. From the compact disk, the images were displayed individually on a computer and edited using Adobe Photoshop 4.0 software, then converted to page format using Adobe Pagemaker 4.0.

Fin-Tissue Collection and Preservation

As noted above, we removed the adipose fin (or occasionally a caudal fin snippet of about the same size) from each fish collected, attempting at all times to retain the fin-clip on the scissors and avoid touching it with our fingers. We found this easiest to do if one of us held the fish and gently arched the back, thus presenting the adipose fin, while another person clipped the fin at the base, always approaching with the scissors from behind the fin. The fin-clip, now on the scissor tips, was then quickly transferred into a 2-mL vial filled with denatured 95-percent ethanol (Shiozawa et al. 1992). We used capped cryo-storage vials for this purpose, which had been pre-filled with ethanol and labeled with the site code and specimen number. We kept the tissue vials in a Coleman cooler while still in the field, and stored them in a home freezer at –20° C until they could be transported to the genetic laboratory for analysis.

Collection Site Physical Description and Habitat Data

After completing our protocols for measuring, weighing, tissue collection and fish photography, we then recorded some basic measurements and observations of stream, riparian, and upland habitat condition at each collection site. Photographs were taken of the site at the outset, and subsequent photos were taken to pictorially record significant habitat features of each stream. One of these photos was chosen for each collection site to accompany the fish photos displayed in the appendix (Appendix B). We measured water temperature with hand-held thermometers at several times over the course of the day and recorded the range, and in addition we placed temperature loggers in the stream which remained in the water for the duration of our stay at the site. Gradient was measured with a Peco hand-held Abney level on one or more stream sections chosen as being typical of the overall collection reach. We also calculated stream discharge on the day of collection from measurements of water velocity and wetted channel width and depth at several points across a

transect. Water velocity readings were taken with a Global Systems "Flow Probe" hand-held flowmeter with the propellar immersed as best we could position it at 0.6 x water depth from the surface. We also measured bankful width at many of the sites on the same transect used to compute discharge, using criteria set forth in Leopold (1994, p 131-133) and Leopold et al. (1995).

In order to further describe the sites as we found them, qualitative rankings of fourteen additional habitat parameters relating to riparian vegetation, streambank condition, bottom substrate, and channel condition were made by visual estimation using a three-page Habitat Assessment Field Data Form derived from the Environmental Protection Agency's Rapid Bioassessment Protocols (Barbour, et al., 1999). Each habitat parameter (for both left and right banks where appropriate) was evaluated from a choice of four comparative values, and each of the four values was given a numerical score within a five point range: Poor (1-5), Marginal (6-10), Suboptimal (11-15), and Optimal (16-20). The maximum possible total score for a site was 360 points. Although subjective, this system was rapid and easy to use in the field, and provided a numerical means to compare individual habitat parameters among collection sites, and also to compare cumulative scores for each collection site.

A copy of the Habitat Assessment Field Data Form, showing the fourteen habitat parameters evaluated, is provided as Appendix A in our FY-98 Report on populations of the upper Yakima Basin and available at the BPA Resident Fish Website. For consistency, the same person completed the ranking for all sites.

Calculation of Fish Condition Index

Condition indices are widely used to assess robustness or physiological well-being of fishes. We chose to calculate Relative Weight, $\mathbf{W_r}$ (Wege and Anderson 1978) as our measure of condition index due to its freedom from length-related and species-related biases (Cone 1989; Murphy et al. 1991). $\mathbf{W_r}$ is given by the formula:

$$\mathbf{W_r = (W/W_s) \times 100} \qquad (1)$$

where **W** is the weight of each individual fish (in grams) and $\mathbf{W_s}$ is a length-specific standard weight which is computed from one of these equations (Kruse and Hubert 1997; Simkins and Hubert 1996):

For interior cutthroat trout (lotic populations) $\mathbf{\log_{10} W_s = -5.189 + 3.099 \log_{10} TL}$ (2)

For rainbow trout (lotic populations) $\mathbf{\log_{10} W_s = -5.023 + 3.024 \log_{10} TL}$ (3)

where **TL** is total length (in mm).

In equations (2) and (3), length is specified as total length, **TL**. Since we recorded fork length, **FL**, in the field, we converted using these formulae:

For interior cutthroat trout (Kruse and Hubert 1997) $\mathbf{TL = (FL + 1.850)/0.977}$ (4)

For rainbow trout (Simpkins and Hubert 1996)) $\mathbf{TL = -0.027 + 1.072FL}$ (5)

Thus, to compute $\mathbf{W_r}$ from our field data, we applied equations (2) and (4) to the cutthroat trout specimens and equations (3) and (5) to the rainbow trout specimens to first compute $\mathbf{W_s}$ for each fish, then we plugged those values into equation (1) to compute the respective $\mathbf{W_r}$ values.

Statistical Analysis

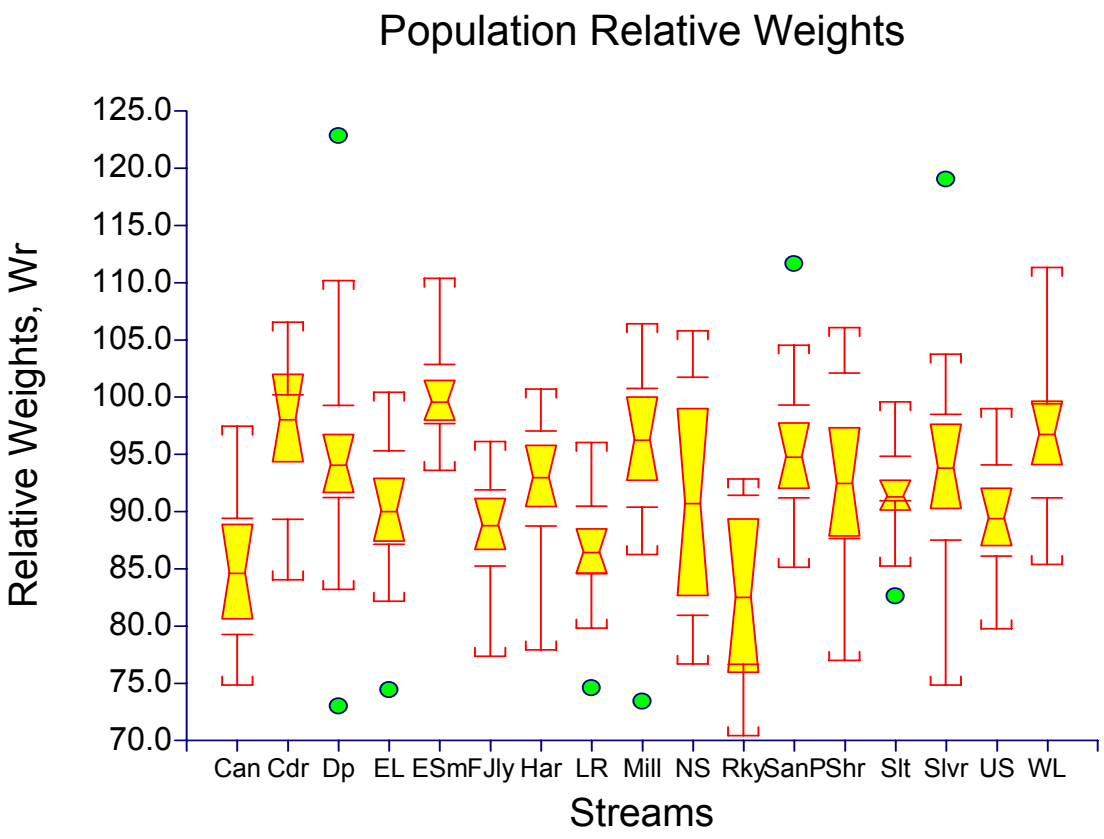
Several statistical procedures were run on the collected data to screen our sampling methods for bias and to test for differences in mean condition index among sampled populations. Statistical analyses were performed using the NCSS 2000 statistical software package (Hintze 1999).

Because the Relative Weight index is dimensionless and is expressed as a proportion or percentage (= proportion *100), the W_r's cannot be expected to be normally distributed, nor can the variances of the samples be expected to be equal. W_r expresses fish condition independent of fish size (weight, length); for example, resident westslope cutthroat of 120 mm and 250 mm fork-lengths may both have a W_r of 90 (0.90). Hence, even if the original length distributions are

normal and all sample populations have equal variances, one should expect the W_r's of a randomly sampled local population to have a repulsed distribution, with more individual values clustered close to the population mean value than in a normal distribution. The distributions of sample proportions can often be rendered normal by arcsine transformation. However, since W_r can have values greater than 100 (proportion greater than 1.0), arcsine transformation could not be used to attempt to normalize the repulsed data or equalize the sample variances. This generally invalidates the use of Analysis of Variance, which strictly requires that the sample populations to be analyzed have normal distributions and that their variances be equal.

Nevertheless, several Anovas were run on the W_r data to compare sample means, since the F-test is robust to mild violations of the normality and equality of variance requirements, provided the groups analyzed are random samples from their respective populations. Viewed in conjunction with Box Plots of the W_r data (Figure 3) and Anovas on the length data of the samples (see below), and with the above caveats in mind, the analyses present a fairly reliable picture of the data.

FIGURE 3



Notched Box Plots of Relative Weights of the sampled stream populations.
The top and bottom of the "box" are the 25th and 75th percentiles and are indicated by horizontal lines nearest the box but attached to the T-shaped lines which extend away from the box. The length between these two lines on either side of the box itself is thus the interquartile range (IQR, the middle 50% of the data).
The line drawn through the middle of the box is the median (the 50th percentile). The outer limits of the notched box itself display the 95% confidence limits of the median, and are constructed using the formula: *Median* ± 1.57*[(IQR)/√n].
Adjacent values are displayed as T-shaped lines that extend beyond each end of the box. The upper adjacent value is the largest observation that is less than or equal to the 75th percentile plus 1.5 times IQR. The lower adjacent value is the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times IQR. Values outside the upper and lower adjacent values are called outside values; those that are under three IQRs from the 25th and 75th percentiles are called "mild outliers" and are shown as green dots.

The following tests were run on the W_r data. A One-Way Analysis of Variance on sample means was run to test for difference among the mean relative weights of the sampled populations. Because our collections spanned the entire summer season, we also conducted a Nested Anova using the NCSS GLM Anova tool with bi-weekly period of sampling as the fixed factor and

stream sample population as the nested factor to test for the influence of collection date on differences in sample populations mean W_r values.

Length data from the sampled populations provides a better indication of the reliability (randomness) of our sampling methods than W_r data. The length data of each sample was screened for normality using the NCSS Descriptive Statistics tool, and One-Way and Nested Anovas were run on the length data as they were for the relative weight data.

We also tested whether W_r values were correlated with length, since a strong correlation would indicate bias in the standard weight formula (W_s), bias in sampling method, or an unexpected length-related causal condition. Least squares regressions were run on each sampled population with individual W_r values as the dependent variable and individual length values as the independent variable, and scatter plots with the least square regression line through them were produced and inspected. Under ideal conditions and random sampling, there should be no correlation between W_r and length, and the slope of the regression line should be zero.

Finally, even though our collection site habitat condition scores (see above) were only qualitative, we tested for the relationship between population mean condition index and collection site habitat quality with linear (least squares) regression and scatter plots, as we did for W_r and length.

Genetic Analysis

We used paired interspersed nuclear elements (PINEs) to identify species and subspecies of the fish collected, and to assess the extent of hybridization that might have occurred in the populations (Spruell et al. 1999). PINEs use pairs of primers that are complementary to the sequences of elements that are interspersed throughout the nuclear genome. Using the polymerase chain reaction (PCR), the fragments of DNA between these elements are amplified. When these amplified fragments are run on an electrophoretic gel, it is possible to reliably distinguish species based on the presence or absence of diagnostic bands. We used markers amplified by the same primer pairs to

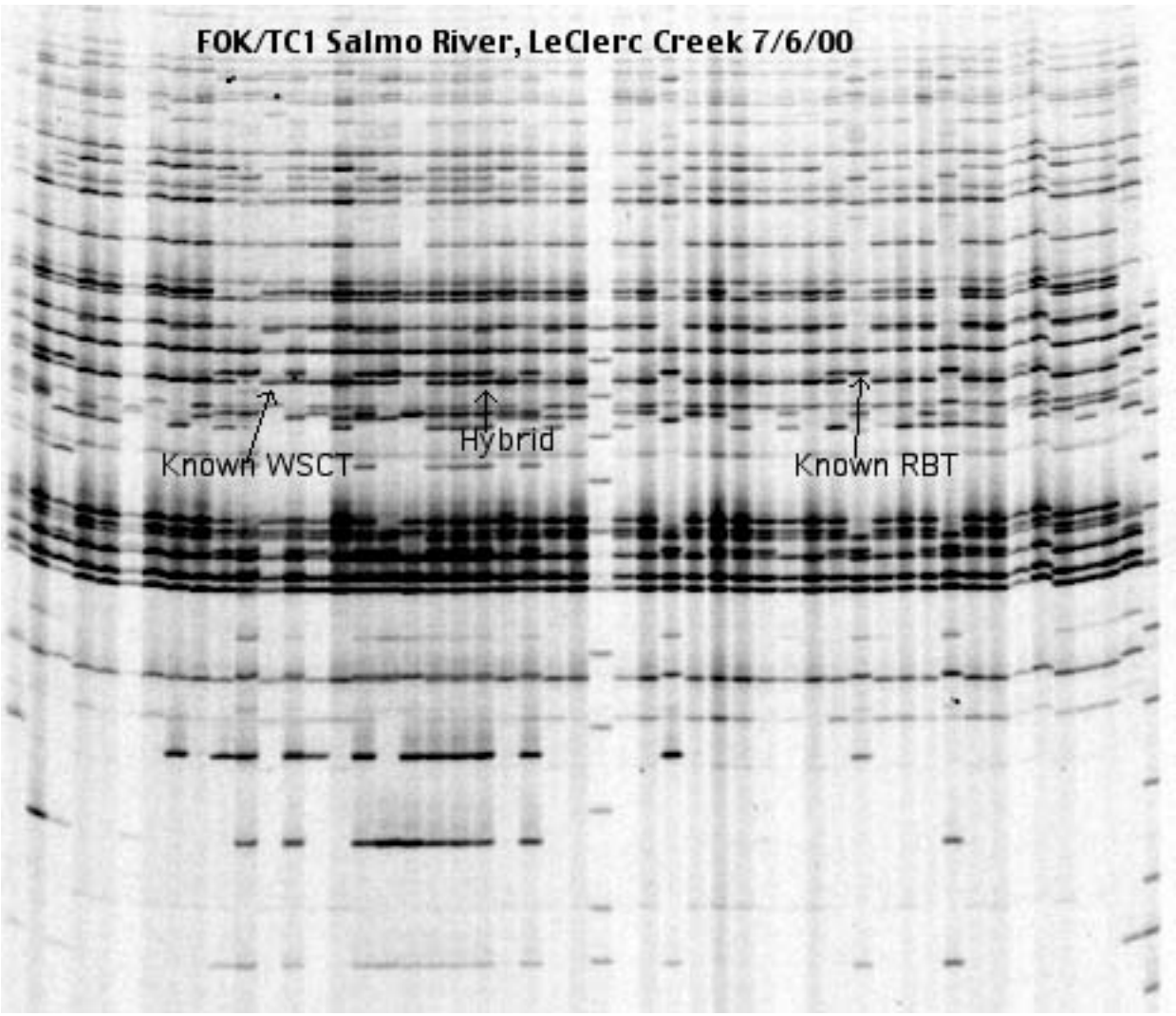
differentiate between coastal, Yellowstone, and westslope cutthroat trout and between coastal and inland rainbow trout.

With regard to hybridization between rainbow and cutthroat trout, PINEs do not always allow a distinction between inland and coastal forms of the rainbow trout component. This is because there are shared bands between the forms, and which of these bands will be expressed in the hybrid is random. Therefore, when levels of hybridization with rainbow trout are low, we cannot assign the hybrid influence to either form with certainty.

Fin clips stored in 95 percent ethanol, as described above, were transported to the University of Montana where DNA was extracted using guidelines provided with the Puregene™ DNA Isolation Kit. DNA was amplified using primers labeled with fluorescent dyes to allow visualization of the product. Each population was analyzed using a minimum of three primer pairs. PCR reagent volume was maintained at 10 μL. Reactions contained the following: approximately 25 ng of genomic DNA, 1 μL 10X Perkin-Elmer PCR buffer, 4.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5.0 pmoles of primer, and 0.5 U Stoffel Taq. Reactions were completed in a MJ Research PTC-100 thermal cycler. All reactions except those including the primer 33.6+2 used the following profile: 3 minutes at 95°C, 30 cycles of: 1 minute at 93°C, 1 minute at 60°C, 2.5 minutes at 72°C, and finally an additional 2.5 minutes at 72°C. For reactions that included the primer 33.6+2, the 60° annealing temperature was increased to 61°. Products were then refrigerated until analysis on an electrophoretic gel. Amplified products were run on a 4.5% polyacrylamide gel for 50-75 minutes at 65 watts. DNA products were then visualized using a Hitachi FMBIO-100™ fluorescent imager.

Each gel was visually inspected to identify DNA fragments that were diagnostic for interior or coastal rainbow trout, or for westslope, Yellowstone, or coastal cutthroat trout. The size of these bands was confirmed using MapMarker LOW size standard and FMBIO software. All gels also included at least one known individual from each species and subspecies in question to be used as a reference for the unknown samples. An example of a PINE gel is shown in Figure 4.

FIGURE 4



Genetic Purity Rating

As we did in our FY-98 work (Trotter et al. 1999), we assigned genetic purity ratings to each of the collected populations using the following modification of an approach originally developed by Binns (1977) for cutthroat trout populations in Wyoming:

A. Pure stock. All individuals examined carry only markers of the species or subspecies of interest, and there is no history of stocking the water with hatchery fish of the same species or subspecies.

B. 1-9 percent of individuals examined carry bands from another species or subspecies, but appearance-wise, all are "good" representatives of the species or subspecies of interest. Also applied to populations with no detectable hybridization, but where a history exists of stocking the water with hatchery fish of the same species or subspecies.

C. 10-19 percent of the individuals examined carry bands from another species or subspecies, but all are still "good" visual representatives of the species or subspecies of interest.

D. 20 percent or more of the individuals examined carry bands from another species or subspecies, but all are still "good" visual representatives of the species or subspecies of interest.

E. A population never examined by a taxonomist or by any genetic method.

F. 20 percent or more of the individuals examined carry bands from another species or subspecies, and the specimens are questionable to poor visual representatives of the species or subspecies of interest. This designation would also apply to populations that are hybrid swarms.

As originally promulgated (Binns 1977), a lower purity rating was assigned if the stream from which the specimens came had any kind of a stocking history. Because we can easily detect foreign DNA bands in the specimens we examine, we determined that there was no need to downgrade purity based on stocking history alone if the only record of stocking was with a different species or subspecies, e.g., rainbow trout stocked in a cutthroat trout stream, or Yellowstone cutthroat stocked in a westslope cutthroat stream. However, we did downgrade based on stocking history alone if: (1) the record showed that non-indigenous fish of the same species or subspecies had been stocked, e.g., Twin Lakes strain westslope cutthroat stocked in a stream where we found pure westslope cutthroat trout; or (2) the stocked fish were the same species but were not identified as to subspecies or origin, e.g., fish identified only as "cutthroat trout" stocked in a stream where we found pure westslope cutthroat.

In scoring, we rounded percentages to the nearest whole number. For example, if 9.1 percent of the individuals in a population carried foreign bands, we scored it 9 percent. If 9.5 percent of the individuals carried foreign bands, we scored it 10 percent.

RESULTS

General Characteristics of Collection Sites

Site coordinates and elevations, reach physical measurements, and habitat scores of our FY-99 collection sites are given in Table 1.

Table 1. Coordinates and Stream Reach Data for Collection Sites

Stream Name	Map Coordinates	GPS Coordinates	Reach Altitude	Stream Order	Reach Gradient	Water Temperature	Habitat Score
S. Fk. Sanpoil R.	36N 34E s10	N48 38.15' W118 32.25'	1265m	1	11	8.1 C-8.3 C	258
Deep Creek	40N 36E s12	N48 58.69' W118 10.76'	579m	3	2.5	10.0 C - ND	231
Mill Creek	35N 44E s33	N48 29.33' W117 15.56'	658m	3	4	11.1 C - 12.2 C	280
Silver Creek	39N 42E 8/9	N48 54.36' W117 31.13'	939	3-4 confl.	4	8.6 C - 10.6 C	230
S. Fk. Salmo R.	40N 45E s11	N48 58.85' W117 04.17	1265m	ND	2	10.0 C - ND	327
W. Br. LeClerc Cr.	36N 44E s18	N48 37.27' W117 17.42'	774m	3	3	12.8C - ND	289
E. Br. LeClerc Cr.	36N 44E s23	N48 36.84' W117 12.49'	914m	3	2	10.0 C - ND	186
Fourth of July Cr.	35N 44E s4	N48 33.59' W117 15.28	857m	2	1	9.4 C - 11.7C	239
Harvey Creek	38N 44E 28/29	N48 46.09' W117 15.81'	1006m	4	2	10.3 C - 11.1 C	261
N. Fk. Sullivan Cr.	39N 43E s23	N48 51.69' W117 19.57'	732m	2	12.5	9.7 C - ND	324

Upper Sullivan Cr.	39N 45E s10	N48 53.92' W117 05.05'	1256m	3	3	9.0 C-12.0 C	276
Rocky Creek	37N 41E s26	N48 40.38' W117 36.05'	939m	3	2	8.3 C-10.6 C	192
Slate Creek	40N 44E s30	N48 56.58' W117 17.02'	829	3	3	9.4 C-9.7 C	291
Cedar Creek	38N 42E s14	N48 47.69' W117 27.47'	853	3	5	11.4 C-12.8 C	225
E. Fk. Smalle Cr.	33N 43E s6	N48 23.19' W117 25.63'	1085	2	1	6.7 C-7.5 C	295
Lone Ranch Creek	40N 34E s24	N48 57.20' W118 27.46'	793	3	2.5	10.6 C-11.1 C	288
Canyon Creek	35N 36E 35/36	N48 34.63' W118 14.39'	744	3	5	9.9 C-ND	223
Upper Sherman Cr.	36N 35E s23	N48 36.55' W118 22.23'	975	4	2.5	9.2 C-ND	212
Thirteenmile Creek	35N 32E s31	N48 28.80' W118 43.68'	610	ND	1	8.3 C-ND	ND

Fish Abundance, Condition Indices, and Other Parameters of the Fish Collections

Table 2 lists the proportions of different fish species encountered at each collection site, along with CPUE of the target species and computed population condition indices.

Table 2. Proportion of principal species and other salmonids encountered, CPUE for target species, and relative weights, FY-99 collections.

Collection Site	Date	Principal species	Other salmonids encountered	Target CPUE, fish/angler-hr	Population W_r
S. Fk. Sanpoil R.	8/13/99	CT 100%		5.7	94.96
Deep Creek	7/22/99	CT 80%	RB 8%; Hybrid 12%	5.4	95.67
Mill Creek	8/27/99	CT 28.6%	RB 4.8%; Hybrid 57.1%; Brook 9.1%	8.0	95.56
Silver Creek	7/28/99	CT 76%	Brook 24%	4.8	93.37

S. Fk. Salmo R.	8/29/99	CT 34.8%	RB 4.3%; Hybrid 60.9%	15.5	ND
W. Br. LeClerc Cr.	7/29/99	CT 55.2%	RB 3.4%; Hybrid 17.2%; Brook 24.1%	8.8	96.19
E. Br. LeClerc Cr.	7/29,30/99	CT 50%	Hybrid 5%; Brook 45%	2.7	91.00
Fourth of July Cr.	8/26/99	CT 100%		5.1	88.24
Harvey Creek	8/11/99	CT 100%		3.7	91.59
N. Fk. Sullivan Cr.	8/11/99	CT 100%		3.8	91.54
Upper Sullivan Cr.	8/10/99	CT 100%		3.9	90.25
Rocky Creek	7/23/99	CT 76.2%	Brook 23.8%	1.7	83.10
Slate Creek	8/24/99	CT 82.6%	Brook 17.4%	4.9	91.89
Cedar Creek	8/25/99	CT 90.4%	Hybrid 4.8%; Brook 4.8%	6.1	95.97
E. Fk. Smalle Cr.	8/31/99	CT 100%		9.2	100.45
Lone Ranch Creek	8/12/99	RB 100%		6.6	86.81
Canyon Creek	7/20/99	RB 100%		2.5	84.46
Upper Sherman Cr.	7/21/99	RB 36.7%	CT 6.7%; Hybrid 33.3%; Brook 23.3%	1.9	93.94
Thirteenmile Creek	8/28/99	RB 25%	Brook 75%	0.3	ND

Of the nineteen collection sites, we had expected to find westslope cutthroat trout as the principal species at fifteen sites and rainbow trout as the principal species at the other four sites. This expectation was largely confirmed, except that hybrids between rainbow and westslope cutthroat predominated at two of the presumed westslope cutthroat sites (South Fork Salmo and Mill Creek) and hybrids were nearly as abundant as rainbow trout at one of the presumed rainbow sites (Upper Sherman Creek).

As noted in Table 2, brook trout *Salvelinus fontinalis* were present in nine of our collection streams, and comprised a significant proportion of the salmonid population in each stream where they occurred (range from 4.8 percent to 75 percent of the total fish captured). We never found a site where brook trout had completely displaced either rainbow or cutthroat, although brook trout

outnumbered rainbow trout by 3 to 1 in Thirteenmile Creek, presumed initially to contain predominately rainbow trout. In East Branch LeClerc Creek, where we expected to find predominately westslope cutthroat trout, brook trout were nearly as numerous as the cutthroat.

Although several of the streams where we collected were said to also contain bull trout *Salvelinus confluentus* (T. Shuhda, U. S. Forest Service, personal communication 1999), none were encountered.

Most of our CPUE values were quite good and indicated high levels of target fish abundance at most of the collection sites. Where we experienced low CPUE (values less than 2 fish of the target species per angler-hour were recorded at the Rocky, Upper Sherman, and Thirteenmile creek sites), other factors such as exceedingly small stream size or severely degraded stream habitat were also evident and could have accounted for the low numbers of target fish.

Condition Index Results

Relative Weight, $\mathbf{W_r}$ for each fish captured (n = 356) was calculated from the raw fork length and weight data, as explained above. In the case of individual rainbow-westslope cutthroat hybrids a decision was required as to which standard weight ($\mathbf{W_s}$) and fork length-to-total length formulas to employ. The decision was made as follows: if the raw genetic data indicated a predominance of rainbow markers, the formulas appropriate to rainbow were employed; if westslope markers were predominate, the formulas appropriate for westslope were employed. Occasionally a hybrid individual displayed equal percentages of both markers. In this case the formula was employed that was applicable to the majority of the individuals in the population.

Relative Weights were determined for populations in seventeen of the nineteen streams sampled. One stream, Thirteenmile Creek only yielded one rainbow and so no population level data were obtained. A second stream, South Fork Salmo River in the Salmo-Priest Wilderness area required a hike of over three miles in order to access. Only one of us (McMillan) made this hike which prevented our transporting our normal complement of sampling equipment to this site. While DNA samples were obtained no fish were weighed and fish lengths were only estimated.

Mean Relative Weights for the seventeen populations are given in Table 2 above and in Table 3 below together with mean fork-lengths, standard deviations, and coefficients of variation.

Table 3. Sample Population Mean Fork Length Statistics and W_r

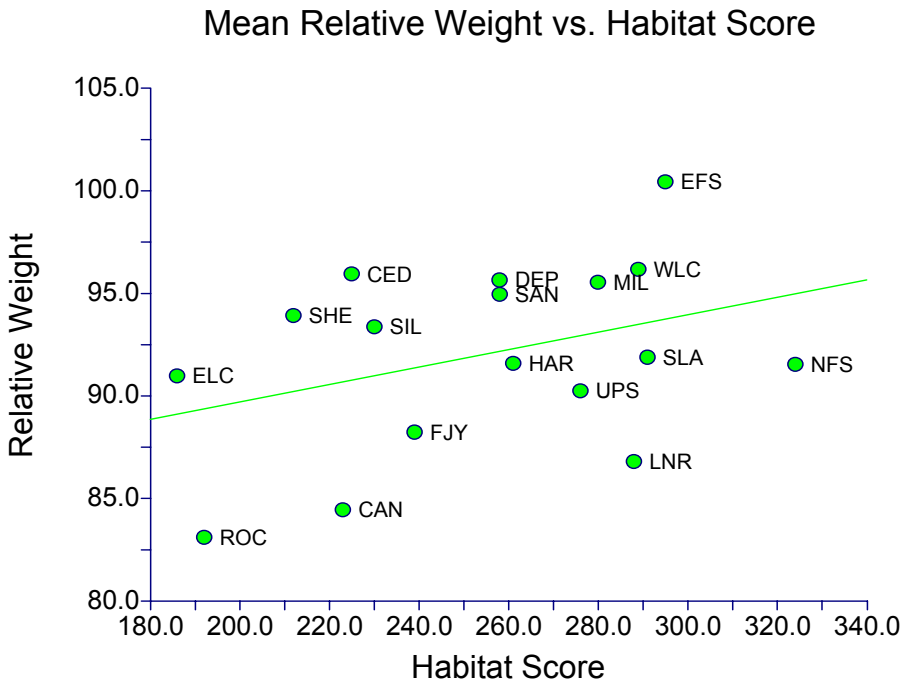
Stream	Fk. Length(mm)	Std. Dev.	C.V.	W_r
CAN	130.67	23.61	.18	84.46
CDR	143.45	37.46	.26	95.97
DEP	156.72	36.53	.23	95.67
EFS	144.64	17.91	.12	100.45
ELC	167.41	34.73	.21	91
FJY	160.86	33.51	..21	88.24
HAR	163.8	32.09	.20	91.59
LNR	141.09	28.81	.20	86.81
MIL	154.25	20.07	.13	95.56
NFS	153.19	31.86	.21	91.54
ROC	122.83	28.13	.23	83.10
SAN	157.3	23.05	.15	94.96
SHR	158.04	31.33	.20	93.94
SIL	139.95	25.98	.19	93.37
SLT	156.68	29.05	.19	91.89
UPS	167.24	31.76	.19	90.25
WLC	179.45	43.13	.24	96.19

Statistical tests rejected the null hypothesis that the mean relative weights (W_r) of all populations are equal at the significance level $\alpha = 0.05$ (One-Way Anova; df (degrees of freedom) = <16, 339>, F-Ratio = 7.67, p = 0.000000, power = 1.0) and the null hypothesis that population medians are equal (Kruskal-Wallis One-Way Anova on Ranks; df = 16, Chi-Square = 102.1583, p = 0.000000).

Sampling occurred within a six week period (July 20 to August 31). A Nested One-Way Anova with stream population relative weights nested within bi-weekly sampling periods failed to reject the null hypothesis that the mean relative weights of sampling periods were equal (df Fixed Factor: Sampling Period = 2, Random Factor: Streams = 16, error Term = 337; F-Ratio, Sampling Period/Streams = 0.41, p = 0.66; F-Ratio, Streams/Error = 6.76, p = 0.000000). Due to the small number of fixed factors (3) this test admittedly has low power (0.11) to detect a difference. However, given the relative homogeneity of environmental conditions during the narrow period of sampling, it is reasonable to expect little difference in mean W_rs between biweekly sampling periods and the nested anova appears to confirm this expectation. Means and sample sizes by biweekly period were: July 20 - July 31, W_r = 93.37, Std. Error = 0.59 (n = 141); August 1 - August 15, W_r = 92.07, Std. Error = 0.67 (n = 109); August 16 - August 31, W_r = 90.21, Std. Error = 0.68 (n = 106).

Neither did there appear to be any correlation between mean relative weights and elevation or habitat quality score. Figure 5 shows the plot of population mean relative weights against habitat score. The correlation coefficient between the two variables is 0.368 and is not statistically significant at $\alpha = 0.05$ (R-squared value = 0.135; F-value = 2.34, df <1,15>, p = 0.147, power = 0.30).

FIGURE 5.



Results of descriptive statistics run on the length data of the samples support our conviction that our method of sampling produced random samples of the population. Of the 17 sampled populations from which length data was obtained, 16 had normal distributions according to the D'Agostino Omnibus normality test at the $\alpha = 0.05$ significance level. As shown in Table 3, mean fork lengths ranged from 122.83 to 179.45 mm., sample standard deviations ranged from 17.91 to 43.13, and coefficients of variation ranged from 0.12 to 0.26. The one population that failed the Omnibus test for normality, North Fork Sullivan creek, failed because of the presence of one individual of 240 mm. which was over 2.5 standard deviations larger than the population mean of 153.19.

Anovas on fork length data paralleled the Anovas on the Relative Weight data. The null hypothesis that all sample means were the same was rejected at the significance level $\alpha = 0.05$ (One-Way Anova, df = <16,339>, F-Ratio = 3.87, p = 0.000001, power = 0.999922). In addition, the Modified-Levene Equal-Variance Test accepted the null hypothesis of equal variance among the samples at the $\alpha = 0.05$ level (p = 0.21). The nested Anova found no difference among

population means grouped by bi-weekly period of sampling (df: Sampling Period = 2, Streams = 16, Error Term = 337; F-Ratio, Sampling Period/Streams = 2.00, p = 0.14, power = 0.40; F-Ratio, Streams/Error = 3.76, p =0.000002).

Results of least squares Regressions of Relative Weight on Fork Length for each population (n = 17) indicated no significant dependence of relative weight on fork length for 16 of the 17 populations at the $\alpha = 0.05$ level. P-values for the null hypothesis that the slope of the regression is equal to zero ranged from 0.07 (West Branch LeClerc Creek) to 0.75 (North Fork Sullivan Creek) with power ranging between 0.06 and 0.43. The one exception, Slate Creek (n= 22), showed a mild negative correlation of Relative Weight with Fork Length (-0.064; R-square = 0.236); the null hypothesis was rejected with a P-value = 0.02 and power = 0.66.

Photo Catalog of Trout Specimens

The photo catalog of live specimens representing each of the collected trout populations, along with a photograph of each respective collection site, is included in Appendix B.

DNA Analysis

Results of the DNA analysis of collected specimens from each population are tabulated in Table 4. Shown here are the total number of specimens examined from each population, the number of genetically pure westslope cutthroat individuals, the number of genetically pure rainbow individuals, and the number of hybrid individuals found in each population.

Table 4. Genetically pure and hybridized individuals in FY-99 collections

Collection Site	Number of Specimens	Number of Cutthroat	Number of Rainbow	Number of Hybrids
S. F. Sanpoil R.	20	20	0	0
Deep Creek.	25	20	2	3

Mill Creek.	19	6	1	12
Silver Creek	22	22	0	0
S. F. Salmo R.	23	8	1	14
W. Br. LeClerc Cr.	22	16	1	5
E. Br. LeClerc Cr.	22	20	0	2
Fourth of July Cr.	22	22	0	0
Harvey Creek	25	25	0	0
N. F. Sullivan Cr.	16	16	0	0
Upper Sullivan Cr	25	25	0	0
Rocky Creek	12	12	0	0
Slate Creek	22	22	0	0
Cedar Creek	20	19	0	1
E. F. Smalle Cr.	23	23	0	0
Lone Ranch Cr.	23	0	23	0
Canyon Creek	15	0	15	0
Upper Sherman Cr.	23	2	11	10
Thirteenmile Cr.	1	0	1	0

Nine of our collection sites were free of hybrids and contained only cutthroat trout. Three sites were free of hybrids and contained only rainbow trout. Of the cutthroat subspecies tested for, only westslope cutthroat markers were found in collected specimens, be they pure specimens or hybrids. Unfortunately, we were not able to clearly distinguish between interior and coastal rainbow trout in this study. As explained in the Methods section, owing to shared bands between the two forms, the PINEs methodology does not always allow a distinction between the two.

Stocking History of Collection Streams

We found stocking records for 16 of our 19 FY-99 collection streams. Three streams—South Fork Salmo, Rocky Creek, and Canyon Creek—had no stocking history of any kind that we could locate. For three additional streams—South Fork Sanpoil, Lone Ranch Creek, and Thirteenmile Creek—the stocking history showed only brook trout. Ten of our FY-99 collection streams had histories of one or more cutthroat trout stockings, and in five of these streams, records showed that hatchery rainbow trout had also been released at one time or another. Eight of our FY-99 collection streams had histories of one or more rainbow trout stockings (presumably coastal origin rainbow trout based on Crawford's (1979) history of State hatchery broodstocks). Of these, five were streams that had also received hatchery cutthroat trout and two were streams which had also received brook trout.

With regard to brook trout, we found records that this species had been stocked in the past in eleven of the streams from which we collected. As Table 5 shows, we captured brook trout in nine streams, including six of the eleven streams with stocking records, and three streams for which we could find no record of brook trout having ever been stocked. Brook trout were not present at our collection sites in five of the streams where they had been previously stocked.

Table 5. Brook trout stocking records and FY-99 status

Collection Site	Year of Brook Trout Stocking	Number Captured, FY-99
S. F. Sanpoil R.	48	0
Mill Creek.	34-37, 40	2
Silver Creek	No record found	6
W. Br. LeClerc Cr.	33, 35, 36, 41, 62, 64	7
E. Br. LeClerc Cr.	34-37, 41, 62	19
Harvey Creek	64, 71-73, 81	0
Upper Sullivan Cr	33-35	0

Rocky Creek	No record found	5
Slate Creek	No record found	4
Cedar Creek	33-35, 37, 40-41,44, 46-47, 49, 54, 59	2
E. F. Smalle Cr.	33-38, 46, 48, 80, 81	0
Lone Ranch Cr.	48, 81	0
Upper Sherman Cr.	33-38, 40, 59, 62,70-74	9
Thirteenmile Cr.	65, 66	3

Genetic Purity Ratings

Tables 6 and 7 combine stocking history with the results of the DNA analysis to yield the Binns genetic purity ratings for each of the FY-1999 trout collections. Table 6 shows the ratings for the fifteen cutthroat collections, and Table 7 shows the ratings for the four rainbow collections.

Table 6. Hybridization, summary of stocking history, and purity ratings for FY-99 westslope cutthroat collections

Collection Site	Percent Hybrids	Record of Stocking with CT (or RB)	Modified Binns Class
S. F. Sanpoil R..	0	No	A
Deep Cr.	12	CT 38, 40, 48 (No record of RB)	C
Mill Creek	63	CT 38, 39, 41, 44, 47, 49. YCT 38. (RB 40)	F
Silver Creek	0	No. (RB 39)	A
S. F. Salmo R.	60.8	No	F

W. Br. LeClerc Cr.	23	CT 37, 47, 49. (RB 39, 40)	D
E. Br. LeClerc Cr.	9	CT 37, 47, 49. (RB 40)	B
Fourth of July Cr.	0	CT 49	B
Harvey Creek	0	CT 34, 37, 39, 41, 43, 45, 47, 49.	B
N. F. Sullivan Cr.	0	CT 49	B
Upper Sullivan Cr.	0	CT 43-45, 47-49. YCT 36, 37. (RB 35-37, 39, 46)	B
Rocky Creek	0	No	A
Slate Creek	0	CT 40, 41, 45.	B
Cedar Creek	5	(RB 39)	B
E. F. Smalle Creek	0	No (RB 47)	A

We conclude that westslope cutthroat populations in South Fork Sanpoil River and Rocky Creek merit A-ratings for genetic purity owing to freedom from hybridization and the absence of any record of past cutthroat stocking. We also conclude that the westslope cutthroat populations of Silver Creek and East Fork Smalle Creek merit A-ratings owing to freedom from hybridization, even though rainbow trout were planted in the past in each of these streams. These four populations thus are deemed the most likely to be native stocks untainted by contact with stocked rainbow trout or by interbreeding with stocked cutthroat trout from outside their respective basins.

Seven additional populations, those in Fourth of July Creek, Harvey Creek, North Fork Sullivan Creek, Upper Sullivan Creek, and Slate Creek as well as the populations in Cedar Creek and East Branch LeClerc, were given B-ratings. The Cedar Creek and East Branch LeClerc populations were rated B because of the small percentage of rainbow x cutthroat hybrids detected in our analysis. These may have resulted from a single stocking with rainbow trout that occurred in Cedar Creek in

1939, and in the case of East Branch LeClerc, from rainbow trout stocked in 1940. The other five populations appear to be genetically pure westslope cutthroat trout, but records of cutthroat stocking exist for all five streams. Therefore, interbreeding with non-indigenous westslope cutthroat stocks cannot be ruled out.

One population, Deep Creek, was rated C and one population, West Branch LeClerc, was rated D owing to the proportionally higher percentages of individuals hybridized by rainbow trout, even though our field calls indicated that fish in these populations are visually good representative of the westslope cutthroat phenotype. The Deep Creek population poses something of a mystery in that no stocking record for rainbow trout could be found to account for the 12 percent of hybrid individuals present in this population.

Two populations, South Fork Salmo River and Mill Creek, were rated F because of very high percentages of individuals hybridized with rainbow trout, and also because a number of the field calls made at each site indicated that many fish were not good visual representatives of either rainbow or westslope cutthroat phenotypes. In the case of the South Fork Salmo, limited access restricted us to a single site which we suspected might be a zone of overlap between native rainbow which occupy lower reaches of this stream, and native cutthroat which occupy upper reaches. In other streams shared by these native species, natural hybridization can and often does occur in these zones of sympatry. Our South Fork Salmo collections appear to have come from such a zone. At Mill Creek, again because of limited access, we found ourselves collecting low in the system. Here we suspect that our results reflect a great deal more influence from hatchery rainbow stocking than the record shown in Table 6 indicates.

Table 7. Hybridization, summary of stocking history, and purity ratings for FY-99 interior rainbow trout collections

Collection Site	Percent Hybrids	Record of Stocking with RB (or CT)	Modified Binns Class
Lone Ranch Creek	0	No	A
Canyon Creek	0	No	A

Upper Sherman Cr.	44	RB 35-37, 39-48, 51, 56. (CT 56, 62)	F
Thirteenmile Creek	0	RB 38	Not rated

We conclude that rainbow trout populations in Lone Ranch and Canyon creeks merit A-ratings for genetic purity owing to freedom from hybridization and the absence of any record of past stocking with rainbow trout. Because of the absence of any record of past stocking, we also conclude that these populations represent genetically pure populations of the interior form of rainbow trout. The Upper Sherman Creek population was rated F owing to the high percentage of hybrid individuals in the population coupled with the extensive stocking record for this stream. The Thirteenmile Creek population was not given a rating because only one specimen of the target species was collected from this stream.

DISCUSSION

Overlap and Comparison with Joint Stock Assessment Project

While we were in the field collecting for this study, we learned that a team from WDFW was also making fin-tissue collections from westslope cutthroat populations for a cooperative Joint Stock Assessment Project with the Kalispel, Spokane, Coeur d'Alene, and Colville Confederated Indian tribes (Shaklee and Young 2000). Seven of their collection sites were located on streams where we also collected; these were Mill, East Branch LeClerc, West Branch LeClerc, Cedar, Upper Sullivan, North Fork Sullivan, and Slate creeks. They also included in their analysis both the Kings Lake and Twin Lakes hatchery strains of westslope cutthroat trout, as well as a hatchery strain of Yellowstone cutthroat trout from Montana. Their field specimens were collected by backpack electroshocking. Although their study was designed to elucidate the level of genetic variation among populations while ours was simply to answer the question of genetic purity or, if hybridized, with what, the overlap of collection streams invites a comparison of their results with ours.

For openers, six of the seven overlapping streams have a record of stocking with hatchery cutthroat trout, and Cedar Creek and East Branch LeClerc have records of stocking with rainbow trout. We classified Slate, Upper Sullivan and North Fork Sullivan creeks as B-populations using the modified Binns genetic purity rating based on their cutthroat stocking history. We also classified Cedar Creek and East Branch LeClerc as B-populations because we found small percentages of individuals hybridized with rainbow trout in each. We classified West Branch LeClerc, and Mill creeks as D- and F-populations respectively based on our finding of progressively higher percentages of specimens hybridized with rainbow trout in these populations and, in the case of Mill Creek, a number of specimens that we regarded as questionable representatives of westslope cutthroat appearance-wise. The Shaklee and Young (2000) study did not take account of rainbow-cutthroat hybridization.

With regard to Upper Sullivan and North Fork Sullivan, where hybridization with rainbow trout was not an issue, Shaklee and Young (2000) found that these populations are quite distinct from either the Kings Lake or the Twin Lakes hatchery strains of westslope cutthroat and even more distinct from the hatchery Yellowstone cutthroat strain. Furthermore, these populations are also distinct from one another, indicating that they exist as reproductively isolated, separate stocks. To us, these findings justify upgrading these two populations to A-purity on the grounds that despite stocking, introgression with hatchery origin cutthroat trout has not taken place.

Shaklee and Young (2000) found that only one population—that in Slate Creek—is genetically similar to the Kings Lake hatchery strain. They suggested that this similarity may be the result of historical introductions of Kings Lake cutthroat into a stream that lacked a native trout population for some reason, giving the hatchery fish an opportunity to establish in the absence of wild fish. We regard this as reasonable and as good an explanation as any. Hatchery cutthroat introductions did occur in 1940, 1941, and 1945, although the records do not indicate that these were Kings Lake cutthroat. A brief account of the King's Lake Hatchery broodstock is provided as Appendix A.

Non-native Brook Trout and Competition with Native Species

According to Tom Shuhda, U. S. Forest Service (personal communication 1999), planting of brook trout in Colville National Forest waters ceased some 14 years ago, and there have been attempts to eradicate them with bag limit increases that now allow anglers to keep as many as 50 brook trout. Shuhda took encouragement from some of our collection results, noting that we found only westslope cutthroat trout in a few streams where brook trout had predominated in earlier U. S. Forest Service surveys. Shuhda feels that improved forest practices over the past 15 years combined with the cessation of planting of brook trout on the Forest have begun to benefit native trout populations to some degree in Colville National Forest.

This may very well be the case, but our habitat scores do not cast much light on this subject. In fact, the only trend that appears in our data would seem to be in the opposite direction—provided that the trend is indeed real. What we see is that collection sites with brook trout average about 12 points *higher* than the mean for all sites (i.e., slightly better habitat conditions than the average) whereas sites without brook trout average about 12 points *lower* than the mean for all sites (i.e., slightly poorer habitat conditions than the average). These differences are not statistically significant and the power of our data to detect real differences is low. However, if this were indeed a real trend, it would suggest that brook trout may fare poorer in competition situations, or conversely, native trout may fare better, in sites with poorer rather than improved habitat. This needs to be looked into further.

Our data do suggest that there may be an elevation threshold above which planted brook trout may not be able to compete successfully with native cutthroat trout in Colville National Forest streams. We found brook trout in nine streams, including six of the eleven streams with stocking records for this species plus three additional streams (Rocky, Silver, and Slate creeks) for which we found no record of brook trout stocking. Brook trout were not present at our collection sites in five of the streams which had been planted with this species in the past. These sites were:

South Fork Sanpoil, elevation 1265 m (4150 ft);
Upper Sullivan Creek, elevation 1256 m (4121 ft);

East Fork Smalle Cr., elevation 1085 m (3560 ft);
Harvey Creek, elevation 1006 m (3300 ft);
Lone Ranch Creek, elevation 793 m (2602 ft).

Of these, the top four are cutthroat trout streams and Lone Ranch Creek, the lowest elevation site, has an A-purity population of presumably native rainbow trout. All streams where we found brook trout co-existing with cutthroat trout ranged in elevation from 610 m (2000 ft) to 939 m (3080 ft). These data suggest that for the cutthroat streams, there may be a threshold at about the 1000 m (3280 ft) elevation above which introduced brook trout do not compete well against the native cutthroat and do not become established.

Such an altitudinal threshold would be consistent with other published studies from the Rocky Mountain west, where Griffith (1988) has reported that many of the remaining populations of interior cutthroat trout are found in small high elevation streams above the upstream limit of brook trout. Reasoning from observations that stream temperatures are generally cooler and water velocities faster at upstream sites dominated by cutthroat trout, Fausch (1989) hypothesized that these factors may provide a competitive advantage to cutthroat trout in high elevation streams.

Since we also had data for water temperature, reach gradient, and water velocity (used to compute discharge) for our collection sites, we went back and tested for differences in these parameters between sites where introduced brook trout became established and sites where they apparently did not. Aside from South Fork Sanpoil River which was steep where we collected, there was no difference between the two groups of sites in reach gradient or water velocity. However, there was about a 2^{O} C difference in mean water temperature, with the higher elevation group being colder, and this difference was significant using a one-tailed Student t-test at the $\alpha = 0.05$ level. So our data, although meager, support the observations of Griffith (1988) and Fausch (1989) at least insofar as elevation and water temperature are concerned.

Further on the matter of water temperature, De Staso and Rahel (1994) found that brook trout and Colorado River cutthroat trout *Oncorhynchus clarki pleuriticus* were nearly equal competitors at 10° C, but brook trout showed a clear competitive dominance over cutthroat trout at 20° C in a

laboratory stream. De Staso and Rahel (1994) did not test water temperatures below 10° C, but their results, taken with the other results discussed above, suggest the likelihood that even colder waters tip the competitive balance in favor of cutthroat trout.

With regard to competition with native rainbow trout (for example, in lower elevation presumably warmer streams such as Lone Ranch Creek), brook trout may again be at a competitive disadvantage. Published evidence from elsewhere in North America indicates that rainbow trout have a greater niche breadth (Cunjak and Green 1981) and are adapted to a greater range of channel gradients (Platts 1976) than are brook trout. There is also evidence that in sympatry, growth of subyearling brook trout may be suppressed following emergence of rainbow trout, which in turn may lead to increased overwinter mortality of the brook trout (Rose 1986). This may be one mechanism by which brook trout are excluded by rainbow trout, but nothing definite has been pinned down (Clark and Rose 1997). One body of literature that has examined brook trout-rainbow trout interactions is from the Appalachian Mountain region where southern brook trout are native and introduced rainbow trout have displaced them in all but the headwater reaches of mountain streams (Larson and Moore 1985; Larson et al. 1995; Clark and Rose 1997; Strange and Habera 1998). However, this literature does not elucidate specific causal mechanisms and concludes only that distributional limits of brook trout and rainbow trout will ebb and flow over time in Appalachian mountain streams.

Similarities with Missouri River Basin Westslope Cutthroat Populations

Recently, Shepard et al. (1997) estimated that westslope cutthroat trout of at least 90 percent genetic purity presently inhabit less than 3 percent of the former range within the upper Missouri River drainage, which has long been regarded as the heart or core of this subspecies' historic distribution (Behnke 1992). Of 16 upper Missouri subbasins still supporting at least one population, 14 contain populations at moderate or high estimated risk of extinction, and almost all of these remaining populations exist in isolation from one another in high elevation mountainous stream fragments less than 10 km long. Shepard et al. (1997) identified existing and future land use activities and introduced species as the principal threats to these upper Missouri populations

If we apply the same 90 percent genetic purity criterion that Shepard et al. (1997) used to the populations we examined in Colville National Forest streams, then 11 of our 15 cutthroat populations qualify, including our four A-purity populations and our seven B-purity populations. As noted earlier, one of our B-populations, Slate Creek, appears to have originated from hatchery introductions based on the findings of Shaklee and Young (2000), so we exclude it from the present discussion.

We have no information on how many stream km each of the remaining 10 qualifying populations occupy, but the elevations of our collection sites range from 732 m (2402 ft.) to 1265 m (4150 ft.) which we would classify as high elevation relative to the basin relief of the Colville National Forest. Also, based on the work of Shaklee and Young (2000), we could say that these populations exist as reproductively isolated, separate stocks.

So in these regards, it would appear that the high purity westslope cutthroat populations of the Colville National Forest share the status of their upper Missouri conspecifics, and probably similar estimated risks of extinction as well. As noted already, our data provide little basis for commenting on the threats of existing and future land use activities to these populations. However, we observe from the record that each of our Colville National Forest populations has already been subjected to exposure to introduced species, and in many cases also to stocking with hatchery origin cutthroat trout. The heartening thing is that several of these populations have shaken off these adverse influences. For example, two of the four A-purity populations (South Fork Sanpoil and East Fork Smalle) and two of the B-purity populations (Upper Sullivan and Harvey Creek) have outlasted introduced brook trout, and two of the B-purity populations (Upper Sullivan and North Fork Sullivan) have survived introductions of hatchery cutthroat trout without introgression (Shaklee and Young 2000). The Upper Sullivan population has even withstood stocking of hatchery rainbow trout without interbreeding (this study).

But introduced species continue pose a threat to some of the other high-purity populations. Brook trout are present and are well established among three of the populations, including two of the A-purity populations (Silver and Rocky creeks) where brook trout comprise 24 percent of the total trout present at each site, and at one of the B-purity sites (East Branch LeClerc) where they comprise 45

percent of the total trout present. Brook trout are also present at the Cedar Creek site cohabiting with the B-purity cutthroat population, but here brook trout comprise less than 5 percent of the total trout present.

ADDITIONAL MATERIAL

For completeness, we also searched the record for stocking histories of the 25 sites in our study area which had been collected previously for earlier genetic studies (Leary 1997; Kanda and Leary 1998, 1999). We found stocking records for 13 of these sites, but the other 12 sites had no history of hatchery stocking that we could locate. For three of the 12 sites lacking stocking records, the genetic results—and here we refer you to the original reports (Leary 1997; Kanda and Leary 1998, 1999)—indicate that stocking must have occurred there nevertheless, since the populations at these sites were hybrid swarms (coastal rainbow trout hybridized with native interior rainbow in each of these cases). In addition, for nine of the sites where we did find stocking records, those records failed to account for all of the species or subspecies identified in the hybrid swarms. For example, for three streams with three-way hybrid swarms between coastal rainbow, native interior rainbow and (presumably) native westslope cutthroat trout, no stocking record could be found to account for the coastal rainbow component. In another interesting case, a hybrid swarm between native interior rainbow trout and Yellowstone cutthroat trout was found in one creek, but no stocking record could be found to account for the Yellowstone cutthroat.

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APPENDIX A.

A Brief History of the Kings Lake Westslope Cutthroat Broodstock

According to Crawford (1979), during the late 1930s the Washington Department of Game (as it was known at the time) was researching a suitable cutthroat broodstock to use in the Spokane area to *replentish the once abundant native cutthroat* [emphasis ours].[(1)] The Twin Lakes strain of westslope cutthroat, whose probable origin is the Stehekin/Lake Chelan drainage (Crawford 1979), was tested in the Spokane and Pend Oreille areas but failed to perform well. So an arrangement was made with the State of Idaho for 107,000 eggs from Granite Creek[(2)] and 12,288 eggs from Kalispel Creek, both tributaries of Priest Lake. Priest Lake, it should be noted, drains via Priest River into the Pend Oreille River, so this stock is at least native to a portion of the area where it was intended to be used.

The eggs from Granite and Kalispel creeks were received in Washington in May, 1940, and were taken to the Pend Oreille Hatchery (located at Usk; water supply Skookum Creek; built 1921, rennovated 1951, closed 1967), where the hatched-out fish were held until the spring of 1941. At that time, they were stocked in Kings Lake (Pend Oreille County, T 33N, R44E, sec. 1 and 2), which had been treated with rotenone in the meantime to remove all other fishes.[(3)] The first egg take from this Kings Lake broodstock took place in May, 1943. At present, fry are reared at the Colville Hatchery, where we took our genetic tissue samples.

According to Crawford (1979), the Kings Lake broodstock has been kept free from mixing with other cutthroat stocks, and *therefore remains representative of the native cutthroat found throughout northeastern Washington and northern Idaho* [emphasis ours]. However, we would also point out that in using these fish to stock waters where wild native cutthroat may already be present, no consideration has been given to any genetic changes that might have occurred due to domestication selection over the period of captivity and hatchery rearing.

Footnotes

(1) The westslope cutthroat trout is native to northeastern Washington and northern Idaho, having recolonized formerly ice-covered habitats from the Lake Missoula refugium (Behnke 1992). Even

so, according to Crawford (1979) the old U. S. Bureau of Fisheries stocked cutthroat trout into many northeastern Washington lakes and streams beginning as early as 1895. Many, perhaps most, of these were "black spotted trout" from Yellowstone Lake, a totally different subspecies from the native westslope cutthroat. Yellowstone Lake cutthroat belong to the subspecies *bouvieri*, whereas westslope cutthroat belong to the subspecies *lewisi* (Behnke 1992).

An interesting hybrid strain, known locally as "carrot trout" for the distinctive overall orange coloration of the fish, may have resulted from one of these old U. S. Bureau of Fisheries stockings, although we could find no corroborating record. "Carrot trout" occur in East Deer Creek, a Kettle River tributary, in and possibly also upstream of a reservoir that serves as a water supply for the small community of Orient Washington. According to Leary (1997), who examined these fish by protein electrophoresis, the population is a hybrid swarm resulting from the interbreeding of native interior redband rainbow *O. mykiss gairdneri* and introduced Yellowstone cutthroat *O. clarki bouvieri*.

Regarding the early use of (presumably) native westslope cutthroat trout for stocking northeastern Washington waters, records compiled by Crawford (1979) indicate that the old Washington Department of Fisheries and Game obtained cutthroat eggs from a station located at Sullivan Lake, Pend Oreille County, in 1915, 1921, 1922, and 1926. These eggs went to the Little Spokane Hatchery for rearing and distribution for stocking. Unfortunately, from the standpoint of out-of-basin transfers, the Little Spokane Hatchery also received cutthroat eggs from the Stehekin Hatchery at Lake Chelan before and during this period (Washington State Fish Commissioner. 1905, 1907….through 1919).

(2) Native westslope cutthroat trout from Priest Lake spawned in several of the lake's tributary streams, but of all of these, Bjornn (1957) considered Granite Creek to be the best. The spawning run into Granite Creek was indeed large enough to support egg taking operations from 1939 through 1947. Although the fish trap for this operation was located 9.6 km (6 miles) upstream from the lake and the large majority of the fish spawned in the lower 4.8 km (3 miles) of stream, the run was nevertheless large enough most years for close to 2,000 spawners to ascend to the trap. In the spring of 1947, the final year of egg taking, 1,660 spawners were captured at the trap. Presently, Granite Creek (which actually heads up in Washington) is closed to angling in both Washington and Idaho as a spawning and stream-rearing sanctuary for westslope cutthroat and bull trout.

(3) The rotenone treatment of Kings Lake has the (dubious) distinction of being the first such lake rehabilitation to be carried out in the State of Washington (Crawford 1979).

APPENDIX B

Photographic Record of Collections in the Colville National Forest in 1999

Contents

Photos B1 1 -7: East Fork Smalle Creek

Photos B1 8 - 14: Lone Ranch Creek

Photos B1 15 - 21: Canyon Creek

Photos B1 22 - 29: Upper Sherman Creek

Photos B2 1 - 9: South Fork San Poille River

Photos B2 10 - 16: Deep Creek

Photos B2 17 - 23: Mill Creek

Photos B2 24 - 29: Silver Creek

Photos B2 30 - 36: South Fork Salmo River

Photos B2 37 - 44: West Branch LeClerc Creek

Photos B2 45 - 46: East Branch LeClerc Creek (no fish photos taken)

Photos B2 47 - 54: Fourth of July Creek

Photos B2 55 - 61: Harvey Creek

Photos B2 62 - 68: North Fork Sullivan Creek

Photos B2 69 - 76: Upper Sullivan Creek

Photos B2 77 - 83: Rocky Creek

Photos B2 84 - 91: Slate Creek

Photos B2 92 - 100: Cedar Creek