Genetic relationships among anadromous and non-anadromous *Oncorhynchus mykiss* in Cedar River and Lake Washington - implications for steelhead recovery planning

FINAL REPORT
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ABSTRACT

The goal of this research project was to understand genetic population structure of Cedar River resident and anadromous *O. mykiss* to assist with conservation and recovery strategies. Cedar River drains into Lake Washington, which is artificially connected to Puget Sound by a shipping channel and lockage system. Landsburg Dam at Cedar River mile 21 had blocked anadromous adults from 17.5 mainstem miles and associated tributary habitat from 1900 until September 2003 when a fish ladder became operational. Restoration of steelhead to the upper watershed was intended but steelhead abundance had been critically low during the last 14 years. In contrast, resident or non-anadromous *O. mykiss* were present throughout the river, and appeared abundant in below-dam areas. To evaluate genetic relationships between anadromous and resident populations, we sampled 180 resident phenotypic *O. mykiss* in below- and above-dam Cedar River areas, 24 phenotypic *O. mykiss* smolts from a lower Cedar River trap, and 57 putative Cedar River steelhead that had been captured at the shipping locks. We also sampled wild and hatchery steelhead in the adjacent and historically-connected Green River, lake-resident *O. mykiss*, and non-native hatchery rainbow trout stocks. We sampled adult and smolt *O. clarki* in Cedar and nearby rivers in order to identify *O. mykiss*/*O. clarki* genetic hybrids in samples. We collected data for 22 microsatellite DNA loci in all samples and used six nuclear DNA loci for additional species identification. We found that nearly all resident adult *O. mykiss* sampled in Cedar River below- and above-dam areas were native-origin and not introduced hatchery trout. Below- and above-Landsburg Dam resident *O. mykiss* samples were divergent from each other, but above-dam fish were genetically more similar to below-dam resident *O. mykiss* than to wild steelhead. This suggested that above-dam *O. mykiss*, which had a long isolation from steelhead, could get downstream successfully prior to the fish ladder. Below-dam resident *O. mykiss* as a group were divergent from steelhead, but individual genotypic analyses showed that many resident fish were most likely derived from native steelhead. Based on genetic assignment tests, some smolts had higher likelihoods of originating from resident *O. mykiss* than from anadromous *O. mykiss*. The percentage of smolts estimated to have resident fish ancestry varied depending on type of assignment test and populations included in baselines. Among all fish sampled in Cedar River, regardless of phenotype, we found about 14.5% *O. mykiss*/*O. clarki* genetic hybrids. We speculate that the resident life-history exhibited by some Cedar River *O. mykiss* may have become recently more common due to modified fish communities and freshwater habitats, coincidental to poor steelhead returns. Similar to other studies, our results suggested that non-anadromous *O. mykiss* may contribute to reducing extinction risk for steelhead. However, to improve the status of steelhead, resident phenotypes must produce smolts that have successful marine migrations.

INTRODUCTION

We initiated this research project in 2002 to investigate genetic aspects of *Oncorhynchus mykiss* populations in the Cedar River watershed (Figure 1). Two major factors prompted this work. Steelhead, the anadromous form of *O. mykiss*, were at low abundance, and fish passage was being added to Landsburg Dam (river mile 21; Figure 1), which was expected to improve conditions for steelhead by greatly expanding spawning and rearing habitat. Non-anadromous or resident *O. mykiss* (rainbow trout) were common throughout the basin and were the only form present above Landsburg Dam. We designed the study to gain knowledge about genetic relationships between Cedar River anadromous and resident *O. mykiss*, and to assess population genetic characteristics of steelhead via adult samples. This type of information was essential for developing effective actions for conservation and restoration of the steelhead resource.

Cedar River wild steelhead spawner abundance had varied widely since 1983-84, and experienced acute declines in 1993-94 and since 1999 (Figure 2). In 2002, resident *O. mykiss* appeared abundant in the river below Landsburg Dam, the existing anadromous zone, although no population size estimates were available. Genetic relationships, such as interbreeding or shared ancestry, between anadromous and below-Dam resident *O. mykiss* were unknown. The nature of this relationship is important for understanding the status of steelhead. For example, if anadromous and resident fish share common ancestry, interbreeding is common, and progeny from any pairing of life-history types express either phenotype, then the population dynamics of steelhead is very different from a case of no interbreeding and no phenotypic plasticity.

In September 2003 the Landsburg Dam fish ladder was completed by Seattle Public Utilities. This action was part of the 50-year Cedar River Habitat Conservation Plan (HCP) approved in 2000. Improvements also were made to assist downstream migration of juveniles or other fish over the dam and past water diversion in-takes. The dam had blocked anadromous adult fish passage since 1900, eliminating access to approximately 17.5 miles of mainstem habitat and additional tributary habitat. For a few years from the mid-1980's to 1990 fry produced by breeding wild steelhead intercepted at Ballard Locks (Figure 1) were released upstream of Landsburg Dam in an effort to gain anadromous production from the blocked habitat (WDFW unpublished records). Presuming that some smolts would pass over the dam successfully, any adult steelhead returning to areas just below the dam were to be transported upstream. We have not found records on whether or how many adults were transported, but some may have been. This is the only activity we have information about regarding possible genetic contributions of anadromous fish above Landsburg Dam since its installation.

Restored upstream access via the fish ladder was expected to benefit steelhead, and their use of upstream habitats was expected to occur through natural processes. There was concern, however, that full advantage of the new conditions might not be realized due to recent low steelhead abundance. Resident *O. mykiss* below Landsburg Dam also could utilize upstream habitat, assuming they could travel up the fish ladder successfully. Migration of any *O. mykiss* above the dam would likely result in ecological and reproductive interactions with the upstream resident population, which had been nearly completely isolated from immigrants. The upstream

population, however, has had the potential to contribute to downstream populations by fish moving over the dam.

In general, Puget Sound streams below migrational barriers appear to be inhabited predominantly by anadromous *O. mykiss* populations, in contrast to inland Columbia Basin rivers where anadromous and resident fish typically co-exist. Pacific coastal and inland *O. mykiss* populations appear to share this contrast coast-wide (Busby et al. 1996). Thus we expect that the steelhead life-history was the most common form of *O. mykiss* in Cedar River when it was a Green River Basin tributary prior to 1912. In that year the Cedar was artificially diverted into Lake Washington (Figure 1). The shipping channel constructed between the lake and Puget Sound at Shilshole Bay became the new ocean migration route, meaning Cedar River anadromous fish had to travel through at least 20 miles of lacustrine habitat and a lockage system. Given the substantial environmental changes, resident *O. mykiss* below Landsburg Dam could be examples of an ecophenotype (Zimmerman and Reeves 2000) that becomes common under particular conditions. Recent freshwater and ocean conditions may have favored fish expressing a resident life-history. Quinn and Myers (2004) suggested that high proportions of rainbow trout compared to steelhead in northern Alaska rivers may be determined more by growth opportunities (abundant freshwater food supply) than opportunity for marine migration.

Several studies (Docker and Heath 2003; Pearsons et al. *in press*; McCusker et al. 2000; Zimmerman and Reeves 2000) have shown that native resident and anadromous *O. mykiss* within a drainage were closely related, and likely to interbreed at some level. In this study we expected to be able to genetically detect whether Cedar River resident trout were native or related to non-native (California-origin) hatchery rainbow trout that had been released historically in area waters, especially in Lake Washington. We examined genetic heritage by comparing below- and above-Dam resident adults to each other, and both to anadromous adults presumed destined for Cedar River, to ancestrally-related Green River steelhead and resident *O. mykiss*, and to relevant hatchery populations. If wild resident fish were genetically similar to non-native hatchery strains, resident trout would not be potential contributors to anadromous population restoration.

If resident fish are of native origin and produce anadromous offspring there is support for their potential to play a role in rebuilding the anadromous fish population. It is relatively well-documented that non-anadromous precocious males occur in steelhead populations. In a Hood River, Oregon steelhead reproductive success study using DNA pedigree analysis, researchers estimated that about 40% of returning steelhead had non-anadromous male parents (Ardren 2003; Blouin 2003). In an on-going breeding study using Grande Ronde Basin (OR) steelhead and resident trout, crosses between male and female trout and between trout and steelhead all produced seaward-migrating smolts (Ruzycki et al. 2003). In an Alaskan study, lake rainbow trout derived from steelhead were bred with contemporary steelhead and all possible crosses produced smolts (Thrower et al. 2004). If Cedar River resident *O. mykiss* are recently derived from steelhead, it is likely that resident adults are capable of producing smolts. We evaluated this genetically by estimating smolt origins relative to all potential source groups, including the above-dam resident population.

Hybridization and other interaction with cutthroat trout could affect the status of Cedar River *O. mykiss* populations. Cutthroat trout (*O. clarki*) are abundant in Lake Washington and its smaller

tributary streams, and appear to favor and succeed at an adfluvial life-history strategy (migration between streams and lake) versus anadromy (Nowak et al. 2004; Seiler et al. 2003). Cutthroat trout are known to hybridize with *O. mykiss* in anadromous zones of Puget Sound and other western Washington streams (Campton and Utter 1985; Hawkins 1997; Wenburg and Bentzen 2001; Young et al. 2001; Ostberg et al 2004). In un-modified natural habitats cutthroat trout and *O. mykiss* are ecologically distinct and remain reproductively isolated through geographic and temporal differences in spawning behavior. Previous genetic analysis (see below) found juvenile inter-specific hybrids in Cedar River. The relatively recent increase in Lake Washington cutthroat trout abundance (see Nowak et al. 2004) could increase the incidence of hybridization. Also, the area's significant habitat changes could be disrupting natural species isolating mechanisms. Hybridization with cutthroat trout is a significant alteration of the *O. mykiss* gene pool, and would likely affect anadromous traits, especially the long-distance ocean migrations innate in steelhead. We designed our genetic analysis to identify and characterize *O. mykiss*/*O. clarki* hybrids in all study samples.

Cedar River steelhead may be affected by other sources of genetic change besides hybridization. For example, genetic change could result from selection against particular life-history trajectories, or from a consistently small number of successful spawners. If resident and anadromous *O. mykiss* are reproductively isolated, a small and declining anadromous population is more likely to experience a reduction in genetic diversity. Low genetic diversity is considered a limiting factor for a population's long-term viability. We evaluated Cedar River adult steelhead genetic diversity by comparing data between these fish and steelhead of the wild Green River population, which is larger and rated in the Washington Salmon and Steelhead Stock Inventory (SaSI) as of 'healthy' status (WDFW, 2002). We also compared genetic diversity among resident *O. mykiss* population and steelhead samples.

A previous genetic study of Cedar River juvenile (age 1 parr) *O. mykiss* provided useful data for development of this study and background on population relationships. Genetic analyses were conducted on juvenile *O. mykiss* sampled during May 1993 and 1994 in mainstem Cedar River (Maple Valley area) below Landsburg Dam, on juveniles sampled in 1994 above Landsburg Dam, and on rainbow trout sampled in 1994 from Chester Morse Lake (Figure 1; Phelps et al. 1994; Phelps and Baker 1995; Phelps et al. 1997). Parr below Landsburg Dam were presumed to be steelhead progeny based on an assumption that steelhead was the primary life-history form in that area. Genetic data were collected by analyzing allelic variation at allozyme (protein) gene loci. Significant temporal variability in allele frequencies occurred between the two lower Cedar River juvenile samples, but the annual samples were more similar to each other than to other *O. mykiss* samples included in comparative analyses (Phelps et al. 1997; WDFW unpublished data). Below-dam juvenile *O. mykiss* were genetically closer to Green River juveniles than they were to *O. mykiss* upstream of the dam or in Chester Morse Lake. Phelps and Baker (1995) described Cedar River *O. mykiss* above Landsburg Dam as being relatively similar to downstream *O. mykiss*, except that the population appeared to contain alleles common in non-native rainbow trout hatchery strains. Also, three above-dam fish appeared to be *O. mykiss*/*O. clarki* hybrids. Phelps and Baker (1995) concluded that Chester Morse Lake *O. mykiss* likely had some ancestry from an exotic rainbow trout hatchery strain.

The use of pre-smolt juveniles in the previous study precludes an understanding of relationships between resident and anadromous *O. mykiss* below Landsburg Dam. Sampled juveniles could have included offspring from parents of either life-history. However, the juveniles as a group did appear to be native Puget Sound-origin, suggesting little if any natural production by hatchery trout strains. Resident *O. mykiss* most likely produced the juveniles sampled in the river above Landsburg Dam, unless steelhead were transported upstream in the early 1990's in response to the brief artificial production program described earlier. Genetic results for these juveniles suggested persistence of a legacy from native *O. mykiss* that remained upstream after dam construction, with perhaps some influence of migrants from Chester Morse Lake. Regarding the relatively unique composition of Chester Morse Lake fish, we expected that microsatellite DNA loci employed in this study and data from trout populations in other lakes and nearby basins would identify their origins more clearly.

For the purposes of this study, we defined resident *O. mykiss* in Cedar River below Landsburg Dam or in Lake Washington as fish of larger sizes and older ages than those expected in juvenile (pre-smolt) steelhead offspring, and which, by scale pattern analysis, appeared to have not migrated to the ocean. Trapped Cedar River *O. mykiss* smolts had an average length of 17.8 cm (range 14.7-22.5) in 2000 (Seiler et al. 2003), and 20.1 cm (range 15.5-25.5) in 2001 (Seiler et al. 2004). Puget Sound steelhead smolt ages typically range from 1 to 3 years with the largest proportion of fish smolting at age 2. We planned to sample *O. mykiss* 30 cm and larger in the river after the steelhead spawning period because we thought fish this size would be at least 3 years old, of reproductive age (adults), and non-anadromous. Any *O. mykiss* upstream of Landsburg Dam prior to 2004 were considered resident trout, or at the very least had recent resident trout parents. Hatchery-origin, non-native rainbow trout, which have been released in area lakes for recreational fishing opportunity, are expected to be non-anadromous, and if they occurred in our study area would likely be larger than 25 cm, given size at release (J. Uehara, WDFW, personal communication). Pearsons et al. (*in press*) also used size, sampling location, scale pattern, and sexual maturity criteria to distinguish between steelhead and resident rainbow trout in their study of gene flow between the two life-history types in the Yakima Basin.

The goal of this project was to understand genetic population structure of Cedar River watershed *O. mykiss* so that managers can design and implement strategies that effectively conserve and recover native steelhead and resident trout resources. Research objectives were:

1) Determine genetic relationships between adult anadromous and resident *O. mykiss* throughout the Cedar River watershed

2) Estimate genetic origins of Cedar River outmigrating *O. mykiss* smolts relative to all potential parent groups

3) Determine genetic relationships between Cedar River *O. mykiss* and Green River wild and hatchery steelhead populations

4) Identify *O. mykiss* and *O. clarki* hybrids and the incidence of hybrids in all sampled groups

5) If possible and appropriate, estimate effective population size for Cedar River steelhead

METHODS

Field Sampling

We planned to sample at least 12 groups or populations of *O. mykiss* and *O. clarki* throughout the Cedar River watershed and nearby drainages or hatcheries for this study. The major targets for field sampling were wild anadromous *O. mykiss* (steelhead) from Cedar and Green rivers, wild resident *O. mykiss* and *O. clarki* from Cedar River above and below Landsburg Dam, residents of both species in Lake Washington, Chester Morse Lake *O. mykiss*, Cedar River smolts, Green River resident *O. mykiss*, and hatchery stocks of winter and summer steelhead released in Green River basin. Our sample size goal was 50 fish per wild fish group in each of two years, and was 50 to 100 for hatchery populations. We assumed 100 fish would adequately represent a population's genetic diversity and that sampling over two years would improve representation of genetic diversity in small populations and/or in those that had a dominant year class when sampled.

Most field sampling occurred between April and July in 2003 and 2004. We acquired some *O. clarki* samples in other months and in 2005. We sampled adult resident fish in river and lakes by angling. Criteria for targeting adults (≥ 30cm) are described and explained in Introduction above. Adult steelhead were trapped at the Ballard Locks fish ladder because biologists and managers agreed that, based on steelhead homing tendencies, few incidences of marked non-Cedar River steelhead, and lack of correlation between annual Cedar River run sizes and nearby Green River run sizes, the vast majority of trapped fish were likely to be returning Cedar River fish. Juveniles were sampled in Cedar River using a downstream migrant screw-type trap (Seiler et al. 1981), and similar trapping was used to sample *O. clarki* in Big Bear Creek (Sammamish River tributary; Figure 1). Smolts were distinguished from other similar-sized fish by their silvery appearance and lack of colored bands or markings. Some fish of both species were sampled in Lake Washington via gill-net or purse-seine incidental catches resulting from other research or harvest activities. Samples for hatchery populations were collected during broodstock spawning or from rearing progeny. Some non-Cedar River/Lake Washington samples were acquired prior to 2003 through other research or hatchery projects.

A very small (approx. 0.5cm^2) clip of ventral fin tissue, a group of scales dorsal to the lateral line, and a fork length measurement was taken from each angled fish while it was held in the water, after which the fish was immediately released. The phenotypic identification for species was made and if a phenotype was judged intermediate, the fish was noted as a potential inter-specific hybrid. Steelhead at Ballard Locks were sampled similarly, except they were held in tubes or cradles and anaesthetized. Juveniles were anaesthetized with MS-222 prior to taking a fin-clip sample and length, and were revived and released. Fin-clips, scales, and measurements were taken from adult fish in other study samples either after death or prior to release from capture. Fin tissue was immediately placed in plastic vials containing 100% ethanol, and all samples were stored in ethanol at room temperature. Scales were placed on traditional paper scale cards, which we used to record sampling data for each fish. As mentioned above, we used some samples that were not acquired by us or under our direction. Fin or opercle tissues had been sampled for DNA analyses, but scales were not always taken.

Due to poor sampling success at Ballard Locks in 2003 and the very low abundance of steelhead subsequently on Cedar River spawning grounds, we chose to include steelhead sampled at

Ballard Locks in 1997, 1998, and 1999 to serve as our anadromous Cedar River population sample. These fish, presumed to originate from Cedar River as described above, had been used as wild broodstock for experimental hatchery production aimed at steelhead restoration in the Sammamish River basin. They had been scale-sampled at spawning time and we used the dried, preserved scales as our tissue source for DNA.

Laboratory Analyses

Scale Pattern Analysis

Scale pattern analysis (Davis and Light 1985; Shapovalov and Taft 1954) was used to determine total age, including time periods for juvenile freshwater rearing and marine residency, and to determine spawning history for all sampled adults. Scale preparation and analysis was conducted by WDFW staff.

DNA extraction and microsatellite loci amplification

Genomic DNA was extracted from tissue samples using silica membrane kits (Clontech Incorporated). We tested 25 microsatellite loci (short sequence-repeat DNA markers) for amplification in *O. mykiss* and *O. clarki* and used 22 loci for analyses of these samples. These loci had few to no artifacts or detectable null alleles and generally displayed Hardy-Weinberg equilibrium. Microsatellite alleles (alleles vary in number of core unit DNA sequence repeats) were amplified using fluorescently labeled primers and the polymerase chain reaction (PCR; see Table 1 for detailed PCR information). PCR's were conducted on a MJResearch PTC-200 thermocycler in 10 µl volumes employing 1 µl template with final concentrations of 1.5 mM $MgCl_2$ and 1X Promega PCR buffer, and two to five loci were pooled ("multiplexed") for processing (Table 1). Number of PCR cycles and dye concentrations were altered for *O. clarki* samples, and differences are indicated in Table 1. Samples were run on an ABI 3730 DNA analyzer. Allele mobilities were determined by allele length (number of basepairs) and alleles were sized to basepairs (bp) using an internal lane size standard (GS500LIZ 3730 by Applied Biosystems), and ABI computer program Genemapper v3.0. Raw allele mobilities were binned into discrete allele bins according to allele frequency histograms in Genemapper. Final allelic genotypes at all loci for each fish were recorded in electronic databases.

Some PCR amplifications of loci were unsuccessful in some fish, resulting in missing genotypic data. Missing data can yield inaccurate results for various analyses. We decided an individual had to have a minimum of eight loci with genotypic data to be included in analyses. Thus, although analyses were conducted based on 22 loci, some individuals did not have data for all loci. Analyses involving genetic data collected previously from WDFW hatchery rainbow trout samples were conducted with 14 loci common to all samples.

DNA species markers for hybridization tests

Individuals that were identified phenotypically or genotypically through microsatellite DNA genotypic profiles (see *Identifying hybrids*.. section below) as potential *O. clarki*/*O. mykiss* hybrids were examined for hybridization using nuclear DNA markers with species-specific patterns. We used three simple sequence repeat (SSR) markers, OCC-16, OCC-28 and OM-35 (Ostberg and Rodriguez 2002), and restriction fragment length polymorphism (RFLP) analysis of

three single copy genes, ribosomal internal transcribed spacer (ITS), gonadotropin-releasing hormone (GnRH), and proto-oncogene p53 (p53-7; Baker et al. 2002). The SSR's were PCR-amplified following methods of Ostberg and Rodriguez (2002) and DNA fragments were separated and visualized on 1.5% agarose gels stained with 1% SYBR Gold. Fragments were sized in comparison to a 100bp ladder. For RFLP analysis, DNA fragments were PCR-amplified, restriction-digested and separated on agarose gels following methods of Baker et al. (2002).

We also chose a large set of individuals believed to be pure *O. clarki* or *O. mykiss* based on phenotype, biological data, sampling location, and microsatellite genotypes to analyze for all six nuclear DNA markers. We expected that results from this analysis would verify species-specific fragment patterns in tandem to verifying individuals as pure species. Ostberg and Rodriguez (2002) did not include any Puget Sound *O. mykiss* or *O. clarki* samples in marker development so it was possible that fragment patterns might differ in the distinct populations of this region.

Statistical analyses

Within-sample genetic variation

Statistical tests were conducted on microsatellite genotypic data per locus and per sample (across all loci) to assess conformation to Hardy-Weinberg expectations for genotypic equilibrium (HWE), linkage disequilibrium, and genotypic heterogeneity using GENEPOP3.3 (Raymond and Rousset 1995) and FSTAT2.9.3 (Goudet 2001). We tested for HWE in loci and samples across all loci using FSTAT2.9.3 and across all samples using GENEPOP3.3. Tests for HWE assess if observed genotypic heterozygosity in populations deviates from expected heterozygosity. Deviations from expectations may indicate sampling error, data collection error, or that a perturbing process is affecting the population. We assessed linkage disequilibrium (non-random genotypic associations between all possible pairs of loci) using GENEPOP 3.3 (200 batches, 1000 iterations). Allelic richness (average number of alleles corrected for sample size), gene diversity (expected heterozygosity corrected for sample size), and *F*-statistics (allelic correlations within or among population subdivisions) were calculated using FSTAT2.9.3. All statistical results were corrected for multiple simultaneous tests of the same hypothesis (Bonferroni correction) to an overall alpha level of 0.05 (Rice 1989).

In the initial analysis of our first group of samples ("2004 group", Table 2) we conducted the above statistical tests on some samples that contained hybrids or mixed species. In most cases we did not know when samples contained true hybrids and/or misidentified species. We were interested to see how results from tests on potentially mixed samples would compare with those from samples of 'pure' species, such as adult steelhead. Subsequent to these within-sample statistical analyses we identified hybrids and verified species in the first group of samples using methods described below. All other statistical analyses of samples, including within-sample variation tests for the second group of samples ("2005 group", Table 2), and those evaluating among-sample relationships, were conducted on samples that had hybrids removed and contained fish of one species. It was important to remove genetic hybrids from samples for analyses comparing putative populations by species, otherwise their inclusion could distort relationships.

In further analyses of within-sample or population variation we combined temporal (annual) samples of single species (genetically identified) within distinct localities (e.g., above Landsburg Dam *O. mykiss*; Ballard Locks steelhead) or from putative independent populations (e.g., Green River wild steelhead). Hybrid individuals were removed from temporal samples, and we tested temporal samples (within location) for genetic differentiation, if sample sizes were large enough (30 or more fish per sample), prior to combining them (see below). We calculated *F*-statistics for combined population or locality samples and other putative population samples (e.g., 2005 Big Bear Creek *O. clarki*).

Identifying hybrids and species within samples

We used STRUCTURE 2.1 (Pritchard et al. 2000) with microsatellite genotype data to estimate the proportion of species ancestry shared among samples and to examine hybridization between *O. clarki* and *O. mykiss* in individuals. In this program, sampled fish are tested for membership in a series of user-defined hypothetical populations. For example, to test if *O. clarki* and *O. mykiss* are reproductively isolated, two groups (the two species) were hypothesized among all sample data. The program calculates the percentage of membership in either group for an individual, which provides an estimate of ancestry. Presuming our total samples contained both pure species, a fish that was purely *O. clarki* would belong predominately to the group containing other *O. clarki*, and vice versa if it was purely *O. mykiss*. A hybrid individual would show ancestry in both groups, with percentage of ancestry varying by generation of hybridization event. A first-generation (F1) hybrid would be expected to have 50% ancestry in both groups (species). Unequal levels of ancestry are expected in offspring of F1 hybrids that breed with pure species or with second- and higher-order hybrids. Ancestry was calculated per individual and over samples. Results for sample groups and sampled populations are reported as the means of individual ancestries.

If estimated ancestry in individuals was greater than 98% in either species, we designated these fish as 'pure' species. To confirm this designation some of these individuals were analyzed for the six species-specific nuclear DNA markers (described above). All fish that appeared to be hybrids based on STRUCTURE 2.1 results were included in the DNA markers analysis. We designated fish as genetic hybrids if, based on their microsatellite genotype, they were estimated to have less than 98% ancestry in one species, and had a mixture of species-specific DNA marker alleles. If DNA marker genotypes had missing data and/or there was uncertainty about the species-specificity of some observed alleles, we designated fish as species or hybrids based on the microsatellite results. We believed making choices based on these results alone should be highly accurate based on large allele frequency differences observed between 'pure' *O. clarki* and *O. mykiss* population samples at many of the 22 microsatellite loci. However, it is possible that some hybrid fish could be missed using this approach if there were missing data at key loci.

We used GENETIX (Belkhir et al. 2004) to view hybridization between *O. mykiss* and *O. clarki* based on microsatellite genotypic data. GENETIX performs a factorial correspondence analysis in which composite axes are generated that maximize differences among individuals, and then plots individuals in three dimensions according to their genotype. Hybridization is hypothesized when individuals plot between the ranges of the two species or when individuals presumed to be of one species plot within the range of the other species.

Among-sample relationships

Genetic relationships among samples (hybrids removed) were examined with pair-wise tests, and with ordination and cluster analyses. All possible sample pairs (temporal and spatial) were tested for differences in genotypic distributions across all loci using FSTAT2.9.3 with 450,000 permutations. Samples were also tested for genetic differences with pairwise F_{ST} tests using ARLEQUIN ver3.0b (Schneider et al. 2000) with 10,000 permutations. Temporal samples from the same locality (or putative population) that were not significantly different from each other were combined and pair-wise F_{ST} tests were conducted again. For ordination and cluster analyses genetic distances (chord distances, Cavalli-Sforza and Edwards 1967) among samples were estimated using the GENDIST program in PHYLIP 3.5c (Felsenstein 1993) and distances were plotted in neighbor-joining dendrograms using the NEIGHBOR program in PHYLIP. In analyses examining *O. clarki* and *O. mykiss* relationships we included previously collected genetic data (22 loci) from Minter Creek *O. clarki* (sampled in 2002, WDFW code 02BP, N=91) for comparison with a Puget Sound anadromous cutthroat trout population.

Samples were examined for relationships with hatchery rainbow trout by including genetic data collected previously by WDFW from non-native rainbow trout broodstocks propagated at four WDFW hatcheries. In cluster and ordination analyses we used compatible microsatellite DNA genotype data for 14 loci common to these hatchery samples and our study samples. The rainbow trout hatchery samples were: Spokane Hatchery (sampled in 2000, WDFW code 00DF, N=96), Goldendale Hatchery (sampled in 2001, WDFW code 01JB, N=48), Eells Springs Hatchery (sampled in 2001, WDFW code 01OA, N=89), and South Tacoma Hatchery (sampled in 2002, WDFW code 02BK, N=50). Rainbow trout in these hatcheries are predominately from stocks descended from California-origin *O. mykiss* (Crawford 1979). These stocks have been, and are, widely released throughout the state.

We used GENETIX to view divergence among anadromous and resident *O. mykiss* and *O. clarki*, introgression between wild *O. mykiss* and hatchery rainbow trout stocks, and to identify which groups Lake Washington and Chester Morse Lake *O. mykiss* were closest to genetically. So few *O. mykiss* were sampled in Lake Washington that we could not evaluate them as a sample group. Introgression, the introduction of genes from one genetically distinct group to another, is hypothesized when individuals from one population plot within the range of another population.

Assignment of origin tests

We conducted assignment tests for Cedar River *O. mykiss* smolts using GENECLASS2 (Piry et al. 2004). We examined the likelihood that, based upon a fish's genotype and allele frequencies in samples designated as baseline data, the fish originated in a population represented by the samples. We conducted these tests under the assumption that the Ballard Locks steelhead sample represented anadromous Cedar River *O. mykiss*, and considered the possibility that Green River steelhead may stray and spawn in the Cedar River. In the first test, the baseline included Ballard Locks steelhead, Green River steelhead, Cedar River below-Landsburg Dam *O. mykiss*, Cedar River above-dam *O. mykiss*, and Chester Morse Lake *O. mykiss*. The latter two groups were included to examine if *O. mykiss* in non-anadromous zones had produced smolts. In a second test, the baseline only included Ballard Locks steelhead and Cedar River below Landsburg Dam *O. mykiss*. We reported results from the Bayesian method of Rannala and

Mountain (1997) as implemented in GENECLASS2 (prior distribution for allele frequency at a given locus is equal to 1/k, with k equal to the total number of different allelic states at this locus over all reference populations), with annual samples combined for baseline groups.

Assignment scores generated by GENECLASS2 are relative values in which negative log likelihoods of assignment to each baseline sample are first calculated. The top-ranked baseline population is given an assignment score equal to the log likelihood (10^{-log}) of the best-matching baseline population over the sum of the likelihoods for other populations (Piry et al. 2004). We rated assignments as positive and un-ambiguous if the assignment score ("score 1") was at least 100 times more likely than the second most likely score. We also used the frequency method (Paetkau et al. 1995) and the Bayesian method by Baudouin and LeBrun (2000) available in GENECLASS2, as well as a maximum likelihood method implemented in WHICHRUN (Banks and Eichert 2000) to test if smolt assignments varied according to the method employed.

We also used STRUCTURE 2.1 to explore the ancestry of Cedar River smolts. We included above and below-dam resident *O. mykiss*, Ballard Locks steelhead, Green River steelhead, Chester Morse Lake *O. mykiss* and the smolts in the test's data set. We chose "K" values (number of possible distinct populations) from 2 to 5 and examined the proportion of ancestry that smolts shared with the different hypothetical groups (possible populations) created, using 5 iterations each with 50,000 burn-ins and 950,000 runs.

We conducted assignment tests for Cedar River below-dam resident *O. mykiss* using GENECLASS2 to assess whether these fish were derived from steelhead or from resident *O. mykiss* above Landsburg Dam. The baseline included Ballard Locks steelhead and Cedar River above-dam *O. mykiss*.

RESULTS

Sampling/Samples

We provided results on 2003 Cedar River sampling activities and genetic analyses involving those samples in a progress report for this contract (Marshall et al. 2004). Some of these results are reported below for continuity and ease of comparison with results from 2004 and 2005 Cedar River samples. The 2003 samples and those included in analyses completed in 2004 ("2004 group") are shown in Table 2, except for one sample, Lake Washington non-anadromous adult *O. mykiss* (03BE; N=4), which was too small to use in genetic diversity and population-level analyses. Also, the "03BJ" sample shown in Table 2 does not include 12 fish phenotypically identified as potential hybrids that genetically were either *O. mykiss* or hybrids (in Marshall et al. (2004) data for all fish in the original 03BJ sample were presented).

In 2004 we sampled or acquired samples of *O. mykiss* and *O. clarki* in the Cedar River below and above Landsburg Dam, *O. mykiss* smolts from lower Cedar River smolt trap, Chester Morse Lake *O. mykiss*, and *O. mykiss* and *O. clarki* in Lake Washington (Table 2). Field samplers noted potential *O. mykiss*/*O. clarki* phenotypic hybrids among adult size fish. We acquired samples of Soos Creek Hatchery winter-run steelhead (Chambers Creek stock) and summer-run steelhead (Skamania stock, Columbia Basin-origin) that had been collected by WDFW staff in 2003 (Table 2). Soos Creek Hatchery (Figure 1) is on a tributary to the Green River. We were

provided samples of *O. mykiss* from the upper Green River above Howard Hanson Dam (Figure 1) that had been collected by NMFS staff in 2003 (Table 2). In 2005 we obtained *O. clarki* smolt samples from a lower Cedar River trap, and *O. clarki* adult and juvenile samples from Big Bear Creek and in Lake Washington (Table 2). Samples described in this paragraph were analyzed in 2005 and collectively are called the "2005 group" (Table 2).

In total we sampled 316 putative *O. mykiss* and *O. clarki* (genetic hybrids identified later) in Cedar River. Of these, 239 were non-anadromous adults or juveniles not in smolt condition, and 77 were smolts. Only 17 *O. mykiss* were sampled in Chester Morse Lake. We had 88 total *O. clarki* and *O. mykiss* sampled in Lake Washington, and 57 steelhead from Ballard Locks. Our Green River comparative samples included a total of 121 wild steelhead, 145 hatchery steelhead and 44 non-anadromous *O. mykiss*. Among all tissue samples analyzed for this study, we successfully extracted genomic DNA and amplified at least eight microsatellite loci for 870 individuals (Table 2). The average number of loci scored successfully was 20.6 per individual.

Genetic analyses

Initial evaluation of within-sample genetic variation

As stated previously, we initially did not know when samples contained true hybrids and/or misidentified species. We were reasonably sure some samples, such as hatchery-origin steelhead and adult wild steelhead, contained 'pure' species, although past hybridization was still possible. We analyzed genetic variation within samples of the 2004 group prior to genetic identification of hybrids and verification of species because we wanted to see how results for truly mixed samples might serve as indicators of such samples. Following this initial evaluation (results shown in Table 2), we used genetic results to remove hybrids and misidentified species from all 2004 group samples. Genetically identified hybrids and non-target species were removed from all samples (except 04IU) of the 2005 group prior to analysis of within-sample variation (Table 2).

All 2004 and 2005 group samples deviated from Hardy-Weinberg expectations (HWE) with homozygote excess except for Chester Morse Lake *O. mykiss* (Table 2). Gene diversity and allelic richness were comparable among most putative *O. mykiss* samples (Table 2). The Chester Morse Lake sample had the lowest diversity and allelic richness and also had a small sample size (Table 2). The 2005 *O. clarki* samples had lower gene diversity and allelic richness compared to *O. mykiss* samples (Table 2). The 2003 sample of phenotypic cutthroat trout ("03BJ") that actually contained *O. mykiss*, *O. clarki*, and hybrids had high diversity and allelic richness (Table 2) as might be expected in a mixed species sample. These data for this mixed sample showed that phenotypic identification of adult cutthroat trout in Cedar River was inaccurate and that high allele diversity may be an indicator of the inclusion of *O. mykiss* in a cutthroat trout sample. The Lake Washington gill net sample ("04IU"), which was analyzed as a mixed sample, did not show relatively high levels of allelic diversity (Table 2) due to a small proportion of *O. mykiss* (8.3%) in the total sample.

Samples varied widely in the number of locus pairs in genotypic (linkage) disequilibria from 0 in 2003 below-Landsburg Dam *O. mykiss*, to 13 in Puyallup Hatchery winter steelhead (Table 2). With 190 possible pair-wise locus tests within samples, and an assumption that 5% of tests could yield significant results by chance, 10 or more significant linkage disequilibria tests per sample would be an overall significant result. Linkage disequilibrium can result from physical linkage

between loci (such as occurrence on the same chromosome), non-random sampling, non-random mating, or mixed-origin fish. The loci *Omm-1070* and *Sco-103* showed linkage in all within-sample tests. No other locus pairs showed consistent significant test results among samples.

O. mykiss and O. clarki species and hybrids identification

Based on microsatellite DNA genotype data for all samples, we identified species composition of samples and individuals using STRUCTURE 2.1 with the criterion of only two possible populations (the two species). Green River wild adult steelhead from 2002 and 2003 collectively had 99.4% ancestry with 'Population 1' (Table 3). We thus assumed that "Population 1" represented *O. mykiss* genotypic distributions (the *O. mykiss* group) and that "Population 2" represented the *O. clarki* grouping. The 2003 Cedar River phenotypic *O. clarki* sample had ancestry at similar levels in both hypothetical populations (Table 3), and estimated ancestry for individuals indicated this sample contained 11 *O. clarki*, 10 *O. mykiss* and 11 hybrids (Table 4a, "03BJ" individuals with 'Ocl' phenotypes). Cedar River fish identified in the field as hybrids in 2003 had only 7% ancestry with *O. clarki* ('Population 2') and the rest with *O. mykiss* (Table 3; see Table 4a results for "03BJ" individuals with 'Hyb' phenotype).

In the 2005 sample group, STRUCTURE 2.1 analysis showed six of seven putative *O. mykiss* samples collectively had 97% or greater ancestry in the designated *O. mykiss* group ('Population 1'; Table 3), and most individuals had 98% or greater ancestry within *O. mykiss* (individual data not shown). Putative *O. mykiss* sampled above Landsburg Dam in 2004 had the highest average percentage of ancestry within the *O. clarki* group (15.3%; Table 3), and 12 fish had various levels of mixed ancestry, indicating hybridization ("04AZ" individuals in Table 4b). Cedar River phenotypic *O. clarki* sampled in 2004 had the highest average *O. mykiss* ancestry of four putative *O. clarki* samples (40.8%; Table 3), and seven fish had mixed ancestry ("04BD" individuals in Table 4b), and one was genotypically *O. mykiss*. Most individuals in the three other *O. clarki* samples had 98% or greater membership within the *O. clarki* group (individual data not shown).

We used STRUCTURE 2.1 results to choose individuals for hybrid and species identification with the nuclear DNA markers. From the 2004 sample group we chose all phenotypic hybrids, all phenotypic *O. clarki* sampled in 2003, all phenotypic *O. mykiss* with estimated mixed ancestry, and some phenotypic *O. mykiss* with 'pure' *O. mykiss* ancestry (e.g., adult steelhead). From the 2005 sample group we chose all individuals with estimated mixed ancestry (most likely hybrids), estimated 'pure' *O. clarki* from several samples, eight hatchery steelhead and eight Chester Morse Lake *O. mykiss*. The percentages of ancestry in *O. mykiss* and *O. clarki* estimated with STRUCTURE 2.1 for individuals chosen for the DNA marker assays from the 2004 and 2005 sample groups are shown in Tables 4a and 4b, respectively.

For the RFLP and SSR markers, patterns labeled A or A' were most common in *O. mykiss* and patterns labeled B or B' were most common in *O. clarki* in the studies that developed the markers (Baker et al. 2002; Ostberg and Rodriguez 2002). In the 2004 sample group, species identification results often did not match field-recorded phenotypes. Ten phenotypic *O. clarki* had predominantly *O. mykiss* genotypic patterns (Table 4a). Twelve phenotypic *O. clarki* were clearly hybrids with mixed species RFLP and SSR patterns as well as mixed proportions of microsatellite ancestry from both species (Table 4a). Only ten phenotypic *O. clarki* appeared to

have *O. clarki* genotypic patterns (Table 4a), if we assume that GnRH "AB" is a variant pattern in the species based on genotypes seen in Big Bear Creek *O. clarki* ("05BA" fish in Table 4b).

Among 10 fish identified phenotypically as potential hybrids in the 2004 sample group, nine appeared to be genetically *O. mykiss* and one was a hybrid (Table 4a). Four phenotypic *O. mykiss* from below and above Landsburg Dam in 2003 appeared to be genetic hybrids with different levels of the two species contributions (Table 4a). One phenotypic *O. clarki* smolt (03BH0005) was a hybrid (Table 4a). Nine of 17 adult steelhead had all *O. mykiss* "A" patterns in their RFLP and SSR genotypes, and the other eight had heterozygous genotypes at one locus, showing a "B", "B'" or "C" pattern at either OM-35, p53-7 or OCC-16 (02BI, 03BK and 03BU coded fish, Table 4a). These heterozygous genotypes may indicate that *O. mykiss* in this region has natural allelic variability for *O. clarki*-like RFLP and SSR loci patterns (B or B' fragments), or that these steelhead had *O. clarki* ancestors. The OM-35 "C" pattern or fragment (125 base-pairs in length) was observed in this study and not reported by Ostberg and Rodriguez (2002).

In the 2005 sample group, all individuals with mixed microsatellite ancestry results also had mixed nuclear DNA marker profiles (Table 4b). Although few fish in this group were identified in the field as hybrids, we identified 40 genetic hybrids (Table 4b). Hybrid individuals occurred below and above Landsburg Dam, in Lake Washington gillnet and purse seine catches, among Cedar River phenotypic *O. clarki* smolts (6 of 50), and among Big Bear Creek phenotypic *O. clarki* (Table 4b). One phenotypic *O. mykiss* smolt was a hybrid. Most of the tested individuals with *O. clarki* phenotypes and 99% *O. clarki* microsatellite ancestry had *O. clarki*-type "B" or "B'" patterns, and 11 had "A" patterns at either GnRH, p53-7, OCC-16, or OCC-28. The GnRH locus showed the most variability among these putative pure *O. clarki* with seven "AB" genotypes and one "AA" genotype (Table 4b). Also, no "B" patterns at OM-35 were resolved in these *O. clarki* (Table 4b), which is consistent with Ostberg and Rodriguez's (2002) results for their coastal cutthroat trout sample. Soos Creek Hatchery steelhead (putative pure *O. mykiss*) all had an AB' pattern at the p53-7 locus and A or A' alleles at the other five loci (Table 4b). Chester Morse Lake *O. mykiss* all had a BB' pattern at the p53-7 locus and A or A' alleles at the other five loci, and this same six-locus genotype occurred in two Lake Washington gillnet-caught fish (Table 4b). These results may indicate that *O. mykiss* has natural allelic variability at the p53-7 locus for patterns reported to be *O. clarki*-specific.

Genetic variation within population samples

Subsequent to genetic verification of species and identification of hybrids in samples, we removed hybrids from samples and, if necessary, sorted species into their correct sample group. For example, genetic *O. mykiss* that had been mistaken for *O. clarki* or hybrids in the 2003 below-dam Cedar River sample "03BJ" were grouped with other 2003 below-dam Cedar River *O. mykiss* ("03BD" samples) to form the sample of the targeted group. The following results of genetic analyses were thus obtained from putative single-species population or locality samples.

In pair-wise genotypic and F_{ST} tests comparing temporal samples from same population or locality, we found no significant differences in the following pairs: 2003 and 2004 Cedar River below-dam *O. mykiss*, F_{ST} = -0.0003, P = 0.378; 2003 and 2004 Cedar River above-dam *O. mykiss*, F_{ST} = 0.0069, P = 0.037; and 2002 and 2003 Green River wild steelhead, F_{ST} = 0.0013, P = 0.158 (P values for genotypic tests not shown but were similarly not significant). Based on these results, we combined temporal samples to represent each population or group for some

further analyses. We could not test for temporal variation among Cedar River *O. clarki* sampled from 2003 to 2005 because of small annual sample sizes for adults. We combined all Cedar River *O. clarki*, adults and smolts, for other analyses.

In allelic correlation tests for combined temporal and single population samples there were 52 significant F_{IS} values at individual loci before Bonferroni corrections. After corrections, 10 values remained significant in *O. mykiss* samples and 12 remained significant in *O. clarki* samples (Table 5). Only Chester Morse Lake *O. mykiss* showed equilibrium for within-sample allelic correlations before and after the multiple test correction. Significant F_{IS} values for *Omy-77* in most *O. mykiss* samples (Table 5) suggests a locus-specific, versus a sample-specific, allelic condition for the species. *Omy-77* was shown to have null alleles in Snow Creek steelhead (Olympic peninsula population; Ardren et al. 1999). The significant F_{IS} values for *Omm-1070* and *Omy-1011* in the three *O. clarki* samples also suggest a locus-specific condition. Significant positive F_{IS} values are indicative of excess homozygous genotypes. Total F_{IS} values (all loci) were significant and positive in most samples (Table 5).

Among-sample genetic relationships

A cluster analysis of genetic distances calculated among annual samples and single population or locality samples (Table 6) produced the neighbor-joining dendrogram shown in Figure 3. Genetic distance between Green River wild steelhead annual samples was smaller than distances between either of these and other *O. mykiss* samples (Table 6) and they plotted closely on the dendrogram (Figure 3). Genetic distances among Ballard Locks and Green River wild steelhead annual samples were relatively small (Table 6), and these four samples, along with Green River resident *O. mykiss*, grouped together on the dendrogram (Figure 3). Genetic distance between Cedar River below-dam *O. mykiss* annual samples was smaller than distances between either of these and other *O. mykiss* samples (Table 6). All four Cedar River *O. mykiss* samples plotted along a branch that had Chester Morse Lake *O. mykiss* at its terminus (Figure 3). Soos Creek Hatchery and Puyallup Hatchery winter steelhead clustered together, and formed a distinct branch with Soos Creek Hatchery summer steelhead (100% bootstrap value; Figure 3). Cedar River *O. mykiss* smolts (2003 sample) occurred on a branch separate from anadromous and resident fish groupings (Figure 3). Cedar River *O. clarki* samples were distant and distinct from all *O. mykiss* and clustered with other *O. clarki* samples (Figure 3).

We generated another dendrogram with most of the *O. mykiss* samples, three *O. clarki* samples, and the four hatchery non-native rainbow trout samples. In this case we used combined annual samples of wild *O. mykiss* or *O. clarki* per locality or population, and a combined Puyallup and Soos Creek hatcheries winter steelhead sample. Chester Morse Lake *O. mykiss* grouped with the hatchery rainbow trout, and this group was clearly distinct (100% bootstrap support) from the other Cedar River *O. mykiss* samples (Figure 4). This result suggested that Chester Morse Lake *O. mykiss* had a non-native origin.

Genetic divergence between non-native hatchery rainbow trout strains, and wild *O. mykiss* (steelhead and resident fish) of Cedar and Lake Washington localities was shown in a factorial correspondence analysis plot (Figure 5). Most fish of these two types plotted in different sectors of the three-dimensional space, forming discrete clusters. Chester Morse Lake *O. mykiss* plotted within the non-native hatchery *O. mykiss* cluster, further supporting a non-native origin for this population. We also included in this analysis Cedar River genetic hybrids and Cedar River and

Lake Washington-area *O. clarki*. Genetic divergence between *O. clarki* and *O. mykiss* is clearly evident in this plot (Figure 5) where individuals of each species are widely separated. Hybrid individuals plotted diffusely in the zone between the species clusters.

In a second factorial correspondence analysis including only *O. mykiss*, Green River and Ballard Locks wild steelhead clustered together on the right side of the first axis with hatchery stock winter steelhead clustering nearby but separated along the third axis (Figure 6). Soos Creek Hatchery summer steelhead also plotted on the right side but were separated from wild and hatchery winter steelhead along the second and third axes. Cedar River *O. mykiss* from below and above Landsburg Dam plotted in a similar space although some individuals plotted in areas occupied by Chester Morse Lake fish (left side of plot) or by wild steelhead (Figure 6). Resident below- and above-dam *O. mykiss* that plotted relatively closely with Chester Morse Lake *O. mykiss* suggested that lake-origin fish may have dispersed downstream into the river, a one-way gene flow. Additionally, some Cedar River below-dam individuals that plotted among Chester Morse Lake fish could indicate presence of non-native hatchery rainbow trout, which have been released in Lake Washington. Below- and above-dam resident *O. mykiss* that plotted within the wild steelhead cluster indicate genetic similarities with local steelhead. Proportionally more below-dam fish plotted with steelhead than above-dam fish.

In pair-wise genotypic and F_{ST} tests among combined temporal samples and single samples of populations or localities, most *O. mykiss* samples were significantly different from each other with the exception of Ballard Locks and Green R. wild steelhead (only F_{ST} results shown in Table 7). *O. mykiss* from below and above Landsburg Dam had a very low F_{ST} value, but were significantly different (Table 7). Pair-wise values for *O. mykiss* from below Landsburg Dam and Ballard and Green R. steelhead were similarly low but significant, indicating close genetic relationships among samples. The F_{ST} tests showed that the magnitude of differentiation between *O. mykiss* and *O. clarki* samples was much greater than differentiation within species.

Assignment of origin tests

In the first GENECLASS2 test for anadromous and resident origins of 2003 and 2004 *O. mykiss* smolts we considered Ballard Locks steelhead, Cedar River below-dam and above-dam resident *O. mykiss*, Green River wild steelhead, and Chester Morse Lake *O. mykiss* as possible sources. Based on our criterion of a "score 1" to "score 2" ratio of 100 or greater, results for this test showed that six smolts had the highest likelihood of originating from Green River steelhead, and five smolts had the highest likelihood of originating from Cedar below-dam resident *O. mykiss* (Table 8a). One smolt had a positive but ambiguous (high score 1 value but score 1 to score 2 ratio below the criterion) assignment to Green River steelhead, and five smolts had positive but ambiguous assignment to Ballard Locks steelhead (Table 8a). The remaining five smolts had log-likelihood values that did not differ greatly for the two highest scoring baseline samples, indicating a very low probability of making a correct assignment given the baseline data (Table 8a). Although positive assignment for these five smolts was not possible, their two highest scores (scores 1 and 2) were either for steelhead or for steelhead and Cedar below-dam *O. mykiss*. No smolts showed high likelihoods of originating from Cedar River above-dam *O. mykiss* or Chester Morse Lake *O. mykiss* (Table 8a).

In a second GENECLASS2 test we removed from the baseline dataset Cedar River above-dam *O. mykiss* and Chester Morse Lake *O. mykiss*, because of assignment results above, and Green River steelhead because we wanted to evaluate smolt origins without these as an anadromous source population. Thus, the baseline included only Ballard Locks steelhead and below-dam resident *O. mykiss*. Results for this test showed that eight smolts had positive, un-ambiguous assignments to Cedar below-dam *O. mykiss*, and two smolts assigned to Ballard Locks steelhead (Table 8b). Eight smolts had high score 1 values for either source but had score ratios below the criterion, indicating positive but ambiguous assignment (Table 8b). The remaining four smolts could not be assigned positively to either source (Table 8b). The number of smolts estimated to originate from Cedar River below-dam *O. mykiss* increased when Green River steelhead were not included as a potential source population, based on the two GENECLASS2 tests.

In an exploration of origins of Cedar River below-dam resident *O. mykiss* relative to Ballard Locks steelhead and the above-dam resident population, we found that below-dam *O. mykiss* were fairly equally distributed in GENECLASS2 assignments between the two potential sources. Of 106 below-dam *O. mykiss*, 44 had positive, un-ambiguous assignments to Ballard Locks steelhead, and 41 had positive, un-ambiguous assignments to above-dam *O. mykiss*. For remaining below-dam *O. mykiss*, 11 and 10 had positive but ambiguous assignments to Ballard Locks steelhead and above-dam *O. mykiss*, respectively. These results indicated that below-dam resident *O. mykiss* shared ancestry with anadromous fish. This was reiterated in some of the STRUCTURE 2.1 analysis results (below), where Cedar River below-dam *O. mykiss* ancestries were divided between hypothetical groups occupied by steelhead and by above-dam *O. mykiss* (Table 9a, top portion).

Smolt origins were explored by STRUCTURE 2.1 analysis, which included an assessment of the most likely number of distinct population groups among all fish in the baseline data set. The natural log (Ln) of the probability of the number of hypothetical groups suggested that there were three or four genetically discrete groups in the data set (Table 9a, top portion). As an analysis proceeds, Ln values increase as the likely number of populations is approached, with the true number of populations having the highest Ln value (number closest to zero; Pritchard et al. 2000). If an analysis finds multiple solutions, or the Ln value continues to increase slowly with increased hypothetical number of populations (K), beyond the true number of populations, samples become divided among groups (Pritchard et al. 2000; Falush et al. 2003).

With K = 3, Cedar River below-dam *O. mykiss* were mostly subdivided between a group occupied largely by steelhead (group 2) and a group primarily occupied by Cedar River above-dam *O. mykiss* (group 1; Table 9a, top portion). Below- and above-dam *O. mykiss* had some ancestry in group 3, which was dominated (99%) by Chester Morse Lake *O. mykiss* (Table 9a). Subdivided ancestry of below-dam *O. mykiss* may represent anadromous and resident origins, and may indicate emigrants from Chester Morse Lake or non-native rainbow trout releases. With K = 4, the Ln value was marginally higher than for K = 3, and steelhead and smolt samples had greatest ancestry in group 2 (steelhead dominated) and group 4, which contained nearly 30% ancestry for Cedar River below-dam *O. mykiss* (Table 9a, top portion). Groups 1 and 3 were dominated by Chester Morse Lake and above-dam *O. mykiss*, respectively, and the below-dam *O. mykiss* had ancestry subdivided among the four groups (Table 9a). With K = 5, the Ln value decreased (Table 9a). Three or four hypothetical genetic groups seemed similarly plausible based on physical partitioning in the basin, and on the possibility that three or four biologically and genetically distinct groups may occur and also be interacting.

With K = 3, smolts had 16% ancestry in group 1, which was occupied primarily by above-dam resident *O. mykiss*, and had 79% ancestry in group 2, which included primarily steelhead and also Cedar River below-dam *O. mykiss* (Table 9a, top portion). In this K = 3 scenario, three smolts had relatively high percentages of ancestry in groups 1 and 2, and the rest had high percentage ancestry in group 2 (Table 9a, lower portion). With K = 4, most smolt ancestry was subdivided and average smolt ancestry was 51% and 40% in groups 2 and 4, respectively (Table 9a). Results for individuals with K=4 showed that nine smolts had 72% or greater ancestry in group 2 ('steelhead-like' group), two had 97% or greater ancestry in group 4, and seven had relatively high percent ancestry in groups 2 and 4 (Table 9a).

We modified the data set analyzed with STRUCTURE 2.1 to include smolts, Ballard Locks steelhead and Cedar River below-dam *O. mykiss*. This modification paralleled that for the second GENECLASS2 test (above). Our intent was to test the STRUCTURE program's performance for smolt assignment using the steelhead and resident fish groups that we assumed had the greatest potential to actually produce Cedar River smolts. In this case, Ballard Locks steelhead had 91% ancestry in hypothetical group 2, and Cedar River below-dam *O. mykiss* had ancestry in both groups, with 57% attributed to the steelhead group 2 and 43% to group 1 (Table 9b). Smolts had 81% ancestry in group 2, and four individual smolts had ancestry of 65% and higher in group 1, while the majority of individuals had 95% or higher ancestry in group 2 (Table 9b). These results suggested that most smolts and many Cedar River below-dam resident adults had anadromous *O. mykiss* ancestry.

Biological characteristics

Steelhead sampled at Ballard Locks were about twice as large as adult resident *O. mykiss* sampled below Landsburg Dam in 2003 and 2004, while above-Dam *O. mykiss* had the smallest average size (Table 10a). The above-dam sample from 2004 that we acquired from other researchers did not include many *O. mykiss* of adult age classes or sizes. Ballard Locks steelhead and below-dam *O. mykiss* adults had a relatively similar range of total age. Among Ballard Locks steelhead, 21% spent only one year in freshwater prior to outmigrating as smolts, the majority smolted after 2 years in freshwater, and 66% spent two growing seasons ("1+") in saltwater prior to returning (Table 10b).

Among 2003 below-dam resident adults, 28 had readable scales, and these patterns provided information about spawning history as well as age. Five of the 28 had been spawners, three had spawned once, one had spawned twice, and one (an eight year old, 58.4 cm. fish) had spawned three times. Among 2004 below-dam *O. mykiss*, 30 had readable scales and 14 had been spawners and 9 of these had spawned more than once. Among 2003 above-dam *O. mykiss*, 27 had readable scales, and three of these had been spawners. The average age of 2003 above-dam fish was only 2 years old (Table 10a), while the few spawners were four or five years old.

The average age of genetic hybrids from 2003 was 3.6, four of these had spawned once and one had spawned twice. Their average length was 38.2 cm. Among the 10 genotypic *O. mykiss* that had been visually identified as cutthroat trout in 2003 only four had total ages (average = 5), but six had scales that showed them as previous spawners, and three had spawned twice. Average length of these "cryptic" *O. mykiss* was 40.7 cm. Average length of below-dam 2003 genetic

cutthroat trout was 23.2 cm and of aged fish, three were age 2, and one was age 5 that had spawned once.

DISCUSSION

Sampling

We had mixed success in achieving sampling goals. We did achieve an adequate sample of Cedar River resident adult *O. mykiss* below Landsburg Dam. Not all fish could be aged, but based on sizes of aged fish, we think we had at least 80 age 3 or older fish, and age 3 was the youngest age for fish that had been spawners (by scale pattern analysis not field observations). We had difficulty getting a large sample size for adults above Landsburg Dam. Currently, we do not know how much this was due to population size (fewer upstream *O. mykiss*?) or inadequacy of sampling effort. We did not meet the sampling goal for Chester Morse Lake *O. mykiss* due to logistic difficulties with lake access. However, since the fish we did sample in 2004 appeared to be non-native and genetically distinctive, we do not think the small sample size compromised the analysis of resident and anadromous fish relationships.

Sampling of steelhead at Ballard Locks in 2003 was unsuccessful due to low abundance of returning fish, and no sampling was done there in 2004. We were fortunate to be able to obtain and utilize scales from wild steelhead sampled at the Locks in three earlier years. These steelhead were intercepted prior to spawning and we recognize the possibility that all of them may not have been born in Cedar River or may not have become Cedar River spawners. At the time of sampling they were presumed to represent the Cedar River population. These steelhead were used in a short-term program testing artificial production methods for wild broodstock, and aimed at restoring production to Sammamish Basin tributaries.

Genetic data analysis showed high similarity between Ballard Locks and wild Green River steelhead, which, besides being expected based on historical geography, suggested the potential that straying or wandering Green River fish had been trapped at the Locks. The Green River population has much higher abundance and entrance to the Green River Basin is only about eight miles from the Locks, which might increase the likelihood of strays. However, there has not been a correlation in abundance between returns to Green River and Ballard Locks (S. Foley, unpublished data). For purposes of this study, we think the Ballard Locks steelhead sample acceptably represents genetic characteristics expected in Cedar River spawners. Based on earlier genetic data (Phelps et al. 1997), we expected to find little differentiation between Green and Cedar rivers' steelhead, and if no Cedar River spawners could be sampled we had planned to use Green River steelhead genetic data as our anadromous population data set.

We were able to acquire only 24 *O. mykiss* smolts from the lower Cedar River trap. The small sample size precluded use of the sample as a representation of an anadromous population (or sub-population), but did not limit usefulness of individuals for hybrid evaluation or assignment tests. Due to few smolt samples and relatively small numbers Ballard Locks steelhead with age and brood year data, it was not possible to achieve Objective 5, the estimation of effective population size (Ne) for Cedar River steelhead. To estimate Ne from genetic data we needed single brood year samples of an adequate size.

Our small 'pure' *O. clarki* sample size for 2003 was remedied by sampling in other localities besides Cedar River in 2004 and 2005. We are confident that our total sample of 'true' cutthroat trout provided an adequate characterization of genetic diversity among microsatellite and nuclear DNA marker loci in local coastal cutthroat trout for the purposes of this study. These data ensured that the identification of hybrids was accurate. We think the most likely reason adult cutthroat trout were not encountered often during in-river sampling (angling) was due to timing. Adult cutthroat trout that spawn in the river probably do so somewhat earlier than *O. mykiss* (Hawkins 1997), and then leave the river through spring months (Seiler at el. 2004). Nowak et al. (2004) found that other Lake Washington tributary cutthroat trout outmigrated after spawning from February through April to reside in the lake, and left streams as immature fish at age 2. Our sampling may have been too late in spring to encounter many adults. Cutthroat trout smolts sampled in trapping activities were, and have been, relatively abundant (e.g. Seiler at el. 2004).

O. mykiss and O. clarki hybridization

This study was not designed to assess the nature and extent of *O. mykiss* and *O. clarki* hybridization in Cedar River, but we needed to accurately identify hybrids. Our sampling targets were the species themselves, as best as they could be identified phenotypically. We expected hybrids to occur and if a captured fish appeared to be a possible hybrid it was tissue-sampled and noted as such. Genetic hybrids occurred below and above Landsburg Dam, among fish phenotypically identified as either species, as juveniles, and as adults that had spawned. Genetic hybrids seemed to be field-identified as *O. clarki* more often than *O. mykiss*. This conforms with results of Baumstieger et al. (2005) in their study of hybridization in northern coastal California. They found that when hybrids were misidentified by knowledgeable field samplers they were always identified as *O. clarki* instead of *O. mykiss*.

It was very important for us to correctly identify hybrids so that these fish could be excluded from samples of resident *O. mykiss*. Evaluation of relationships between anadromous and resident *O. mykiss* would be inaccurate if hybrid genotypes were included. We also found that genetic species identification was important in order to include resident *O. mykiss* that were misidentified as cutthroat trout in the field. Our total group of *O. clarki* samples provided ample data on genetic divergence between the two species in this region. We found that allelic differentiation was high at multiple microsatellite DNA loci, which gave us an additional and complementary means of species and hybrid identification, relative to the nuclear DNA species markers. We also found that a few of the nuclear DNA marker fragment patterns or alleles were not exclusive to either species in our samples, which diminished their utility for hybrid identification. The *O. clarki*-dominant allele at the *p53-7* locus occurred in samples from two divergent *O. mykiss* populations. Baumsteiger et al. (2005) also found that a *p53* allele was not completely species-specific. The microsatellite DNA analysis for hybrids was particularly informative in cases where nuclear DNA marker loci or data were inadequate.

As mentioned earlier, hybrids between *O. mykiss* and *O. clarki* have been regularly observed in western Washington streams. However, the phenomena of hybridization has not caused the loss of genetically discrete populations of these species within streams. Reproductive isolation is enforced by environmental, behavioral and other intrinsic factors such that sympatric natural populations are relatively independent. About 14.5% of fish sampled in Cedar River during this study were hybrids, and in comparisons with other studies (Baumsteiger et al. 2005; Hawkins 1997) this level did not seem excessive. Our study data do not allow us to draw any conclusions

about whether interbreeding between steelhead or resident *O. mykiss* and *O. clarki* has reduced the success of Cedar River steelhead. Spawning grounds observations and genetic data on redd-specific parents and offspring would address this issue.

Genetic characterization of Cedar River O. mykiss

In this study we accomplished the first genetic characterization of adult non-anadromous *O. mykiss* in this watershed. Genetic analyses showed clearly that most Cedar River *O. mykiss* residing in former and present anadromous zones represented a native gene pool, and were not a result of exotic hatchery trout introductions. Chester Morse Lake *O. mykiss*, which were most similar to hatchery rainbow trout stocks, are upstream of migration barriers but possibly contribute fish to downstream areas. Cedar River above-Landsburg Dam *O. mykiss* were most similar genetically to below-dam resident *O. mykiss*. We expected the above-dam population to diverge from the downstream population through genetic drift, due to long-term blockage of upstream migrants. However, downstream passage over the dam likely has been possible, providing a one-way gene flow that may have slowed genetic divergence between fish above and below the dam. The new fish ladder provides passage and we can expect genetic differences between fish in the two areas to diminish. Based on *O. mykis*s (ages 2 to 4) PIT-tagged in 2005 and released below Landsburg Dam, we now have evidence that these fish have passed upstream and downstream of the dam (George Pess, NMFS, personal communication).

Below- and above-Landsburg Dam *O. mykiss* samples showed significant departures from Hardy-Weinberg genotypic equilibrium, which can be a consequence of samples containing mixtures of fish from genetically distinct populations. Results of factorial correspondence and STRUCTURE 2.1 analyses for the two samples indicated that they likely contained some individuals from isolated upstream areas. Small numbers of annual spawners can produce brood year differences in gene frequencies, and mixed brood year samples, such as ours, may show genotypic disequilibrium. We also found significant disequilibrium in samples presumed to contain fish from single populations such as wild Green River steelhead, the hatchery steelhead, and Big Bear Creek cutthroat trout. Relatively large, randomly mating populations are expected to be in genotypic equilibrium at neutral gene loci. Sampling error, or small numbers of related individuals in broodstock may contribute to significant Hardy-Weinberg disequilibrium.

Genetic relationships among O. mykiss samples and populations

Wild steelhead sampled at Ballard Locks were very similar genetically to wild Green River steelhead in all statistical analyses. Assuming Cedar River steelhead comprised the Locks sample, a close relationship between Cedar and Green rivers' populations is a likely outcome based on the shared ancestry we expect from historical physical connection of the rivers. Any straying between the two rivers since the re-routing of Cedar River would minimize genetic divergence. Although we have not found any tagging data that verify straying, few steelhead are coded-wire tagged in this region. As mentioned above, our results are consistent with previous genetic data from *O. mykiss* parr of both rivers. In answer to Objective 3, the wild steelhead samples were genetically well-differentiated from all hatchery steelhead samples, which is consistent with management strategies for the hatchery populations. To highlight this differentiation, the pair-wise F_{ST} value between Ballard Locks wild steelhead and hatchery winter-run steelhead was more than three times larger than pair-wise F_{ST} between Ballard Locks steelhead and Cedar River resident *O. mykiss* below Landsburg Dam.

The lack of, or very low, differentiation between upper Green River resident *O. mykiss* and Green River steelhead may result from 17 years (1982-1998) of wild Green River steelhead fry releases and 9 years (1992-2000) of wild Green River adult releases into mainstem and tributary locations above Howard Hanson Dam. Even though anadromous adults can not migrate past this dam, any *O. mykiss* that became landlocked by the dam and persisted have not been sequestered from interaction with an anadromous population like fish above Landsburg Dam were. Assuming the fish sampled in 2003 upstream of Howard Hanson Dam were expressing a resident life-history in terms of maturing and reproducing in freshwater, genetic data indicated they share a close relationship with contemporary wild steelhead. Upper Green River resident *O. mykiss* were more similar to Cedar River resident *O. mykiss* below Landsburg Dam than to those above the dam. This finding may serve as an indicator of gene flow between anadromous and resident fish below Landsburg Dam, the area steelhead have always had access to.

Several statistics (e.g. genetic distance, F_{ST}) showed that Cedar River resident *O. mykiss* below Landsburg Dam were relatively similar genetically to Ballard Locks and Green River steelhead. Relatively less similarity occurred between Cedar River *O. mykiss* above Landsburg Dam and Ballard Locks and Green River steelhead, which we expect based on the long-term blocked upstream passage. Steelhead ancestry in below-dam resident adults was indicated in results from assignment tests.

It was also evident that the relationship between anadromous and below-dam resident fish is complicated by the likelihood of individuals from different sources occurring in the lower river. Data analyses indicated that fish from Chester Morse Lake and the above-Landsburg Dam section of Cedar River have dispersed downstream. They appeared as individuals intermingled in below-dam samples, and possibly have interbred with steelhead or resident fish produced by steelhead. The GENECLASS2 assignment test and STRUCTURE analyses gave estimates of a large percentage contribution or ancestry from above-dam *O. mykiss* among fish sampled below the dam. We think it is accurate to describe resident adults present in Cedar River below Landsburg Dam as including *O. mykiss* from anadromous and non-anadromous native population sources, a lake-origin non-native source and possibly ad-mixed (inter-bred) individuals.

The current low abundance of steelhead might be expected to change proportions of spawning interactions among resident and anadromous fish. One outcome could be that fewer steelhead would allow resident males more opportunity to spawn with steelhead females. A pedigree-based study in Snow Creek (Olympic peninsula, Washington) showed that in some years of low steelhead return mature (precocious) non-anadromous males were collectively more successful at producing anadromous offspring than anadromous males (Seamons et al. 2004). In another Snow Creek study, Ardren and Kapuscinski (2003) found that the ratio of effective population size to the actual number of steelhead spawners was significantly higher in years with low steelhead spawner density. Seamons et al. (2004) stated that an explanation for this observed pattern may be a proportional increase in reproductive success of resident males when there are few anadromous males. Therefore, resident males may increase the probability of persistence for a small steelhead population.

Another possible outcome of fewer steelhead could be fewer matings between steelhead and fish with resident life-histories, and relatively more matings among resident individuals. Depending on the extent of heritability of resident life-history traits and survival and reproductive success of

resultant offspring, a resident population could arise with a different evolutionary trajectory than one that regularly interacted with steelhead adults. Cedar River *O. mykiss* are rearing to maturity in freshwater, reaching relatively large sizes, and reproducing at ages 3 and 4. Anecdotally, residency and freshwater maturation is not a common *O. mykiss* life-history in Puget Sound-area rivers, instead, an anadromous life-history is predominant. We speculate that the modified fish communities and habitats of Lake Washington areas as described by Nowak et al. (2004) and of Cedar River may have contributed to the success of a resident life-history.

Nowak et al. (2004) suggested that high growth rates of *O. clarki* produced in Lake Washington area streams and reared in the lake may be responsible for their shift from anadromy to an adfluvial life history. The high growth rate and increased abundance of *O. clarki* was attributed to a much larger prey fish supply since the 1970's (Nowak et al. 2004). We have not measured growth rates for Cedar River resident *O. mykiss* in this study but we did have two age 2+ fish greater than 30 cm. fork-length, which is about 70% larger than average size of smolts, which are typically age 2. We do not know if *O. mykiss* utilize Lake Washington habitats like cutthroat trout do for feeding and rearing to maturity because we had few *O. mykiss* in our lake samples. Although we do not have historical time-series data on abundance, an increase in abundance of Cedar River resident *O. mykiss* below Landsburg Dam is perceived to have occurred more recently than that for Lake Washington *O. clarki* (Nowak et al. 2004), and coincidental to decline in steelhead abundance.

Smolt origins

Estimated ancestry or origins of smolts varied depending on mode of analysis and projected source populations. In all cases smolts appeared to have ancestry or originate from both anadromous and non-anadromous (particularly below-dam *O. mykiss*) groups. In two GENECLASS2 tests, 23% or 36% of smolts had much higher likelihoods for originating from below-dam resident *O. mykiss* than from steelhead. Other smolts either had higher likelihoods for originating from steelhead or had assignment scores that did not clearly distinguish between steelhead sources or a steelhead and resident group source. In the most simplistic STRUCTURE 2.1 analysis 14% of individual smolts had high percent ancestry (>78%) in the hypothetical group that included the largest proportion of resident fish ancestry, while 77% of individual smolts had high percent ancestry (>90%) in the hypothetical group that included fish with anadromous ancestry (Table 9b). In this and other STRUCTURE 2.1 tests some smolts had similar percent ancestry in a resident and steelhead group, which may indicate interbreeding between fish of distinct populations. The low level of genetic differentiation between the Ballard Locks steelhead and below-dam *O. mykiss* samples (F_{ST} = 0.009) likely reduces assignment accuracy in both analytical approaches.

Hybrid smolts also occurred and showed hybridization between *O. mykiss* and *O. clarki* at levels expected in first-generation hybrids and in higher-order hybrids. Most hybrid smolts were found in *O. clarki* smolt samples, and had been phenotypically identified as cutthroat trout. About 13% of sampled *O. clarki* smolts were hybrids, a factor that should be taken into account if cutthroat trout production is estimated from trapping data. We do not know whether hybrid smolts resulted from anadromous or resident parents, and we have no information on marine migration behavior of hybrid smolts. We found adult hybrids in Lake Washington, and scale pattern analysis did not indicate a marine phase. Cutthroat trout populations in Lake Washington

tributaries annually produce smolts, but apparently more of these trout rear to maturity in the lake (adfluvial life-history) than in marine waters.

Steelhead conservation and recovery

This study provides data needed to develop effective management strategies for conservation and recovery of native steelhead, which was the primary goal for the project. Conservation of native resident *O. mykiss* is an important aspect of reducing extinction risk for steelhead. The persistence, and thus status, of steelhead may be improved by occurrence of resident phenotypes. Most resident *O. mykiss* in lower Cedar River were closely related to steelhead, and may be recently derived from them. Genetic data support that resident and anadromous *O. mykiss* are reproductively interacting. Our data indicate that some smolt production from resident fish likely occurs. Production of smolts by resident phenotypes and landlocked resident populations has been demonstrated through breeding experiments (Ruzycki et al. 2003; Thrower et al. 2004), and deduced in pedigree-based studies (Blouin 2003; Seamons et al. 2004). However, for the production of steelhead, smolts must be successful at marine migrations.

There is evidence from local cutthroat trout studies that ecological and environmental changes may have selectively favored an adfluvial versus anadromous life-history in this species (Nowak et al. 2004). Similarly, recent environmental conditions may have favored survival of *O. mykiss* progeny that exhibited resident life-history traits instead of marine migration behavior. If this hypothesis is correct, conditions would have to reverse such that future production included more marine-migrating smolts than freshwater maturing fish, and these smolts had higher survival to adult return. This directional environmental change may not be forthcoming and steelhead abundance could become unacceptably low in terms of healthy genetic and phenotypic diversity among Cedar River *O. mykiss*, and in terms of social interests in rebuilding a steelhead fishery. If in response to low steelhead abundance, managers choose to experiment with transplanting steelhead smolts or adults, Green River wild steelhead are genetically and historically the most closely related population and could be a suitable donor stock. Another possible strategy for increasing smolt production would be breeding native resident adults together and rearing offspring under traditional steelhead smolt culture conditions (accelerate growth to produce yearling smolts) for release into Cedar River.

The above-Landsburg Dam resident *O. mykiss* population that existed prior to 2003 might be expected to produce fewer smolt offspring than below-dam fish, as shown by Thrower et al. (2004) for a lake population derived from steelhead that had been sequestered for 70 years. Assuming reduced smolt production capacity, and the genetic divergence due to long-term isolation, upstream-origin fish may not substantially improve status of the steelhead life-history in the short-term. Except for Chester Morse Lake fish, pre-2003 upstream-origin fish do represent a genetic legacy from steelhead and interbreeding with downstream-origin fish that occurs due to improved passage at the Landsburg Dam fish ladder should at the least increase their genetic diversity. If some above-dam habitat areas and conditions are particularly suitable for steelhead spawning and rearing, these might be interesting areas for any experimental releases considered by managers.

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Figure 1. Map showing primary features of Cedar River watershed, Lake Washington Basin, including migrational route for anadromous fish through Ballard Locks, and nearby watersheds. Localities for study samples are shown. Map taken from City of Seattle, Cedar River Habitat Conservation Plan web site ('Lansburg' should be Landsburg).

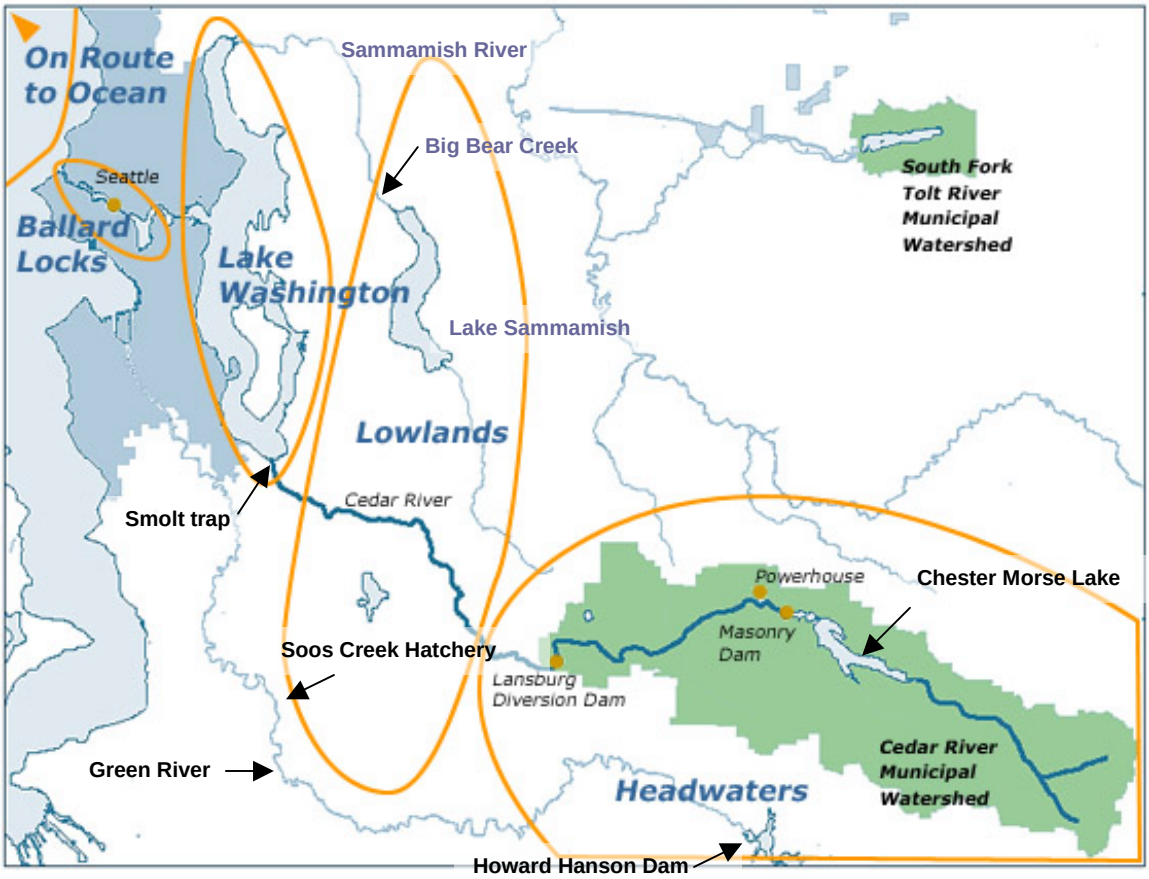

Figure 2. Cedar River wild steelhead annual escapement estimates based on data from WDFW.

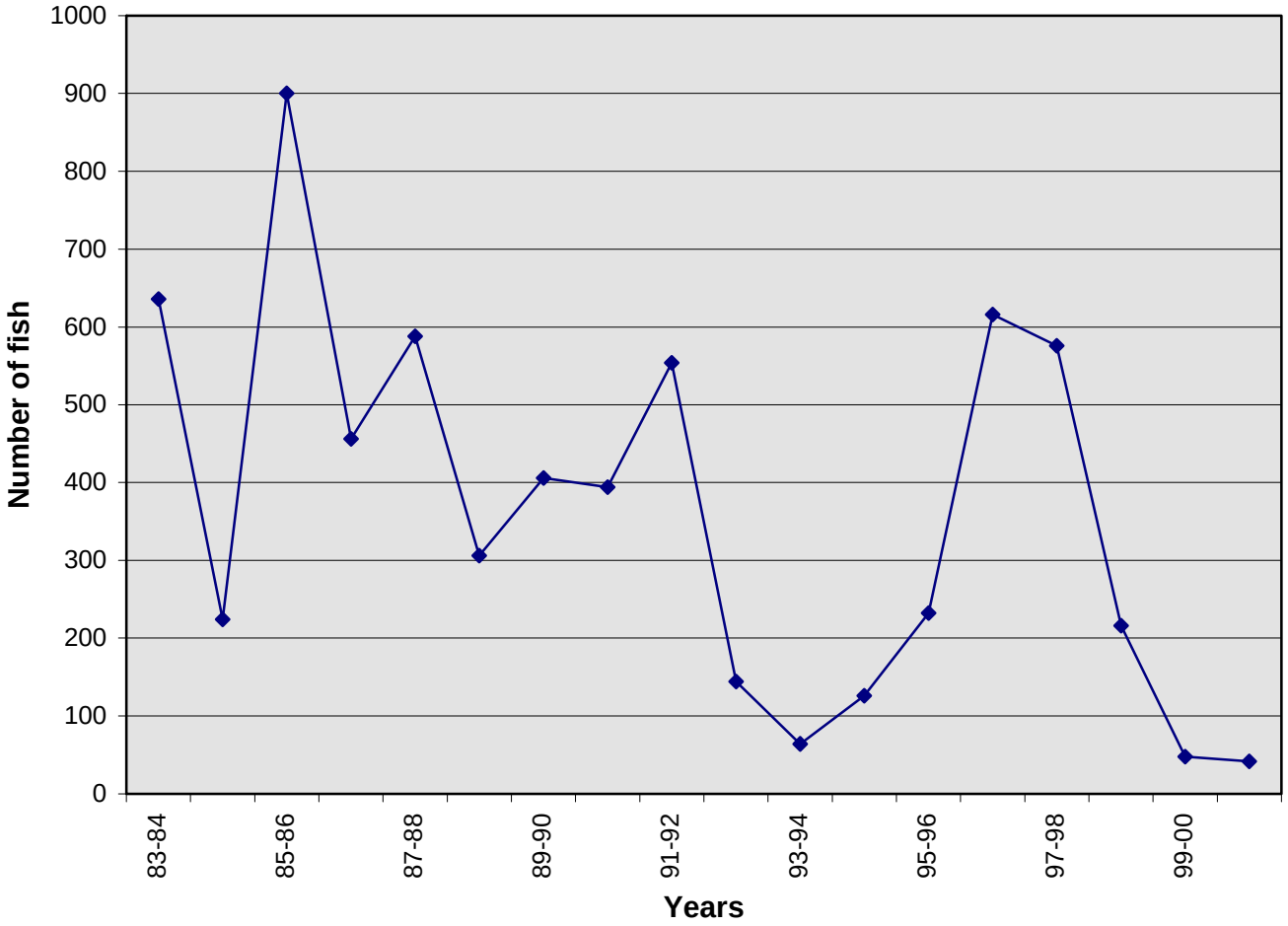

Figure 3. Neighbor-joining dendrogram of genetic distances (scale shown at bottom-left) among samples of resident and anadromous *O. mykiss* and *O. clarki*, with bootstrap node values >95% shown. Numbers in sample names indicate year fish were sampled. Abbreviations: Omy = *O. mykiss*; R. = river; Hat. = hatchery; Ocl = *O. clarki*; Cr. = creek.

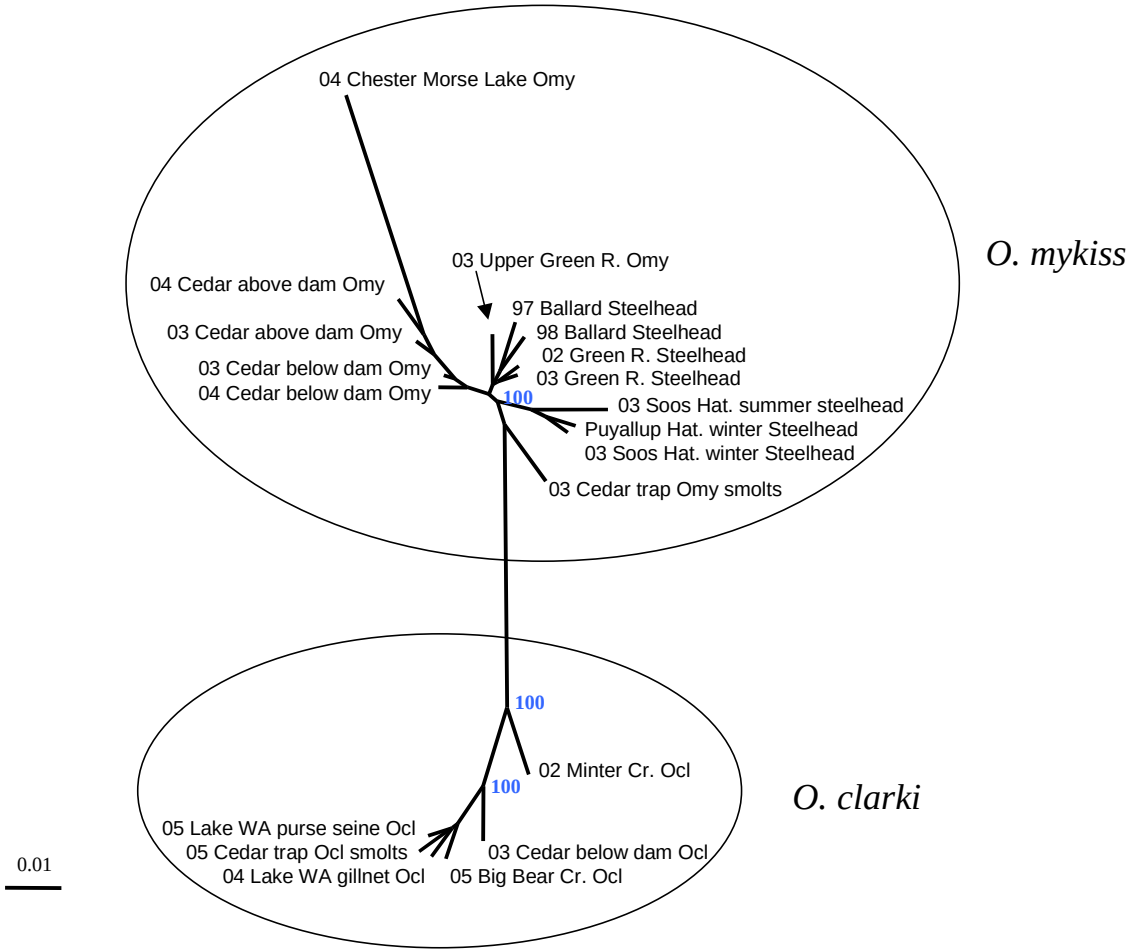

Figure 4. Neighbor-joining dendrogram of genetic distances among resident and anadromous *O. mykiss*, *O. clarki*, and hatchery rainbow trout samples, with bootstrap node values >95% shown. Temporal population or locality samples were combined and Soos Cr. and Puyallup hatcheries winter steelhead were combined (= Hatchery winter steelhead). Numbers in sample names indicate year fish were sampled. Abbreviations: Omy = *O. mykiss*; R. = river; Hat. = hatchery; Ocl = *O. clarki*; Cr. = creek; RBT =rainbow trout.

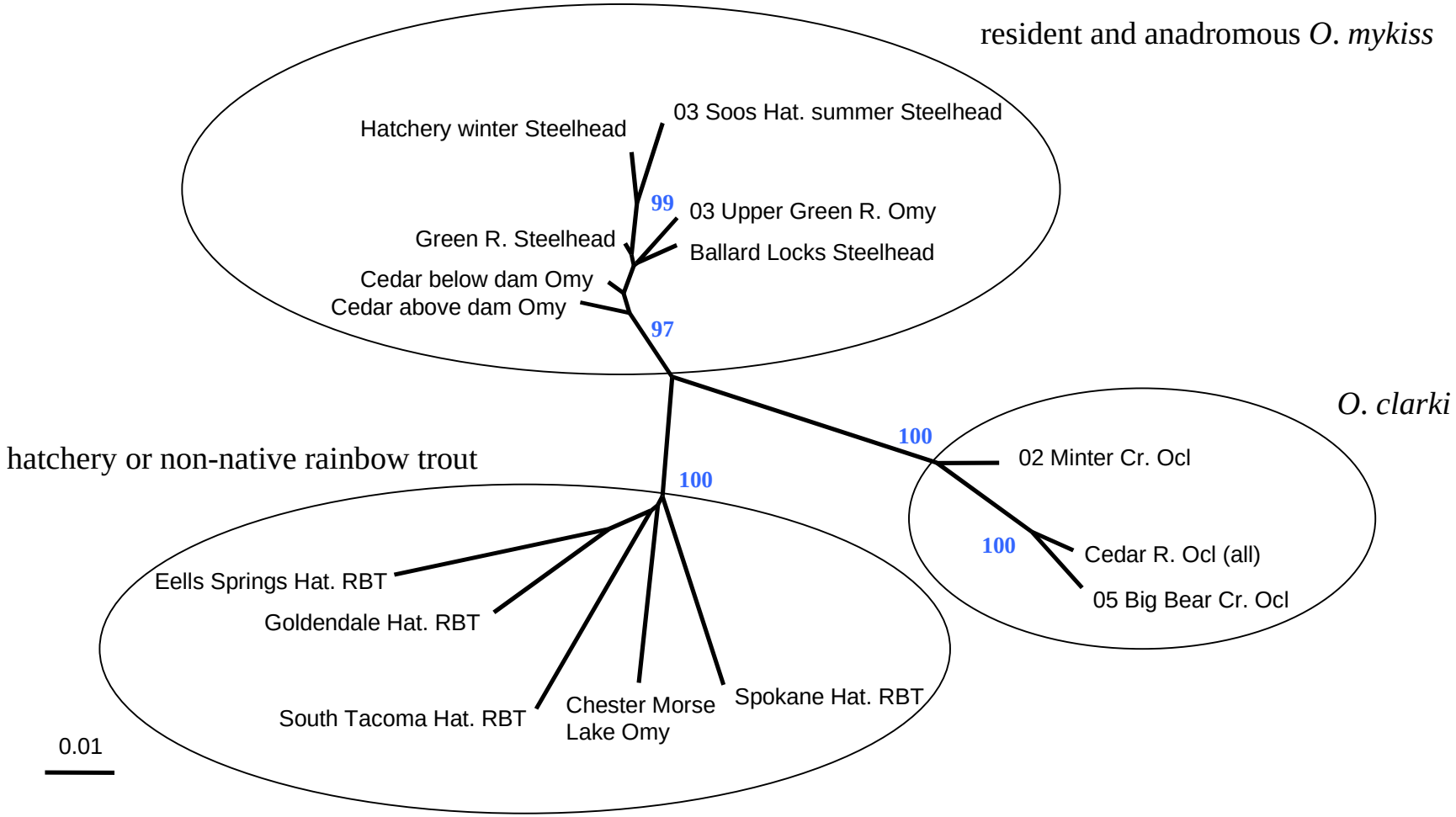

Figure 5. Factorial correspondence plot of individuals from GENETIX. All 2003-05 wild resident and anadromous phenotypic *O. mykiss* and *O. clarki* in Cedar River and Lake Washington area samples, and four hatchery rainbow trout (non-native *O. mykiss*) samples included. Circles were drawn around most individuals within the three groups (wild *O. mykiss*, wild *O. clarki* and hatchery rainbow trout). Genetically identified *O. mykiss* and *O. clarki* hybrids are circled. Individual Chester Morse Lake *O. mykiss* are indicated by red boxes within the circled hatchery rainbow trout cluster.

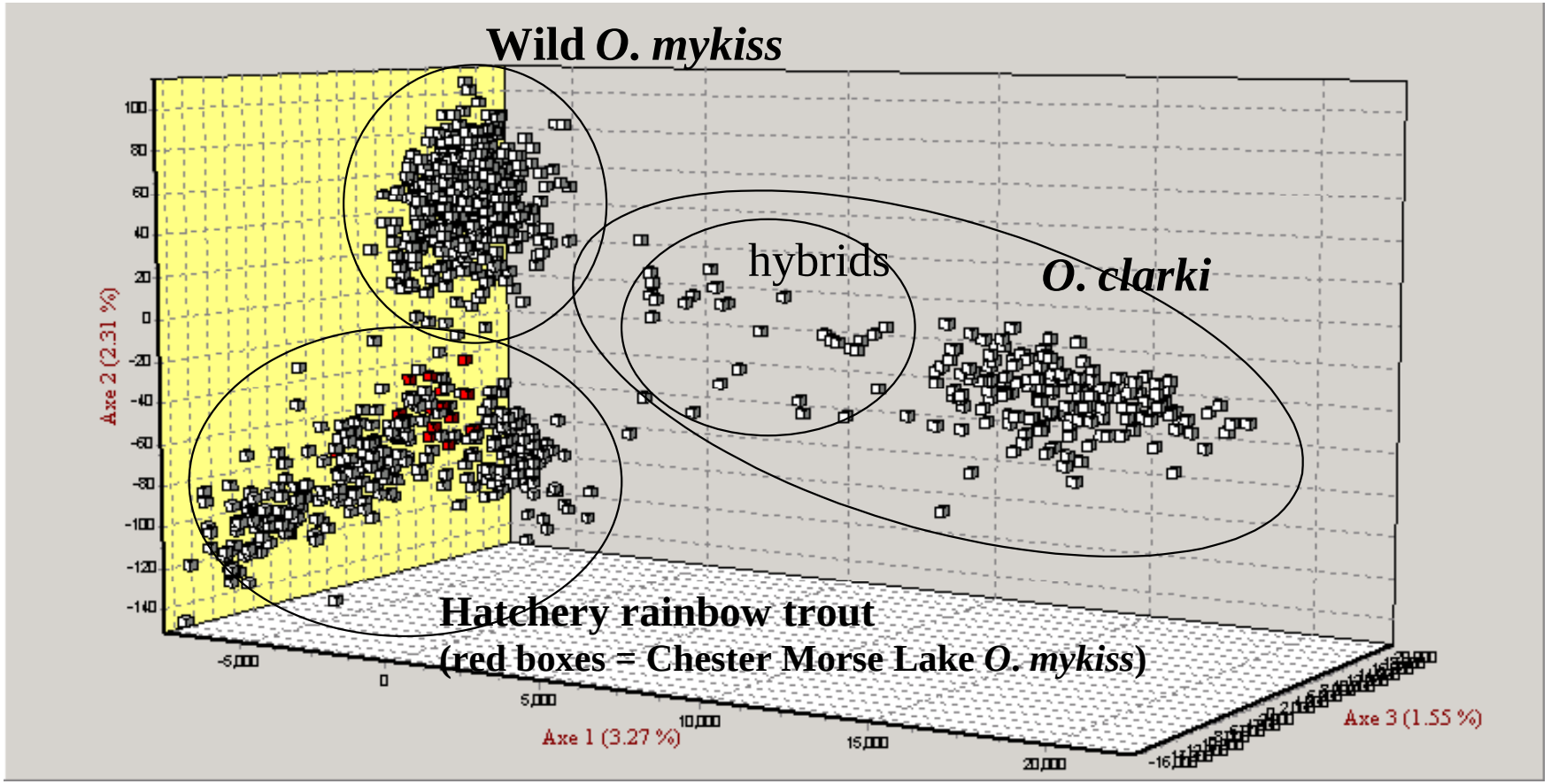

Figure 6. Factorial correspondence plot of resident and anadromous individual *O. mykiss* from all years (genetic hybrids removed) using GENETIX with samples shown by color. Temporal population or locality samples were combined and Soos Cr. and Puyallup hatcheries winter steelhead were combined (= Hatchery winter steelhead). Circles are drawn around most individuals from a population or locality sample. Abbreviations: R. = river; Omy = O. mykiss; Cr. = creek; Hat. = hatchery.

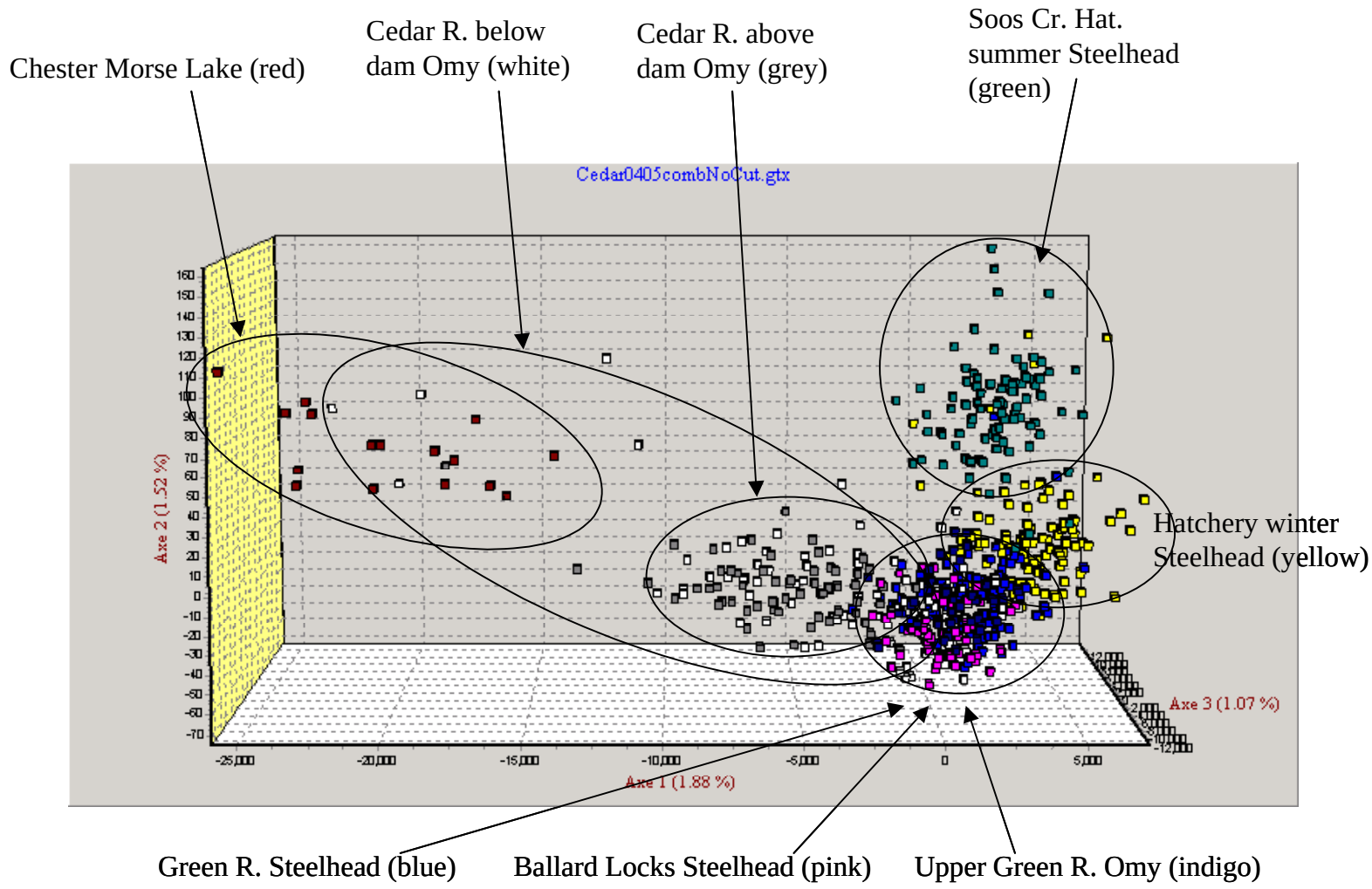

Table 1. Information for multiplexes and 22 microsatellite DNA loci used for study. "Anneal T" is annealing temperature in ^{o}C. Number of PCR cycles for *O. mykiss* is "cycles" with number for *O. clarki* following in parentheses. Dye concentration in μM for *O. mykiss* is under "conc Omy" and for *O. clarki* under "conc Ocl". Number of alleles (#alleles) in study samples is followed by size range and repeat unit size in basepairs. Observed heterozygosity (Hobs) and probability (*P*) value for deviation from Hardy-Weinberg equilibrium (HWE) per locus over all samples is shown. Values out of equilibrium are underlined. Loci were tested for excesses of homozygotes using GENEPOP3.3 (Raymond and Rousset, 1995) with 100 batches and 2000 iterations. "Source" is reference for primer sequences for each locus.

Multiplex	Anneal T	cycles	Locus	conc Omy	conc Ocl	Dye	#alleles	range	repeat	Hobs	HWE *P*	Source
Omy-B2	55	26 (32)	One-102	0.05	0.08	6fam	23	182-269	4	0.883	0.0025	Olsen *et al.* 2000
			One-114	0.05	0.1	vic	25	177-322	4	0.896	0.0028	Olsen *et al.* 2000
			Ots-100	0.04	0.09	ned	24	168-224	2	0.860	0.0676	Nelson and Beacham 1999
Omy-C2	55	28 (32)	One-108	0.02	0.04	6fam	13	148-337	4	0.897	0.1218	Olsen *et al.* 2000
			Ots-103	0.015	0.025	vic	29	56-119	4	0.218	0.3989	Small *et al.* 1998
			One-101	0.02	0.04	ned	7	119-230	4	0.521	0.2748	Olsen *et al.* 2000
Omy-D2	49	25 (29)	Ots-1	0.03	0.07	6fam	27	158-284	2	0.813	< 0.0001	Banks *et al.* 1999
			Omy-77	0.03	0.03	vic	25	97-147	2	0.801	< 0.0001	Morris *et al.* 1996
			Ots-3M	0.02	0.02	ned	22	128-203	2	0.677	0.1814	Banks *et al.* 1999
Omy-E2	62	26 (32)	Omm-1130	0.05	0.06	6fam	33	190-391	4	0.913	0.0932	Rexroad *et al.* 2001
			Omm-1070	0.025	0.04	vic	29	164-291	4	0.843	< 0.0001	Rexroad *et al.* 2001
			Omy-1011	0.045	0.06	ned	27	134-249	4	0.918	< 0.0001	Spies *et al.* 2005
Omy-F2	52	25 (35)	Omy-1001	0.03	0.04	6fam	25	160-242	2	0.878	0.0176	Spies *et al.* 2005
			Omm-1128	0.025	NA	vic	32	211-381	4	0.942	0.1131	Rexroad *et al.* 2001
			One-18	0.02	0.025	ned	9	170-186	2	0.766	0.2267	Scribner *et al.* 1996
			Oki-10	0.02	0.04	vic	16	97-160	2	0.628	0.1050	Smith *et al.* 1998
			Ogo-3	0.06	0.06	ned	11	182-213	2	0.589	0.1136	Olsen *et al.* 1998
Ocl-E2	50	35 (32)	Sco-110	0.12	0.08	6fam	26	150-263	2	0.827	< 0.0001	WDFW unpublished
			One-2	0.06	0.025	ned	45	193-293	2	0.909	0.1714	Scribner *et al.* 1996
Ocl-F2	50	35 (32)	Omm-1138	0.05	0.03	6fam	7	142-159	2	0.669	0.5701	Rexroad *et al.* 2001
			Sco-103	0.1	0.035	vic	26	199-303	4	0.911	0.1013	WDFW unpublished
			Omy-325	0.08	0.04	ned	39	98-180	2	0.891	0.3839	O'Connell *et al.* 1997

Table 2. Samples and descriptive genetic statistics, grouped by year of genetic analysis. Samples pooled for this analysis are shown by two or more identification codes (Code). The number of individuals in original samples (N), the number of individuals that amplified at eight or more loci (worked >8 loci), and the number included in statistical analysis (Stats N) are shown. For samples in 2005 group, except sample coded 04IU, genetically detected hybrids and incorrect species were removed (see sample column) prior to calculating the statistics in this table. Gene diversity (Gene Div) is expected heterozygosity corrected for sample size. Allelic richness (Richness) is average number of alleles/locus corrected for sample size. The number of pairs of loci in genotypic disequilibria is under "Dis" (22 loci - 190 possible pair-wise comparisons). Hardy-Weinberg equilibrium probability values (HWE *P*) indicate whether samples deviated significantly from HWE expectations with an excess of homozygotes; significant values are underlined. Abbreviations: Ocl = *O. clarki*; Omy = *O. mykiss*; Hat. = hatchery; NA = not analyzed.

Sample (Year, location, identity) - 2004 group	Code	N	worked >8 loci	Stats N	Gene Div	Richness	Dis	HWE *P*
01 Puyallup Hatchery winter steelhead	01GB	71	68	68	0.796	8.2	13	0
03 Cedar River below-dam Ocl (mixed[1])	03BJ	32	31	31	0.861	9.94	8	0
03 Cedar River above-dam Omy	03BF	50	50	50	0.786	7.88	2	0.0002
03 Cedar River below-dam Omy	03BD	53	48	48	0.803	8.71	0	0
03 Cedar River trap smolts - Omy & Ocl	03BH	20	18	18	0.791	7.9	1	0.0037
02 Green River wild steelhead (for Keta Hat.)	02BI	40	38	38	0.78	8.17	4	0.0247
03 Green River wild steelhead (for Keta Hat.)	03BK	43	42	42	0.791	8.52	1	0.0007
02, 03 Green River wild steelhead	02BJ+03BU	38	37	37	0.784	8.22	1	0
97-03 Ballard Locks steelhead	97ZY+98ZZ+99ZX+03AV	57	56	56	0.762	7.74	1	0
2005 group								
04 Cedar River below-dam Omy (1 hybrid removed)	04AY	49	49	48	0.800	8.63	5	0
04 Cedar River above-dam Omy (12 hybrids removed)	04AZ	27	27	15	0.783	7.64	2	0.0206
04 Chester Morse Lake Omy	04BA	17	17	17	0.689	5.48	1	0.051
04 Cedar River trap Omy smolts	04BB	7	7	NA	NA	NA	NA	NA
04 Lake WA purse-seine resident Omy	04BC	3	1	NA	NA	NA	NA	NA
03 Upper Green River resident Omy	03NA	44	43	43	0.757	7.35	10	0.0017
03 Soos Creek Hatchery winter steelhead	03LZ	45	45	45	0.821	8.79	5	0
03 Soos Creek Hatchery summer steelhead	03MA	100	90	90	0.803	8.29	8	0
04 Cedar River Ocl (7 hybrids & 2 Omy removed)	04BD	15	15	NA	NA	NA	NA	NA
04 N. Lake WA tribal gill-net Ocl, Omy, hybrids	04IU	36	36	36	0.709	6.74	2	0
05 Lake WA purse seine Ocl (4 hybrids removed)	05JF	47	47	43	0.692	6.56	1	0
05 Cedar River trap Ocl smolts (6 hybrids removed)	05BB	50	50	44	0.678	6.14	2	0
05 Big Bear Creek Ocl (4 hybrids removed)	05BA	49	47	43	0.695	6.55	5	0

[1] This sample included genetic *O. mykiss*, *O. clarki* and hybrids based on later analyses.

Table 3. Species composition of samples or sample groupings based on microsatellite DNA loci data and STRUCTURE 2.1 analysis with two populations hypothesized among all samples. The percentage of ancestry in two hypothetical populations (two "species") is indicated for fish from the 2004 sample group combined by location and field phenotype for this analysis, and for individual samples of the 2005 group (see Table 2). The percentage is averaged over all fish in each sample. Population 1 (Pop 1) and Population 2 (Pop 2) represent *O. mykiss* and *O. clarki* genotypic distributions, respectively. Numbers in sample names indicate year fish were sampled. Abbreviations: N = number of fish analyzed, Ocl = *O. clarki*; R. = River; Omy = *O. mykiss*; Cr. = Creek.

2004 sample group – combined samples or types	Pop 1	Pop 2	N
02 & 03 Green R. wild steelhead	0.994	0.006	118
03 Cedar R. phenotypic Ocl	0.483	0.517	32
03 Cedar R. phenotypic Omy	0.949	0.051	98
03 Cedar R. phenotypic hybrids	0.932	0.068	9

2005 sample group	Pop 1	Pop 2	N
03 Soos Cr. Hatchery winter steelhead	0.998	0.002	44
03 Soos Cr. Hatchery summer steelhead	0.998	0.002	90
03 Upper Green R. resident Omy	0.997	0.003	43
04 Cedar R. below-dam Omy	0.984	0.016	49
04 Cedar R. above-dam Omy	0.847	0.153	27
04 Chester Morse Lake Omy	0.998	0.002	17
04 Cedar R. trap Omy smolts	0.974	0.026	8
04 Cedar R. phenotypic Ocl	0.408	0.592	15
04 Lake WA gillnet, mixed species	0.147	0.853	36
05 Big Bear Creek Ocl	0.029	0.971	49
05 Cedar R. trap Ocl smolts	0.067	0.933	50
05 Lake WA purse seine Ocl	0.042	0.958	47

Table 4a. Species identification results for selected individuals (shown by sample code (Table 2) and number) from 2004 sample group. Phenotypes from field records are Hyb = hybrid, Ocl = *O. clarki*, and Omy = *O. mykiss*. STRUCTURE 2.1 estimates of percentage *O. mykiss* (*Omy*) and *O. clarki* (*Ocl*) ancestry based on microsatellite DNA genotypes shown as "msat ancestry", and values greater than 98% for *Omy* are green-highlighted and for *Ocl* are blue-highlighted. Nuclear DNA marker genotypes are shown under "RFLP" for ITS, GnRH, and p53-7 (markers identified by Baker et al. 2002), and "SSR" for OCC-16, OCC-28 and OM-35 (identified by Ostberg and Rodriguez 2002); A or A' and B or B' patterns were most common in *O. mykiss* and *O. clarki*, respectively, in the two publications. The OM-35 "C" pattern is a DNA fragment (125 base-pairs) observed in this study. Individuals with 'pure' species genotypes and ancestry that did not match with phenotype are shown with *. Individuals classified as genetic hybrids, based on msat ancestry less than 98% in either species and mixed nDNA species marker genotypes, are shown with @.

		msat ancestry		RFLP			SSR		
Sample	**Phenotype**	***Omy***	***Ocl***	**ITS**	**GnRH**	**p53-7**	**OCC-16**	**OCC-28**	**OM-35**
03BH0022*	Hyb	0.998	0.002	AA	AA	AA	AA	AA	AA
03BJ0001@	Hyb	0.490	0.510	AB	AB	AB'	BB	AB	AA
03BJ0013*	Hyb	0.998	0.002	AA	AA	AA	AA	AA	AA
03BJ0021	Hyb	0.997	0.003	AB	AA	AA	AA	AA	AA
03BJ0023*	Hyb	0.999	0.001	AA	AA	AA	AA	AA	AA
03BJ0029*	Hyb	0.998	0.002	AA	AA	AB'	AA	AA	AA
03BJ0031*	Hyb	0.998	0.002	AA	AA	AB'	AA	AA	AA
03BJ0060*	Hyb	0.997	0.003	AA	AA	AA	AA	AA	AA
03BJ0061*	Hyb	0.997	0.003	AA	AA	AA	AA	AA	AA
03BJ0073@	Hyb	0.918	0.082	AA	AB	AB'	AA	AA	AA
03BH0005@	Ocl	0.505	0.495	AB	AB	AB	AB	AB	AA
03BH0013	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
03BJ0002@	Ocl	0.510	0.490	AB	AB	BB'	AB		AA
03BJ0003@	Ocl	0.547	0.453	AB	AB	AB'	AB	AB	AA
03BJ0004@	Ocl	0.432	0.568	AB	AB	BB'	AB	AB	
03BJ0005	Ocl	0.002	0.998	BB	AB	BB	BB	BB	
03BJ0006@	Ocl	0.505	0.495	AB	AB	BB'	AB	AB	
03BJ0007*	Ocl	0.998	0.002	AA	AA	AB'	AA	AA	AA
03BJ0008*	Ocl	0.999	0.001	AA	AA	AB'	AA	AA	AA
03BJ0009*	Ocl	0.998	0.002	AA	AA	AB'	AA	?A	AA
03BJ0010	Ocl	0.003	0.997	BB	BB	BB	BB	BB	
03BJ0011@	Ocl	0.473	0.527	AB	AB	BB'	AB	AB	AB
03BJ0022*	Ocl	0.997	0.003	AA	AA	AA	AA	AA	AA
03BJ0024	Ocl	0.002	0.998	BB		BB	BB	BB	
03BJ0025*	Ocl	0.996	0.004	AA	AA	AA	AA	AA	AA
03BJ0026@	Ocl	0.312	0.688	BB	BB	BB	AB	BB	

Table 4a. Species identification, 2004 group – continued.

Sample	Phenotype	*Omy*	*Ocl*	ITS	GnRH	p53-7	OCC-16	OCC-28	OM-35
03BJ0027*	Ocl	0.999	0.001	AA	AA	AA	AA		AA
03BJ0028@	Ocl	0.531	0.469				AB		
03BJ0032	Ocl	0.002	0.998	BB	AB	BB'	BB	BB	
03BJ0034	Ocl	0.002	0.998	BB	BB	B'B'	BB	BB	
03BJ0035	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
03BJ0064*	Ocl	0.999	0.001	AA	AA	AA	AA	AA	AA
03BJ0065	Ocl	0.001	0.999	BB	AB	BB	BB	BB	AA
03BJ0066@	Ocl	0.497	0.503	BB	AA'	AB	AB	AB	AA
03BJ0067	Ocl	0.001	0.999	BB	AA	BB'	BB	BB	
03BJ0068@	Ocl	0.533	0.467	AB	BB	AB'	AB	AB	AA
03BJ0069	Ocl	0.001	0.999	BB	BB	BB'	BB	BB	
03BJ0070*	Ocl	0.998	0.002	AA	AB	AA	AA	AA	AA
03BJ0071*	Ocl	0.998	0.002	AA		AA	AA	AA	AA
03BJ0072@	Ocl	0.586	0.414	AB	AB	BB'	AB	AB	AC
03BJ0074*	Ocl	0.998	0.002	AA	AA'	AA		AA	AA
03BJ0075	Ocl	0.002	0.998	BB	BB	B'B'	BB		
03BJ0076@	Ocl	0.529	0.471	AB	AB	AB'	AB	AB	AA
03BJ0077	Ocl	0.001	0.999	BB		BB'	BB	BB	
02BI0001	Omy	0.999	0.001	AA	AA	AA	AA	AA	AA
02BI0002	Omy	0.999	0.001	AA	AA	AA	AA	AA	AA
02BI0003	Omy	0.999	0.001	AA	AA'	AA	AA		AC
02BI0004	Omy	0.997	0.003	AA	AA	AB'	AA	AA	AA
02BI0007	Omy	0.999	0.001	AA	AA	AA	AA		AB
02BI0008	Omy	0.999	0.001	AA	AA		AA	AA	AA
02BI0009	Omy	0.999	0.001	AA	AA	AB'	AA		AA
02BI0010	Omy	0.998	0.002	AA	AA'	AA	AA	AA	AA
03BD0010@	Omy	0.749	0.251	AB	A'A'	AA	AB	AB	AA
03BD0038@	Omy	0.634	0.366	AB	AB	AA	AB	AB	AA
03BF0013	Omy	0.986	0.014	AB	AA	AA	AA		AC
03BF0034@	Omy	0.595	0.405	AB	AB	AB'	AB	AB	AA
03BK0002	Omy	0.998	0.002	AA	AA	AA		AA	AA
03BK0003	Omy	0.997	0.003	AA	AA	AA	AB	AA	AA
03BK0005	Omy	0.998	0.002	AA	AA	AA	AB	AA	AA
03BK0007	Omy	0.997	0.003	AA	AA	AA	AA	AA	AA
03BK0008	Omy	0.996	0.004	AA	AA	AA	AB	AA	AA
03BK0012	Omy	0.983	0.017	AA	AA	AA	AA	AA	AA
03BK0013	Omy	0.991	0.009	AA	AA	AA	AA	AA	AA
03BK0014	Omy	0.999	0.001	AA	AA	AA	AA	AA	AC
03BU0001	Omy	0.997	0.003	AA	AA	AA	AA	AA	AA

Table 4b. Species identification results for selected individuals (shown by sample code (Table 2) and number) from 2005 sample group. Phenotypes from field records are Omy = *O. mykiss* and Ocl = *O. clarki*. STRUCTURE 2.1 estimates of percentage *O. mykiss* (*Omy*) and *O. clarki* (*Ocl*) ancestry based on microsatellite DNA genotypes shown as "msat ancestry", and values greater than 98% for *Omy* are green-highlighted and for *Ocl* are blue-highlighted. Nuclear DNA marker genotypes are shown under "RFLP" for ITS, GnRH, and p53-7 (markers identified by Baker et al. 2002), and "SSR" for OCC-16, OCC-28 and OM-35 (identified by Ostberg and Rodriguez 2002); A or A' and B or B' patterns were most common in *O. mykiss* and *O. clarki*, respectively, in the two publications. The OM-35 "C" pattern is a DNA fragment (125 base-pairs) observed in this study. Individuals with 'pure' species genotypes and ancestry that did not match with phenotype are shown with *. Individuals classified as genetic hybrids, based on msat ancestry less than 98% in either species and mixed nDNA species marker genotypes, are shown with @.

		msat ancestry		**RFLP**			**SSR**		
Sample	**Phenotype**	***Omy***	***Ocl***	**ITS**	**GnRH**	**p53-7**	**OCC-16**	**OCC-28**	**OM-35**
04AY0061@	Omy	0.452	0.548	BB	AB	BB'	AB		AA
04AZ0005@	Omy	0.848	0.152	AB	AA	AA	AA		AA
04AZ0007@	Omy	0.726	0.274	AB	AB	BB	AA		AA
04AZ0008@	Omy	0.915	0.085	AB	AA	AA	AA	AB	AA
04AZ0009@	Omy	0.317	0.683	BB	AB	BB	BB	AB	AA
04AZ0010@	Omy	0.494	0.506	BB	BB	AB	BB	AB	AA
04AZ0014@	Omy	0.763	0.237	AB	AA	AB'	AA	AB	AA
04AZ0018@	Omy	0.678	0.322		AB				AA
04AZ0019@	Omy	0.685	0.315	AB	AB	BB'	AB	AB	AA
04AZ0021@	Omy	0.682	0.318	AB	AB	AB	AA	AB	AA
04AZ0022@	Omy	0.519	0.481	AB	AB	AB	AB	AB	AA
04AZ0024@	Omy	0.651	0.349	AB	AA	AB	AA	AA	AA
04AZ0025@	Omy	0.612	0.388	AB	AB	AB'	AA	AB	AA
04BB0018@	Omy	0.819	0.181	AB	AB	AB'	AA	AA	AA
04BD0002@	Ocl	0.524	0.476	AB	AB	AB'	AB	AB	AA
04BD0052@	Ocl	0.524	0.476	AB	AA	BB	BB	AB	AA
04BD0053@	Ocl	0.315	0.685	BB	AB	BB	BB	BB	BB
04BD0058@	Ocl	0.851	0.149		AA		BB		
04BD0059@	Ocl	0.746	0.254	AA	AB	AB'	AB	AB	AA
04BD0060@	Ocl	0.574	0.426	AB	AB	AB'	AB	AB	AA
04BD0061@	Ocl	0.573	0.427	AB		BB'	AB	AB	AA
04IU0006@	Ocl	0.54	0.46	AB	AB	BB'	AB	AB	AA
04IU0009@	Ocl	0.558	0.442	AB	AB	B'B'	AB	AB	AA
04IU0013@	Ocl	0.439	0.561	AB	AB	AB	AB	AB	AA
04IU0029@	Omy	0.518	0.482	AB	AB	B'B'	BB	AB	AA
05BA0008@	Ocl	0.188	0.812	BB	AB	BB	AB	AB	

Table 4b. Species identification, 2005 group – continued.

Sample	Phenotype	*Omy*	*Ocl*	ITS	GnRH	p53-7	OCC-16	OCC-28	OM-35
05BA0019@	Ocl	0.09	0.91	AB	BB	BB	BB		BB
05BA0028@	Ocl	0.535	0.465	AB	AB	BB'	AB	AB	AA
05BA0034@	Ocl	0.449	0.551	AB	AB	AB	AB	AB	AA
05BB0012@	Ocl	0.067	0.933	AB	BB	BB'	BB	BB	
05BB0019@	Ocl	0.393	0.607	AB	AB	BB			AA
05BB0022@	Ocl	0.547	0.453	AB	AB	AB	AB	AB	AA
05BB0030@	Ocl	0.337	0.663	BB	AB	BB'	AB	BB	BB
05BB0031@	Ocl	0.241	0.759	BB	AB	BB	BB	BB	
05BB0037@	Ocl	0.645	0.355	AB	AB	BB'	AB	AB	AA
05JF0005@	Omy	0.823	0.177	AB		BB	AA	AA	AA
05JF0013@	Ocl	0.554	0.446	AB	AB	AB'	AB	AB	AA
05JF0027@	Ocl	0.074	0.926	BB	BB	BB	BB	AB	
05JF0042@	Ocl	0.321	0.679	BB	AB	BB	BB	BB	
04BD0051	Ocl	0.009	0.991		BB		BB		
04BD0054	Ocl	0.001	0.999	BB	AA	BB	BB	BB	
04BD0055	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
04BD0056	Ocl	0.003	0.997	BB	BB	BB	BB		
04BD0057	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
04BD0063	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
04IU0020	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
04IU0021	Ocl	0.001	0.999	BB	AB	BB	BB	BB	
04IU0022	Ocl	0.001	0.999	BB	BB	AB	BB	BB	
04IU0023@	Omy?	0.042	0.958	AB	AB	BB	BB	BB	
04IU0024	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
04IU0025	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
04IU0026	Ocl	0.001	0.999	BB	BB	BB'	BB	BB	
04IU0027	Ocl	0.005	0.995	BB	BB	B'B'	BB	BB	
04IU0028	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
04IU0033	Ocl	0.004	0.996	BB	BB	BB	AB	BB	
04IU0035	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BA0003	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BA0004	Ocl	0.001	0.999	BB	AB	BB	BB	BB	
05BA0005	Ocl	0.012	0.988	BB	BB	B'B'	BB	BB	
05BA0006	Ocl	0.002	0.998	BB	BB	BB'	BB	AB	
05BA0007	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BA0009	Ocl	0.001	0.999	BB	BB	BB'	BB	BB	
05BA0010	Ocl	0.001	0.999	BB	BB	BB	BB	BB	

Table 4b. Species identification, 2005 group – continued.

Sample	Phenotype	*Omy*	*Ocl*	ITS	GnRH	p53-7	OCC-16	OCC-28	OM-35
05BA0015	Ocl	0.001	0.999	BB	BB	BB'	BB	BB	
05BA0016	Ocl	0.001	0.999	BB	AB	BB	BB		
05BA0017	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BA0018	Ocl	0.001	0.999	BB	AB	BB	BB		
05BA0020	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BA0021	Ocl	0.001	0.999	BB	AB	BB	BB	BB	
05BA0023	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BA0024	Ocl	0.005	0.995	BB	AB	BB	BB	BB	
05BB0010	Ocl	0.001	0.999	BB	BB	BB'	BB		BB
05BB0011	Ocl	0.001	0.999	BB	BB	BB	BB		
05BB0013	Ocl	0.001	0.999	BB	BB	BB'	BB	BB	
05BB0014	Ocl	0.005	0.995	BB	BB	BB	BB	BB	
05BB0015	Ocl	0.001	0.999	BB	BB	BB'	BB	BB	
05BB0016	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05BB0017	Ocl	0.002	0.998	BB	BB	BB'	BB		
05BB0018	Ocl	0.001	0.999	BB		BB'	BB		
05JF0026	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
05JF0028	Ocl	0.002	0.998	BB	BB	B'B'	BB	BB	
05JF0030	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
05JF0031	Ocl	0.001	0.999	BB	BB	BB	BB	BB	
05JF0032	Ocl	0.002	0.998	BB	BB	BB'	BB	BB	
05JF0033	Ocl	0.001	0.999	BB	AB	BB	BB	BB	
05JF0034	Ocl	0.002	0.998	BB	BB	BB	BB	BB	
05JF0035	Ocl	0.007	0.993	BB	BB	B'B'	BB	BB	
03LZ0008	Omy	0.999	0.001	AA	AA	AB'	AA	AA	AC
03LZ0009	Omy	0.999	0.001	AA	AA'	AB'	AA	AA	AA
03LZ0010	Omy	0.999	0.001	AA	AA'	AB'	AA	AA	AA
03LZ0011	Omy	0.996	0.004	AA	AA	AB'	AA	AA	AC
03LZ0012	Omy	0.998	0.002	AA	AA'	AB'	AA	AA	AA
03LZ0013	Omy	0.999	0.001	AA	AA	AB'	AA	AA	AA
03LZ0014	Omy	0.998	0.002	AA	AA	AB'	AA	AA	AA
03LZ0015	Omy	0.999	0.001		AA	AB'	AA	AA	AA
04BA0003	Omy	0.999	0.001	AA	AA	BB'	AA	AA	AA
04BA0004	Omy	0.999	0.001	AA	AA	BB'	AA	A'A	AA
04BA0005	Omy	0.999	0.001	AA	AA	BB'	AA	AA	AA
04BA0006	Omy	0.992	0.008	AA	AA	BB'	AA		AA
04BA0007	Omy	0.999	0.001	AA	AA	BB'	AA		AA

Table 4b. Species identification, 2005 group – continued.

Sample	Phenotype	Omy	Ocl	ITS	GnRH	p53-7	OCC-16	OCC-28	OM-35
04BA0008	Omy	0.999	0.001	AA	AA	BB'	AA	A'A	AA
04BA0009	Omy	0.998	0.002	AA	AA	BB'	AA	AA	AA
04BA0010	Omy	0.998	0.002	AA	AA	BB'	AA	AA	AA
04IU0014	Omy	0.999	0.001	AA	AA	BB'	AA	AA	AA
04IU0019*	Ocl	0.997	0.003	AA	AA	BB'	AA	AA	AA

Table 5. Estimates of allelic correlations within individuals within samples, "F_{IS}", for population and locality samples (hybrids removed). Temporal population or locality samples were combined and Soos Cr. and Puyallup hatcheries winter steelhead were combined. Sample F_{IS} values at each locus are underlined if significant before Bonferroni correction, and are in bold type if significant after correction. Last line shows probability (*P*) value for F_{IS} value calculated over all loci ("All") in each sample. Abbreviations: Omy = *O. mykiss*; R. = River; Hat. = hatchery; Ocl = *O. clarki*; Cr. = Creek. N = number of fish included.

Locus	Hatchery winter Steelhead	Chester Morse Lake Omy	Green R. wild Steelhead	Cedar R. below-dam Omy	Cedar R. above-dam Omy	Ballard Locks Steelhead	Soos Hat. summer Steelhead	Upper Green R. Omy	All Cedar R. Ocl	Minter Cr. Ocl	Big Bear Cr. Ocl
One-102	0.091	0.228	0.035	0.048	0.061	0.093	0.062	0.117	0.080	**0.201**	0.098
One-114	-0.031	0.193	0.042	0.046	-0.009	-0.033	-0.038	0.031	0.004	0.139	0.200
Ots-100	0.072	-0.062	0.054	0.019	0.026	0.026	0.034	-0.015	0.028	-0.036	0.091
One-101	0.058	-0.397	0.126	0.059	-0.039	0.284	-0.007	0.206	0.035	0.117	-0.066
One-108	0.014	-0.020	0.046	0.062	0.031	-0.023	-0.012	0.145	-0.062	0.071	0.090
Ots-103	0.026	-0.333	-0.024	0.128	-0.115	0.354	-0.107	-0.012	-0.026	0.006	-0.006
Omy-77	**0.229**	0.129	**0.210**	**0.227**	**0.291**	**0.272**	**0.209**	0.194	0.102	-0.008	-0.025
Ots-1	-0.008	0.141	**0.219**	**0.269**	**0.319**	0.140	0.172	0.062	-0.024	**0.267**	0.149
Ots-3M	0.071	0.000	0.052	0.114	-0.014	-0.030	-0.010	-0.314	-0.045	**0.175**	0.030
Omm-1070	-0.010	-0.136	0.005	0.021	0.068	0.144	-0.040	0.044	**0.467**	**0.288**	**0.457**
Omm-1130	-0.003	-0.074	0.039	-0.003	0.005	-0.024	-0.018	0.065	0.072	0.103	0.327
Omy-1011	-0.024	0.118	0.031	0.100	-0.009	0.040	0.020	0.049	**0.431**	**0.543**	**0.411**
Oki-10	0.051	0.085	-0.081	0.011	0.095	0.221	0.089	-0.047	0.027	-0.023	0.058
Omm-1128	0.007	-0.108	-0.012	0.015	-0.006	-0.003	0.103	0.065	-0.033	**0.224**	0.005
Omy-1001	0.090	0.244	0.055	0.015	0.009	0.060	-0.017	0.106	0.002	0.092	0.015
One-18	0.089	-0.019	0.012	0.020	-0.002	0.032	0.023	-0.060	0.107	0.099	-0.153
Ogo-3	0.018	0.250	0.059	0.008	0.059	NA	0.036	0.001	NA	-0.104	NA
One-2	0.056	0.053	0.036	0.046	0.038	0.012	-0.024	-0.080	0.099	0.052	0.068
Sco-110	0.111	0.074	0.080	0.056	0.082	0.096	0.000	0.056	0.140	0.116	0.250
Omm-1138	-0.004	-0.067	0.123	0.048	0.086	0.077	-0.007	0.043	0.075	0.080	-0.048
Omy-325	0.015	-0.158	0.035	0.092	0.037	**0.191**	0.013	0.003	0.012	**0.456**	0.002
Sco-103	0.014	-0.081	0.050	0.054	0.073	0.159	-0.040	0.070	0.030	**0.280**	0.016
All	**0.042**	0.007	**0.056**	**0.066**	**0.056**	**0.082**	0.023	**0.041**	**0.077**	**0.164**	**0.109**
P value	0	0.3978	0	0	0	0	0.0067	0.0032	0	0	0
N	112	17	117	106	66	55	90	43	60	91	47

Table 6. Cavalli-Sforza and Edwards genetic chord distances calculated among samples and used for plotting dendrogram in Figure 3. Numbers in first column are reference numbers for each sample used for labeling columns of the pair-wise distance matrix. Numbers in sample names indicate year fish were sampled. Abbreviations: Hat. = hatchery; R. = River; Omy = *O. mykiss*; Ocl = *O. clarki*; Cr. = Creek.

	Samples	1	2	3	4	5	6	7	8	9	10
1	01 Puyallup Hat. Steelhead	0.0000									
2	02 Green R. Steelhead	0.0182	0.0000								
3	03 Cedar R. below-dam Omy	0.0264	0.0167	0.0000							
4	03 Cedar R. above-dam Omy	0.0375	0.0254	0.0126	0.0000						
5	03 Cedar trap Omy smolts	0.0367	0.0272	0.0267	0.0320	0.0000					
6	03 Cedar R. Ocl	0.0945	0.0915	0.0922	0.0903	0.0855	0.0000				
7	03 Green R. Steelhead	0.0186	0.0084	0.0143	0.0223	0.0252	0.0896	0.0000			
8	97 Ballard Locks Steelhead	0.0304	0.0158	0.0206	0.0265	0.0303	0.0963	0.0173	0.0000		
9	98 Ballard Locks Steelhead	0.0286	0.0155	0.0180	0.0254	0.0298	0.0936	0.0151	0.0166	0.0000	
10	02 Minter Cr. Ocl	0.0812	0.0796	0.0794	0.0785	0.0767	0.0424	0.0763	0.0848	0.0820	0.0000
11	03 Soos Hat. winter Steelhead	0.0106	0.0196	0.0271	0.0383	0.0347	0.0934	0.0177	0.0323	0.0288	0.0800
12	03 Soos Hat. summer Steelhead	0.0250	0.0287	0.0316	0.0403	0.0398	0.0908	0.0254	0.0423	0.0383	0.0792
13	03 Upper Green R. Omy	0.0257	0.0151	0.0186	0.0243	0.0297	0.0944	0.0137	0.0214	0.0186	0.0811
14	04 Cedar R. below-dam Omy	0.0303	0.0163	0.0107	0.0175	0.0240	0.0898	0.0162	0.0195	0.0207	0.0786
15	04 Cedar R. above-dam Omy	0.0442	0.0333	0.0208	0.0179	0.0412	0.0950	0.0316	0.0346	0.0326	0.0841
16	04 Chester Morse Lake Omy	0.0801	0.0782	0.0529	0.0500	0.0828	0.1057	0.0769	0.0819	0.0795	0.1006
17	04 Lake WA gillnet Ocl	0.0989	0.0969	0.1000	0.0966	0.0915	0.0245	0.0947	0.1019	0.1004	0.0382
18	05 Big Bear Cr. Ocl	0.0974	0.0957	0.0996	0.0962	0.0915	0.0257	0.0932	0.1029	0.0993	0.0352
19	05 Cedar trap Ocl smolts	0.1016	0.0984	0.1005	0.0975	0.0913	0.0230	0.0958	0.1036	0.1004	0.0397
20	05 Lake WA purse seine Ocl	0.0973	0.0941	0.0982	0.0954	0.0900	0.0226	0.0927	0.1000	0.0964	0.0350

		11	12	13	14	15	16	17	18	19	20
1	01 Puyallup Hat. Steelhead										
2	02 Green R. Steelhead										
3	03 Cedar below-dam Omy										
4	03 Cedar above-dam Omy										
5	03 Cedar trap Omy smolts										
6	03 Cedar R. Ocl										
7	03 Green R. Steelhead										
8	97 Ballard Locks Steelhead										
9	98 Ballard Locks Steelhead										
10	02 Minter Cr. Ocl										
11	03 Soos Hat. winter Steelhead	0.0000									
12	03 Soos Hat. summer Steelhead	0.0185	0.0000								
13	03 Upper Green R. Omy	0.0261	0.0325	0.0000							
14	04 Cedar below-dam Omy	0.0277	0.0333	0.0198	0.0000						
15	04 Cedar above-dam Omy	0.0451	0.0459	0.0337	0.0239	0.0000					
16	04 Chester Morse Lake Omy	0.0792	0.0732	0.0771	0.0579	0.0522	0.0000				
17	04 Lake WA gillnet Ocl	0.0993	0.0977	0.0990	0.0980	0.1024	0.1181	0.0000			
18	05 Big Bear Cr. Ocl	0.0964	0.0955	0.0982	0.0970	0.1019	0.1157	0.0125	0.0000		
19	05 Cedar trap Ocl smolts	0.1019	0.0980	0.0998	0.0978	0.1031	0.1179	0.0132	0.0164	0.0000	
20	05 Lake WA purse seine Ocl	0.0975	0.0971	0.0970	0.0960	0.1007	0.1165	0.0110	0.0138	0.0112	0.0000

Table 7. Pairwise F_{ST} values (lower matrix) among *O. mykiss* and *O. clarki* population and locality samples. Upper matrix presents probability (*P*) values for pairwise F_{ST} values being different from 0 based on 10,000 permutations. Yellow (shaded) cells indicate F_{ST} and *P* values that were not significant. Temporal population or locality samples were combined and Soos Cr. and Puyallup hatcheries winter steelhead were combined (= Hatchery winter Steelhead). Numbers in first column are reference numbers for each sample used for labeling columns of the matrix. Abbreviations: Omy = *O. mykiss*; R. = River; Ocl = *O. clarki*; Cr. = Creek; Hat. = hatchery.

	Samples	1	2	3	4	5	6	7	8	9	10	11
1	Chester Morse Lake Omy	0	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
2	Hatchery winter Steelhead	0.1530	0	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
3	Upper Green R. Omy	0.1314	0.0376	0	0.00002	0.00020	0.00010	0.00002	0.00002	0.00002	0.00002	0.00002
4	Ballard Locks Steelhead	0.1624	0.0354	0.0189	0	0.02653	0.00010	0.00002	0.00002	0.00002	0.00002	0.00002
5	Green R. wild Steelhead	0.1418	0.0259	0.0100	0.0035	0	0.00010	0.00002	0.00002	0.00002	0.00002	0.00002
6	Cedar R. below-dam Omy	0.0948	0.0359	0.0117	0.0097	0.0054	0	0.00069	0.00002	0.00002	0.00002	0.00002
7	Cedar R. above-dam Omy	0.0695	0.0581	0.0290	0.0264	0.0226	0.0061	0	0.00002	0.00002	0.00002	0.00002
8	All Cedar R. Ocl	0.3701	0.3036	0.3111	0.3371	0.3135	0.3098	0.3059	0	0.00002	0.00002	0.00238
9	Minter Cr. Ocl	0.3038	0.2350	0.2438	0.2695	0.2471	0.2433	0.2424	0.0486	0	0.00002	0.00002
10	Soos Hat. summer Steelhead	0.1061	0.0373	0.0381	0.0517	0.0409	0.0302	0.0432	0.2781	0.2168	0	0.00002
11	Big Bear Cr. Ocl	0.3709	0.3009	0.3081	0.3372	0.3132	0.3096	0.3035	0.0126	0.0331	0.2786	0

Table 8a. GENECLASS2 assignments for smolts using a baseline that included Green River wild steelhead (Green), Cedar River *O. mykiss* below and above Landsburg Dam (CedarBelow and CedarAbove), Ballard Locks steelhead (Ballard), and Chester Morse Lake *O. mykiss* (Chester) samples. Individual smolts are shown by identification codes. All positive assignments to baseline samples are shown followed by their ranked score. Score ratio is the ratio between the most likely (score 1) and second most likely (score 2) assignment scores. Unambiguous positive assignment values are in bold. Negative log likelihood values (-log(L)) for assignment to each baseline sample are shown on the right.

						Baseline samples				
					Score	Green	CedarBelow	CedarAbove	Ballard	Chester
Smolt ID	**Assignment**	**Score 1**	**Assignment**	**Score 2**	**Ratio**	**-log(L)**	**-log(L)**	**-log(L)**	**-log(L)**	**-log(L)**
03BH0004	Green	97.30	CedarBelow	1.46	67	24.41	26.23	28.99	26.30	54.52
03BH0006	Ballard	70.65	Green	23.47	3	35.38	35.98	45.37	34.90	78.51
03BH0007	**Green**	**100.00**	CedarBelow	0.00	100000	37.96	44.84	53.94	48.05	74.77
03BH0008	Ballard	90.05	CedarBelow	9.64	9	38.92	37.43	43.84	36.46	83.69
03BH0009	**Green**	**99.25**	CedarBelow	0.71	139	22.67	24.82	27.44	26.05	59.53
03BH0011	CedarBelow	63.21	Ballard	33.32	2	29.32	28.06	35.20	28.33	66.89
03BH0012	Green	81.18	Ballard	16.35	5	30.77	32.29	38.83	31.47	67.37
03BH0014	Ballard	77.68	CedarBelow	21.40	4	40.44	36.70	38.07	36.14	64.22
03BH0015	**CedarBelow**	**98.93**	Ballard	0.79	126	38.23	35.39	38.22	37.49	73.37
03BH0016	Green	52.61	Ballard	42.40	1	36.14	37.16	46.07	36.23	78.15
03BH0017	**Green**	**100.00**	Ballard	0.00	49999	28.46	34.62	39.33	33.18	74.91
03BH0018	Ballard	80.19	Green	19.80	4	37.71	40.79	46.27	37.10	80.30
03BH0019	**CedarBelow**	**99.95**	Green	0.05	1960	31.01	27.71	32.38	36.92	52.32
03BH0020	**Green**	**100.00**	CedarBelow	0.00	99999	30.51	35.71	41.94	36.88	53.30
03BH0021	CedarBelow	62.21	Green	37.76	2	32.69	32.47	36.55	35.85	65.36
04BB0009	**Green**	**99.50**	CedarBelow	0.50	200	33.84	36.14	47.08	39.26	74.30
04BB0010	Ballard	94.34	CedarBelow	5.65	17	36.86	34.19	42.28	32.97	64.02
04BB0018	**CedarBelow**	**100.00**	Ballard	0.00	100000	47.74	41.40	49.76	47.50	70.95
04BB0020	**Green**	**99.71**	CedarBelow	0.29	343	32.67	35.21	44.67	40.07	77.47
04BB0021	**CedarBelow**	**99.85**	Ballard	0.13	745	40.47	36.69	45.23	39.56	75.56
04BB0022	**CedarBelow**	**99.85**	Ballard	0.12	832	35.69	32.16	39.96	35.08	68.40
04BB0023	Ballard	55.97	Green	43.87	1	38.10	40.53	52.47	37.99	76.29

Table 8b. GENECLASS2 assignments for smolts using a baseline that included Cedar River *O. mykiss* below Landsburg Dam (CedarBelow) and Ballard Locks steelhead (Ballard) samples. Individual smolts are shown by identification codes. All positive assignments to baseline samples are shown followed by their ranked score. Score ratio is the ratio between the most likely (score 1) and second most likely (score 2) assignment scores. Unambiguous positive assignment values are in bold. Negative log likelihood values (-log(L)) for assignment to each baseline sample are shown on the right.

						Baseline samples	
					Score	CedarBelow	Ballard
Smolt ID	**Assignment**	**Score 1**	**Assignment**	**Score 2**	**Ratio**	**-log(L)**	**-log(L)**
03BH0004	CedarBelow	52.82	Ballard	47.18	1	26.21	26.26
03BH0006	Ballard	92.44	CedarBelow	7.56	12	35.97	34.89
03BH0007	**CedarBelow**	**99.93**	Ballard	0.07	1388	44.77	47.91
03BH0008	Ballard	93.39	CedarBelow	6.61	14	37.41	36.26
03BH0009	CedarBelow	94.44	Ballard	5.56	17	24.82	26.05
03BH0011	CedarBelow	65.08	Ballard	34.92	2	28.05	28.32
03BH0012	Ballard	87.04	CedarBelow	12.96	7	32.28	31.45
03BH0014	Ballard	78.72	CedarBelow	21.28	4	36.62	36.06
03BH0015	CedarBelow	98.75	Ballard	1.25	79	35.38	37.28
03BH0016	Ballard	92.79	CedarBelow	7.21	13	37.15	36.04
03BH0017	Ballard	95.11	CedarBelow	4.89	19	34.42	33.13
03BH0018	**Ballard**	**99.98**	CedarBelow	0.02	4999	40.61	36.91
03BH0019	**CedarBelow**	**100.00**	Ballard	0.00	100000	27.71	36.73
03BH0020	CedarBelow	91.47	Ballard	8.53	11	35.62	36.65
03BH0021	**CedarBelow**	**99.96**	Ballard	0.05	2221	32.47	35.82
04BB0009	**CedarBelow**	**99.82**	Ballard	0.18	545	36.13	38.87
04BB0010	Ballard	94.84	CedarBelow	5.16	18	34.03	32.76
04BB0018	**CedarBelow**	**100.00**	Ballard	0.00	100000	41.34	47.30
04BB0020	**CedarBelow**	**100.00**	Ballard	0.00	49999	35.20	39.97
04BB0021	**CedarBelow**	**99.79**	Ballard	0.21	471	36.68	39.35
04BB0022	**CedarBelow**	**99.86**	Ballard	0.14	693	32.16	35.00
04BB0023	**Ballard**	**99.80**	CedarBelow	0.20	494	40.50	37.80

Table 9a. STRUCTURE 2.1 results for *O. mykiss* smolt ancestry with the number of population groups ("K") hypothesized from 2 through 5. The top portion of the table shows the percentage of ancestry of each baseline sample in the hypothesized groups. Log-likelihood (Ln) values indicate the probability of the number of hypothesized groups. The bottom portion of the table shows the percentage of ancestry of each smolt in the hypothesized groups. Values above 0.2 are in bold type. Abbreviations: Green Sthd = Green River steelhead; Ballard Sthd = Ballard Locks steelhead; CedarBelow = *O. mykiss* below Landsburg Dam; CedarAbove = *O. mykiss* above Landsburg Dam; Chester = Chester Morse Lake *O. mykiss*.

	K = 2		K = 3			K = 4				K= 5				
	1	2	1	2	3	1	2	3	4	1	2	3	4	5
Green Sthd	**0.955**	0.045	0.063	**0.926**	0.012	0.012	**0.680**	0.059	**0.250**	0.014	0.058	**0.250**	**0.479**	**0.199**
Ballard Sthd	**0.954**	0.046	0.096	**0.895**	0.008	0.008	**0.557**	0.079	**0.355**	0.011	0.072	**0.454**	**0.359**	0.104
CedarBelow	**0.684**	**0.316**	**0.279**	**0.577**	0.143	0.142	**0.321**	**0.244**	**0.293**	0.136	**0.209**	**0.300**	**0.197**	0.157
CedarAbove	**0.439**	**0.561**	**0.798**	0.126	0.075	0.085	0.067	**0.722**	0.126	0.107	**0.648**	0.101	0.052	0.092
Chester	0.010	**0.990**	0.008	0.003	**0.989**	**0.981**	0.004	0.011	0.004	**0.973**	0.012	0.005	0.005	0.006
Smolts	**0.864**	0.136	0.157	**0.793**	0.049	0.043	**0.507**	0.052	**0.398**	0.030	0.048	**0.339**	**0.314**	**0.269**
Ln value		-32060.9			-31691.8				-31529.1					-31807.5
03BH0004	**0.983**	0.017	0.018	**0.978**	0.005	0.006	**0.558**	0.012	**0.424**	0.007	0.014	**0.318**	**0.575**	0.087
03BH0006	**0.989**	0.011	0.006	**0.991**	0.003	0.003	**0.921**	0.006	0.069	0.004	0.008	0.101	**0.844**	0.043
03BH0007	**0.980**	0.020	0.012	**0.982**	0.006	0.008	**0.739**	0.011	**0.241**	0.009	0.012	0.047	**0.584**	**0.348**
03BH0008	**0.975**	0.025	0.051	**0.945**	0.003	0.003	**0.252**	0.047	**0.698**	0.004	0.057	**0.867**	0.035	0.037
03BH0009	**0.985**	0.015	0.018	**0.978**	0.004	0.005	**0.426**	0.014	**0.555**	0.006	0.014	**0.929**	0.025	0.026
03BH0011	**0.930**	0.070	0.080	**0.911**	0.008	0.008	**0.722**	0.106	0.163	0.012	0.119	**0.693**	0.114	0.062
03BH0012	**0.985**	0.015	0.008	**0.988**	0.004	0.004	**0.933**	0.009	0.055	0.004	0.008	0.016	**0.962**	0.010
03BH0014	**0.845**	0.155	**0.708**	**0.284**	0.008	0.009	**0.272**	**0.457**	**0.261**	0.011	**0.367**	0.100	0.087	**0.436**
03BH0015	**0.969**	0.031	**0.481**	**0.515**	0.003	0.004	0.162	**0.230**	**0.604**	0.005	**0.353**	**0.323**	0.174	0.144
03BH0016	**0.983**	0.017	0.026	**0.971**	0.003	0.003	**0.832**	0.030	0.135	0.004	0.040	**0.665**	**0.226**	0.066
03BH0017	**0.984**	0.016	0.013	**0.984**	0.003	0.004	**0.640**	0.014	**0.342**	0.005	0.019	**0.843**	0.098	0.035
03BH0018	**0.984**	0.016	0.016	**0.982**	0.003	0.003	**0.797**	0.015	0.185	0.004	0.024	0.142	**0.769**	0.061
03BH0019	**0.561**	**0.439**	**0.432**	**0.410**	0.158	**0.206**	**0.414**	**0.207**	0.173	**0.211**	0.090	0.067	**0.316**	**0.317**
03BH0020	**0.927**	0.073	0.014	**0.970**	0.017	0.015	**0.740**	0.011	**0.233**	0.019	0.017	0.111	0.171	**0.683**
03BH0021	**0.979**	0.021	0.019	**0.976**	0.005	0.005	**0.531**	0.016	**0.448**	0.005	0.023	**0.816**	0.025	0.130
04BB0009	**0.990**	0.010	0.008	**0.989**	0.003	0.003	**0.922**	0.007	0.067	0.004	0.008	0.021	**0.947**	0.019
04BB0010	**0.977**	0.023	0.019	**0.975**	0.006	0.008	**0.395**	0.015	**0.582**	0.009	0.013	**0.423**	0.137	**0.418**
04BB0018	**0.891**	0.109	0.133	**0.851**	0.016	0.005	0.010	0.005	**0.979**	0.006	0.008	0.128	0.016	**0.842**
04BB0020	**0.982**	0.018	0.010	**0.986**	0.004	0.005	**0.584**	0.011	**0.400**	0.005	0.012	**0.776**	0.048	0.158
04BB0021	**0.871**	0.129	0.059	**0.889**	0.052	0.057	**0.828**	0.055	0.060	0.057	0.050	0.018	**0.831**	0.044
04BB0022	**0.980**	0.020	0.019	**0.977**	0.004	0.004	0.020	0.011	**0.966**	0.006	0.016	**0.905**	0.028	0.045
04BB0023	**0.988**	0.012	0.006	**0.990**	0.004	0.004	**0.660**	0.006	**0.330**	0.005	0.007	**0.404**	**0.378**	**0.205**

Table 9b. STRUCTURE 2.1 results for *O. mykiss* smolt ancestry with two population groups hypothesized (K = 2). The top portion of the table shows the percentage of ancestry of each baseline sample and the smolt sample in the two hypothesized groups. The bottom portion of the table shows the percentage of ancestry of each smolt in the hypothesized groups. Values above 0.2 are in bold type. Abbreviations: Ballard Sthd = Ballard Locks steelhead; CedarBelow = *O. mykiss* below Landsburg Dam.

	1	2
Ballard Sthd	0.085	**0.915**
CedarBelow	**0.432**	**0.568**
Smolts	0.193	**0.807**
03BH0004	0.053	**0.947**
03BH0006	0.016	**0.984**
03BH0007	**0.787**	**0.213**
03BH0008	0.016	**0.984**
03BH0009	0.017	**0.983**
03BH0011	0.065	**0.935**
03BH0012	0.012	**0.988**
03BH0014	**0.285**	**0.715**
03BH0015	0.075	**0.925**
03BH0016	0.030	**0.970**
03BH0017	0.024	**0.976**
03BH0018	0.101	**0.899**
03BH0019	**0.940**	0.060
03BH0020	**0.824**	0.176
03BH0021	0.052	**0.948**
04BB0009	0.022	**0.978**
04BB0010	0.079	**0.921**
04BB0018	**0.651**	**0.349**
04BB0020	0.043	**0.957**
04BB0021	0.099	**0.901**
04BB0022	0.031	**0.969**
04BB0023	0.023	**0.977**

Table 10a. Fork-length and age of *O. mykiss* in Cedar River below and above Landsburg Dam and Ballard Locks steelhead samples. Genetic hybrids in samples were excluded. SD = standard deviation; N = number of fish included; n/a = not available.

Sample	**Ave. Length cm (SD; N)**	**Ave. Age (range; N)**
Steelhead- Ballard Locks	72.7 (7.4; 53)	see Table 10b (3 – 6; 56)
Below-dam *O. mykiss*		
2003	36.2 (8.8; 51)	3.6 (2 – 8; 28)
2004	37.6 (9.7; 48)	3.4 (1 – 6; 30)
Above-dam *O. mykiss*		
2003	24.2 (6.1; 49)	2.0 (1 – 5; 27)
2004	18.5 (8.8; 15)	1.2 (0 – 3; 9)
Smolts - all	18.5 (2.2; 24)	n/a

Table 10b. Ages, including freshwater and marine phases, of steelhead sampled at Ballard Locks in 1997, 1998, and 1999, N=56. The percent steelhead in each age class is shown. Numbers preceding the decimal or period indicate freshwater years prior to smolting, and "W1" indicates a wild-type freshwater rearing stage of approximately one year prior to smolting. As an example for translation to total age, a 2.1+ fish is approximately four years old.

Age Class	**W1.1+**	**W1.2+**	**2.1+**	**2.2+**	**2.3+**	**3.1+**	**3.2+**
%	14	7	48	23	2	4	2