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

PROGRESS REPORT

To:

Cedar River Anadromous Fish Committee
and Seattle Public Utilities

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INTRODUCTION

We initiated this research to assist development of a steelhead (anadromous *Oncorhynchus mykiss)* restoration plan for approximately 17.5 miles of previously blocked habitat in Cedar River, a Puget Sound drainage in Washington. Landsburg Dam (RM 21) had blocked anadromous fish passage since 1900. Construction of adult and juvenile fish passage at Landsburg Dam was completed in 2003, and the restored accessibility of upstream habitat was expected to benefit steelhead. Steelhead abundance in this watershed had been critically low during the last 12 years, leaving potentially few fish to naturally re-colonize the opened habitat. Rainbow trout (resident *O. mykiss*) are present throughout the river, and appeared abundant in below-dam areas. Genetic relationships between anadromous and above- and below-Dam resident *O. mykiss* were unknown.

Several studies (Docker and Heath 2003; Pearsons et al. 2003; McCusker et al. 2000; Zimmerman and Reeves 2000) have shown that native resident and anadromous *O. mykiss* within a drainage are closely related, and likely to interbreed at some level. However, non-native (California-origin) hatchery rainbow trout had been released historically in the watershed, especially in Lake Washington. We needed to determine the genetic relationship between Cedar River resident and anadromous *O. mykiss* to understand what role the trout resource might play in restoration of the anadromous population.

Factors most critical to the success of Cedar River steelhead have not been determined, but several habitat-related and migration behavior research projects are underway. Adult and juvenile steelhead must migrate through Lake Washington and approximately five miles of an engineered waterway through Seattle, which includes a shipping locks ("Ballard Locks") and associated fish passage facilities, to reach Puget Sound. Although the recent (1996 to 2002) drop in steelhead abundance was acute, there has been a less severe but steady longer-term decline in four-year (typical generation length) rolling average abundance. Genetic change in Cedar River steelhead may be an important factor in the decline of the anadromous population, as well as various habitat-related problems. For example, genetic change could result from selection against particular life-history trajectories or types, interbreeding with other populations or species, or, if not interbreeding, a consistently small number of successful spawners, which would reduce genetic diversity.

The current low abundance of anadromous adults could be viewed as a severe impediment to population restoration. However, it is conceivable that resident adults are capable of producing seaward-migrating smolts. It is relatively well-known that residual, precocial males occur in steelhead populations. In a Hood River, Oregon steelhead reproductive success study using DNA pedigree analysis techniques, 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 trout, crosses between resident trout and between trout and steelhead all produced out-migrating smolts (Ruzycki et al. 2003). Smolt production capability of

resident Cedar River trout is likely most dependent on genetic heritage and environmental factors. Our genetic study examines their heritage by comparing resident adults from above- and below-Dam areas to anadromous adults presumed destined for Cedar River, from the ancestrally-related Green River population, and several relevant hatchery populations. The environmental capacity to support both life-history types or strategies is a major issue that should be evaluated subsequently.

Although above-Dam *O. mykiss* have been land-locked since 1900, below-Dam resident adults may be a recent ecophenotype (Zimmerman and Reeves 2000) of the steelhead population that is dominant under particular conditions, but could alternatively produce smolts. Puget Sound streams below migrational barriers generally are inhabited nearly exclusively by anadromous *O. mykiss* populations according to WDFW biologists, in contrast to inland Columbia Basin rivers where anadromous and resident fish typically co-exist. The steelhead life-history was likely the dominant natural state of *O. mykiss* in Cedar River when it was a Green River tributary, prior to its diversion into Lake Washington. Recent Cedar River and Lake Washington conditions may have favored fish expressing resident life-histories causing these phenotypes to become abundant coincidentally to a period of poor ocean survival for regional steelhead.

Previously, genetic analyses had been conducted on juvenile (age 1 parr) *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 (Phelps et al. 1994; Phelps and Baker 1995; Phelps et al. 1997). Parr below Landsburg Dam were presumed to be steelhead progeny based on the assumption that steelhead was the primary life-history form in that area. Genetic data were collected by analyzing allelic variation at allozyme gene loci. Significant temporal variability in allele frequencies occurred between the two lower Cedar River juvenile samples, but they 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* in the two upstream Cedar Basin locations. 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 from non-native rainbow trout hatchery strains. Also, three above-Dam fish appeared to be *O. mykiss*-*O. clarki* (cutthroat trout) hybrids. They surmised that Chester Morse Lake *O. mykiss* likely had some ancestry from an exotic rainbow trout hatchery strain.

These earlier genetic results are informative in a number of ways, but do not elucidate the relationship between resident and anadromous *O. mykiss* below Landsburg Dam. The juveniles sampled could have included offspring from resident and/or anadromous parents. Various possible scenarios could be hypothesized (100% anadromous offspring, 50% offspring each from non-interbreeding anadromous and resident populations, 100% resident offspring, etc.) and the results examined under those conditions, but this is not definitive as to the source and behavior of resident *O. mykiss*. Above-Landsburg Dam resident *O. mykiss* necessarily produced the juveniles sampled in 1994. Their genetic

results suggested the persistence of a legacy from steelhead remaining upstream after dam construction.

Hybridization and other interaction with cutthroat trout are important issues relative to the status of *O. mykiss* populations in Cedar River and other Lake Washington drainages. According to WDFW biologists, cutthroat trout are abundant in Lake Washington and appear to favor and succeed at an adfluvial life-history strategy (migration between streams and lake) versus anadromy. Within this system, cutthroat trout are potential competitors with and/or predators on *O. mykiss*. 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). 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. The significant human-induced habitat changes in Cedar River and Lake Washington could have disrupted natural isolating mechanisms. Hybridization with cutthroat trout is a significant alteration of the *O. mykiss* gene pool and likely has an especially negative impact on the steelhead phenotype.

The over-riding goal of this project is to understand genetic population structure of Cedar River/Lake Washington *O. mykiss* so that managers can design and implement strategies that effectively conserve and recover native steelhead and rainbow trout resources. Our study design targets adult fish at or near reproductive age and employs several types of DNA markers expected to be highly informative. Our major research objectives to be accomplished through two years of sampling are:

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

2) Determine genetic relationships between Cedar River outmigrating *O. mykiss* and potential parent groups

3) Evaluate genetic relationships between *O. mykiss* above Landsburg Dam in former anadromous fish habitat and those below the dam

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

5) Estimate extent of cutthroat trout and *O. mykiss* hybridization in all sampled groups

6) Based on population structure inferred from sample data, estimate effective population size for Cedar River steelhead

This progress report summarizes our work on this research project during 2003 and the first half of 2004, including the genetic analysis of all samples collected in 2003. Sampling for 2004 is already underway.

METHODS

Field Sampling

We originally designated 12 groups or populations of *O. mykiss* and *O. clarki* throughout the Cedar River watershed and nearby drainages or hatcheries as our study samples, and field sampling was planned for April through July. Some non-Cedar River samples were acquired prior to 2003. We sampled adult fish in-river by angling. Returning adult steelhead were trapped at the Ballard Locks fish ladder. Juveniles were sampled in Cedar River using a downstream migrant screw-type trap (Seiler et al. 1981). Samples from hatchery populations were collected during broodstock spawning or among rearing progeny.

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. A phenotypic identification of potential hybrids was noted for some fish. Fish at Ballard Locks were sampled similarly, except they were held in tubes or cradles and anaesthetized. Juveniles were anaesthetized with MS222 prior to taking a fin-clip and measurement, and then were revived and released. Fin-clips, scales, and measurements were taken from all adult fish in other study samples. Fin tissue was immediately placed in plastic vials containing 95% ethanol, and all fin samples used for this study were stored in 95% ethanol at room temperature. Scales were placed on traditional paper scale cards, which we used to record sampling data for each fish.

Due to poor sampling success at Ballard Locks in 2003 and the extremely 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 population sample. These fish, most of which were presumed to originate from Cedar River, had been used as broodstock for an experimental hatchery production project. They had been scale-sampled at spawning time and we used the dried, preserved scales as our tissue source for DNA.

Laboratory Analyses

Scale Aging

We used scale pattern analysis (Davis and Light 1985; Shapovalov and Taft 1954) to determine total age, including time periods for juvenile freshwater rearing and marine residency, and to determine spawning history for all sampled adults. John Sneva, WDFW, carried out all scale analyses and provided the results.

DNA extraction and microsatellite loci amplification

Genomic DNA was extracted from tissue samples using chelex resin (Small et al. 1998). We tested 25 microsatellite loci (short sequence-repeat DNA markers) for amplification

in *O. mykiss* and *O. clarki* and used 20 loci for analysis. 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. 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 automated sequencer. Allele mobilities were determined by allele length (number of basepairs) and alleles were sized to basepairs (bp) using an internal lane size standard (GS500 by Applied Biosystems), and ABI computer program Genemapper. 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 a database.

DNA species markers and hybridization tests

Individuals that were identified phenotypically as *O. clarki-O. mykiss* hybrids or through microsatellite DNA genotypic profiles were examined further for hybridization using three simple sequence repeat (SSR) markers, Occ-16, Omm-28 and Omm-35 (Ostberg and Rodriguez 2002). These 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% Sybre Gold. Fragments were sized in comparison to a 100bp ladder. Hybrid individuals were also tested with restriction fragment length polymorphism (RFLP) analysis of three single-locus nuclear DNA markers, ribosomal internal transcribed spacer (ITS), gonadotropin-releasing hormone (GnRH), and proto-oncogene p53 (p53) as described by Baker et al. (2002). DNA fragments were PCR-amplified, restriction-digested and separated on agarose gels following methods of Baker et al. (2002). We also analyzed a set of individuals believed to be of pure species composition for both species at all six nuclear DNA markers.

Statistical analyses for microsatellite data

Within-sample genetic variation

Statistical tests were conducted on genotypic data per locus and per sample (all loci) to assess conformation to Hardy-Weinberg expectations (Hardy-Weinberg 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. HWE tests assess whether observed genotypic heterozygosity in populations deviates from expected heterozygosity. Deviations from Hardy-Weinberg 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 (number of alleles corrected for sample size),

F-statistics (allelic correlations within or among population subdivisions) and gene diversity (expected heterozygosity corrected for sample size) were calculated using FSTAT2.9.3. We used MSA (Dieringer and Schlötterer 2003) to calculate observed and expected heterozygosity.

Among-sample relationships

Genetic relationships among samples were examined with pair-wise tests. All possible sample pairs were tested for differences in genotypic distributions at each locus and across all loci using GENEPOP3.3 with 300 batches and 2000 iterations. Test results were corrected for multiple simultaneous tests to an overall alpha level of 0.05 (Rice 1989). Relationships were also explored with ordination and dendrogram analyses. Genetic distances (chord distances, Nei et al.1983) among samples were estimated using MSA and distances were plotted in a multidimensional scaling (MDS) analysis using NTSYS-pc (Rohlf 1993). Underlying trends in the data set were examined in a principle components analysis using PCAGEN (Goudet 1999).

For among-sample comparisons and introgression and hybridization tests described below, we used compatible microsatellite DNA genotype data collected previously by WDFW staff from several pertinent populations. These included four WDFW hatchery populations (Spokane, Goldendale, South Tacoma and Eell Springs hatcheries) of California-origin rainbow trout, a Packwood Lake, WA collection of *O. mykiss*, and a Dosewallips River, WA (Hood Canal drainage) collection of *O. mykiss*. All these populations were sampled in years prior to this study.

Introgression and hybridization

We used STRUCTURE 2.1 (Pritchard et al. 2000) to estimate the proportion of ancestry shared among samples and to examine hybridization between *O. clarki* and *O. mykiss*. In this program, samples are tested for membership in a series of user-defined hypothetical populations. For example, to test whether *O. clarki* and *O. mykiss* are reproductively isolated, two populations (the two species) are hypothesized among the sample data and percentage of membership in either population calculated for an individual gives an estimate of the individual's ancestry. If the individual is purely *O. clarki*, it would belong predominately to the population group containing other *O. clarki*, and visa versa if the individual is purely *O. mykiss*. A hybrid individual would show ancestry in both groups, with percentage of ancestry varying by generation of hybridization event (1^{st} generation 50:50). Membership is calculated per individual and over samples.

We used GENETIX (Belkhir et al. 2004) to view divergence between anadromous and resident *O. mykiss* and *O. clarki*, hybridization between *O. mykiss* and *O. clarki*, introgression between wild *O. mykiss* and rainbow trout hatchery strains, and to identify which group the Lake Washington resident *O. mykiss* were closest to genetically. GENETIX calculates a center of gravity for a sample of individuals and performs a factoral correspondence analysis on the individuals and sample. Resulting data for individuals and samples are plotted in three dimensions. Hybridization or introgression is

hypothesized when individuals from one species or sample plot within the range of the other species or sample(s).

The program WHICHRUN 4.2 (Banks and Eichert 2000) was used for assignment tests to examine genetic relationships from individual and sample perspectives. The WHICHRUN program implements a jackknife procedure where each individual in turn is removed from the samples dataset (the baseline), allele frequencies of the baseline are re-calculated and the individual is assigned to the most likely sample-of-origin based upon its genotype and the allele frequencies of the samples. Individuals can also be tested for assignment as unknowns. We estimated origins of outmigrating smolts by testing their assignment to Ballard Locks steelhead, Cedar River samples including *O. mykiss* from below and above Landsburg Dam, and to *O. clarki*.

RESULTS

Samples

In 2003 we sampled fish from the following life-history-types or species and locations: *O. mykiss* (steelhead) passing through the Ballard Locks; *O. mykiss* and *O. clarki* and field-identified hybrids in the Cedar River below Landsburg Dam; *O. mykiss* in the Cedar River above Landsburg Dam; juvenile *O. mykiss* and *O. clarki* outmigrants in the Cedar River smolt trap; and *O. mykiss* in Lake Washington (Table 2). We obtained samples of wild adult steelhead in Green River (Table 2) from WDFW biologists involved in a wild broodstock research project during 2002 and 2003. We also obtained samples from the Chambers Creek winter steelhead stock at Puyallup Hatchery (2001 brood).

As mentioned above, our poor sampling success at Ballard Locks (N=1), led us to using scale samples from wild adults sampled in three earlier years as a DNA source. These scales proved to be valuable materials as we succeeded in extracting usable DNA from 55 of 56 sampled fish. Among all tissue samples, we successfully extracted genomic DNA and amplified at least eight microsatellite loci for 400 individuals (Table 2). Only four non-anadromous adult *O. mykiss* were sampled in Lake Washington (near Cedar River) and while we used their genotypic data to test relationships with other samples, we did not use them in the full genetic diversity and population structure analysis.

Genetic analyses- microsatellite DNA loci

Hardy-Weinberg and linkage disequilibrium tests

All annual samples deviated from Hardy-Weinberg expectations (HWE) with homozygote excess (Table 2). The locus *Omy-77* was out of HWE in all samples and was removed from the data set for all other analyses. Gene diversity and allelic richness were comparable among *O. mykiss* samples (Table 2). Before Bonferroni corrections (correction for multiple tests of same hypothesis), there were 39 significant F_{IS} values at individual loci, 29 in tests within *O. mykiss* samples (Table 3) and 10 within Cedar River

putative *O. clarki* (Table 3). After corrections, only two values remained significant in *O. mykiss* collections and four remained significant in the *O. clarki* sample (Table 3). Significant, positive total F_{IS} values occurred in all samples (Table 3).

In genotypic (linkage) disequilibrium tests over all samples, 16 out of 171 tests indicated significant non-random associations between loci. In tests within samples, *Omm-1070* and *Sco-103* showed linkage in all samples suggesting that these two loci were on the same chromosome. *Omm-1070* was removed from the data set for further analyses because statistics employed are based on the assumption that loci are unlinked. For the rest of the significant tests, different pairs of loci were in disequilibria in most samples, suggesting associations due to causes other that physical linkage, such as non-random mating, inbreeding, or mixed-origin fish.

Individual samples varied widely in the number of pairs in disequilibria from 0 in *O. mykiss* from Cedar River below Landsburg Dam, to 13 in *O. mykiss* (steelhead) from Puyallup Hatchery (Table 2). The Cedar River phenotypic *O. clarki* sample had 8 locus pairs in disequilibria (Table 2) and the highest total F_{IS} value among samples (Table 3). We later found that STRUCTURE results indicated this *O. clarki* sample had substantial introgression or contributions from *O. mykiss* (see Table 6). The linkage and F_{IS} values and higher allelic richness (Table 2) also suggest introgression, and/or a mixed sample.

MDS analysis

The multidimensional scaling analysis (MDS) plot (Figure 1) indicated that the Ballard Locks *O. mykiss* were genetically closer to Green River *O. mykiss* than to Cedar River *O. mykiss*. Green River *O. mykiss* samples clustered closely and plotted closer to Puyallup Hatchery *O. mykiss* than to Cedar River *O. mykiss*. Within Cedar River, *O. mykiss* from below and above Landsburg Dam were closest to each other. Cedar River smolts plotted closely to the Cedar River *O. mykiss* samples along the first and second axes and separated from them along the third axis. The phenotypic hybrids plotted closely to Cedar River *O. mykiss* along the first axis, but were distant along the second and third axes. Cedar River *O. clarki* were distant from the *O. mykiss* samples along the first axis.

Principle components analysis

In the principle components analysis (PCA), the global F_{ST} value was 0.044 ($P = 0.01$), indicating high genetic structure, as expected with *O. mykiss* and *O. clarki* samples in the data set. The PCA plot (Figure 2) was similar to the MDS plot, although Green River samples appeared closer to Cedar River samples than to the Puyallup Hatchery sample. *O. mykiss* samples are on the right side of the first axis and the *O. clarki* sample is on the left side (F_{ST} for axis 1 = 0.015, $P = 0.003$ for proportion of inertia in first axis). This suggests that the most substantial trend in the data is the distance between *O. clarki* and *O. mykiss*. Green River and Puyallup Hatchery *O. mykiss* separated along the second axis ($F_{ST} = 0.011$, $P = 0.01$ for proportion of inertia in second axis) indicating that the division between hatchery and wild *O. mykiss* is the next substantial trend.

Factoral correspondence analysis

In the factoral correspondence analysis plot (Figure 3), *O. mykiss* samples are at the left side of axis 1 and each sample forms overlapping clusters with the Puyallup Hatchery cluster the most discrete. Cedar River putative *O. clarki* form a large diffuse cluster with a few individuals plotted within the *O. mykiss* group (too difficult to see or point out on the plot), and three juvenile *O. mykiss* plot within the *O. clarki* cluster (encircled, Figure 3). Cedar River *O. mykiss* from above Landsburg Dam form a tighter cluster than the *O. mykiss* from below Landsburg Dam, but the two groups overlap with *O. mykiss* from Green River and Ballard Locks (Figure 3).

A second factoral correspondence analysis was conducted including four *O. mykiss* hatchery strains, *O. mykiss* from Packwood Lake of uncertain ancestry, wild *O. mykiss* from Dosewallips River, and the four *O. mykiss* individuals collected in Lake Washington near Cedar River (Figure 4). Dosewallips samples plotted closer to Cedar and Green samples than to the hatchery samples. The rainbow trout hatchery groups were discrete and formed a multiple cluster composed of Spokane, Goldendale and Eell Springs samples (Figure 4). Packwood Lake *O. mykiss* was discrete and separate from all other samples. The four *O. mykiss* from Lake Washington plotted within the multiple hatchery cluster, suggesting a hatchery origin for these fish.

Pairwise genotypic tests

In pairwise genotypic tests, most samples had significantly different genotypic distributions (Table 4). In all the comparisons to *O. mykiss*, Cedar River phenotypic *O. clarki* shared the most genotypic distributions with Cedar River smolts (Table 4, upper matrix). Within Cedar River, the smolts shared the most genotypic distributions with *O. mykiss* from below the Landsburg Dam (Table 4), suggesting that they originated in this population. *O. mykiss* from above and below Landsburg Dam were significantly different when results were summed over all loci, although after Bonferroni corrections no genotypic distributions were different (Table 4, upper and lower matrix). There was substantial overlap in genotypic distributions between Ballard Locks steelhead and Green River steelhead and below-dam Cedar River *O. mykiss*, although all differences were significant (Table 4, upper and lower matrices).

Hybridization analysis

All the *O. mykiss* and *O. clarki* were analyzed for genetic ancestry using the program STRUCTURE. To assess hybridization, only two populations were hypothesized for this analysis (Table 5). *O. clarki* belonged predominantly to one of the hypothetical populations, although *O. clarki* shared about 35% of its microsatellite ancestry with the *O. mykiss*-dominated cluster. *O. mykiss* from the Green and Cedar rivers had over 94% ancestry in the *O. mykiss* population group. Cedar River potential hybrids (identified phenotypically as hybrids) had 21% membership with *O. clarki* and the rest with *O. mykiss* (Table 6). Most individuals displayed over 95% membership in a single population (individual data not shown). In Table 6 we show the percentage of

membership in the two populations for individuals selected for the nuclear DNA marker analysis (see next paragraph). These were identified phenotypically as *O. mykiss* or *O. clarki* or as hybrids. Several individuals identified phenotypically as *O. mykiss* or *O. clarki* appeared to have mixed ancestry.

The selected individuals for further hybrid testing with the nuclear DNA markers (RFLP and SSR analyses) included all phenotypic hybrids, all phenotypic *O. clarki*, all phenotypic *O. mykiss* with estimated mixed microsatellite ancestry, and a representative sample of phenotypic *O. mykiss* with pure *O. mykiss* microsatellite profiles (see Table 6). In both RFLP and SSR techniques, the A patterns were identified in *O. mykiss* and the B patterns were identified in *O. clarki* (Baker et al. 2002; Ostberg and Rodriguez 2002). Results were mixed for the three phenotypic categories, *O. clarki*, *O. mykiss* and hybrids. Only eight phenotypic *O. clarki* had purely *O. clarki* genotypic patterns (Table 6). Ten phenotypic *O. clarki* had predominantly *O. mykiss* genotypic patterns (bold in Table 6). Eighteen phenotypic *O. clarki* had varying levels of microsatellite ancestry with at least one *O. mykiss* pattern at RFLP and SSR loci.

Nine of 21 phenotypic *O. mykiss* had purely *O. mykiss* genotypes, three had mixed microsatellite profiles and some *O. clarki* RFLP and SSR patterns, and the rest had *O. mykiss* microsatellite profiles and some *O. clarki* RFLP and SSR patterns (Table 6). Among fish identified phenotypically as potential hybrids, five had pure *O. mykiss* genotypic patterns. Hybrid 03BH0022 had a predominantly *O. clarki* microsatellite profile but *O. mykiss* RFLP and SSR patterns. The rest of the phenotypic hybrids had predominantly *O. mykiss* microsatellite profiles and at least one *O. clarki* RFLP or SSR pattern (Table 6). Of 89 adult trout sampled by angling below Landsburg Dam that had DNA data, 14.6% appeared to be hybrids with intermediate contributions from both species, and another 5.6% were potential hybrids with a low *O. clarki* contribution.

WHICHRUN

Smolt assignments: Although origins are uncertain for steelhead collected at Ballard Locks, they were a possible parent group for Cedar River smolts and were included in the baseline for the smolt assignment test. Genetic data suggested that 10 of the phenotypic *O. clarki* were genotypically *O. mykiss* (see Table 6). Thus the analysis was conducted with and without these genotypic *O. mykiss* within the Cedar River *O. clarki* group. If the genotypic *O. mykiss* were included with Cedar River *O. clarki*, all smolts were assigned to the putative *O. clarki* sample. If genotypic *O. mykiss* were excluded from the *O. clarki* sample, four smolts were assigned to below-dam *O. mykiss*, four were assigned to *O. clarki* and 12 were assigned to Ballard Locks steelhead (Table 7). The smolts assigned to *O. clarki* included 03BH0001, 03BH0005, 03BH0010 and 03BH0013. The first smolt had been identified phenotypically as *O. mykiss* but had some *O. clarki*-type microsatellite alleles (possible hybrid) and the other three had been identified phenotypically as *O. clarki* smolts.

Ballard steelhead assignments: All *O. mykiss* collections (including Ballard steelhead) were included in the baseline for estimating Ballard steelhead assignments. Most Ballard

steelhead were assigned back to their group, several assigned to Green River steelhead, two assigned to Cedar River below-Dam *O. mykiss* and one to above-Dam *O. mykiss* (Table 7). If Ballard steelhead were not included in the baseline and tested as fish of unknown origin, most of them assigned to Green River steelhead, and a few to Cedar below- and above-Dam *O. mykiss* (Table 7).

Cedar River comparisons with Ballard steelhead: An assignment test was conducted with only Cedar River *O. mykiss* and *O. clarki* and Ballard steelhead included in the baseline to examine relationships between anadromous and resident fish presumedly from the same drainage (Table 7). *O. mykiss* from below Landsburg Dam assigned more often to *O. mykiss* from above Landsburg Dam and to Ballard Locks steelhead than back to their group of origin. Above-dam *O. mykiss* had few assignments to Ballard Locks steelhead and more assignments to *O. mykiss* from below the dam (Table 7).

All Cedar River below-dam *O. mykiss*: For this assignment test we used phenotypic *O. clarki* identified genotypically as *O. mykiss* (from 03BJ sample) and the below-Landsburg Dam *O. mykiss* sample and treated them as fish of unknown origin. Resident below-dam *O. mykiss* showed a strong relationship with anadromous *O. mykiss* with 23 assigning to Green River and 5 to Ballard steelhead, and a strong relationship within Cedar River *O. mykiss* with 20 fish assigned to the above-Dam sample (Table 7). The resident phenotypic *O. clarki* with *O. mykiss* genotypes were similarly associated with Green River and Ballard steelhead (Table 7). No resident fish were assigned to the non-native hatchery rainbow trout population.

Biological characteristics

Steelhead sampled at Ballard Locks were about twice as large as adult resident *O. mykiss* sampled below Landsburg Dam, while above-Dam residents had the smallest average size among adults (Table 8a). Ballard Locks steelhead and below-dam resident adults had a relatively similar range of total age (Table 8a.) 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 8b).

Among the 53 resident adult *O. mykiss* sampled below the dam, 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, 584mm, fish) had spawned three times. Among the 50 *O. mykiss* sampled above the dam, 27 had readable scales, and three of these had been spawners. The average age of above-dam fish was only 2 years old (Table 8a) while the spawners were four or five years old, thus we may not have achieved an adequate sample of adults in this river section.

The average age of the clearly genetic hybrids was 3.25 (8 readable scales of 11 fish), and two of these fish had spawned once. Their average length was 388.5mm. Other potential hybrids had also spawned once and ranged in age from 2 to 6 years old. Among the 10

genotypic *O. mykiss* that had been visually identified as cutthroat 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 406.5mm. Average length of the 12 fish we think were pure cutthroat genetically was 233.3mm and only three of these could be aged, with one being a 5 year old that had spawned once.

DISCUSSION

Our first sampling season on the Cedar River has yielded informative and interesting results. To our knowledge, this is the first time wild adult steelhead and resident *O. mykiss* have been genetically characterized in this watershed. For steelhead, our 2003 sampling success was poor but we were fortunate to be able to obtain and utilize sampled steelhead scales from previous recent years. This situation exemplifies the importance of scale sampling fish under study or evaluation in case future work may benefit from some kind of genetic analysis. Properly sampled and preserved, scales are usually a good source of extractable DNA.

Regarding other sampling goals, we only acquired 17 *O. mykiss* smolts from the lower Cedar River trap versus the 50 targeted. This low sampling rate might be expected based on the few steelhead expected to have spawned in the previous 1 to 3 years. Having these few smolts limits the usefulness of their genetic data as a population sample, i.e. some population genetic statistics likely will not be accurate, but the data were useful for the factoral correspondence analysis, hybrid evaluations, and assignment tests.

Our cutthroat sample turned out to be much smaller than field-sampling data indicated originally. It appears we had only 12 genetic cutthroat among 32 fish sampled as cutthroat. This sample size is a major weakness for our analysis of hybridization and introgression because it is unlikely that we have a complete and accurate characterization of genetic diversity among microsatellite and nuclear species markers DNA loci in coastal cutthroat of this region. It is important for us in the coming year to sample and analyze at least 50 cutthroat that are both phenotypic and genetic cutthroat so that we can be highly confident in hybrid analysis results.

We think we have an excellent start at characterizing resident or non-anadromous *O. mykiss* in both Cedar River areas, given the 50 or higher sample sizes and the very high percent of samples that provided usable DNA. Both samples showed significant departures from Hardy-Weinberg genotypic equilibrium, which, discounting effects of hybridization, could be due to the range of broodyears within each sample. Relatively large, randomly mating populations are expected to be in genotypic equilibrium at gene loci unaffected by natural selection. Because each annual group of spawners is likely to be unique, broodyear differences in gene frequencies can be expected, and mixed broodyear samples may show disequilibrium. Analysis of 2004 samples that are currently being collected will allow us to evaluate the extent and source of disequilibrium. It is also possible that these samples contained mixtures of fish from genetically distinct and reproductively isolated populations, such as might occur if fish

produced upstream got below Landsburg Dam. Further sample analysis should help resolve this issue.

We did not attempt to acquire samples from any steelhead returning in 2004 and we plan to use data for the Ballard Locks steelhead sample presented in this report as our anadromous population baseline data set in continuing work. Our results showed that steelhead from prior Ballard Locks sampling were relatively similar genetically to Green River steelhead samples in terms of genetic diversity and genotypic distributions. Generally, we think that increasing sample size or adding to broodyear representation for the anadromous population is probably not necessary for the completion of this study. However, if other scales samples were to be located, we would like the opportunity to assess including them in further genetic data collection.

The among-sample comparative analyses showed clearly that Cedar River resident *O. mykiss* represented a native gene pool and were not a result of exotic hatchery trout introductions. They also were not closely related to the Chambers Creek-origin winter steelhead stock that is widely produced in Washington hatcheries. Above-Landsburg Dam adult *O. mykiss* shared the closest genetic relationship with below-dam resident *O. mykiss*. This would be expected if the ancestry of above-dam fish is primarily from native resident and juvenile *O. mykiss* that became landlocked. We did not have as many hybrids in the above-dam sample compared to below-dam, but we also did not sample putative cutthroat in the upper watershed. We plan to analyze cutthroat sampled by NOAA Fisheries staff from upper basin tributaries during 2004 lab analyses.

Although resident *O. mykiss* in Cedar River anadromous areas had a relatively distinctive genetic profile compared to that of Ballard Locks steelhead, they were closely allied with them. In assignment tests, a high percentage of below-dam residents were assigned to Ballard steelhead, indicating genotypic affinity. A nearly equal percentage was assigned to the above-dam population. It seems likely that both sources could be contributing to the resident or non-anadromous population below Landsburg Dam. In smolt assignment tests we found that 25% of *O. mykiss* smolts were estimated to originate from the below-dam population. Alternatively, we estimated that a smaller percentage of Ballard Locks steelhead originated from resident populations. These results indicate that resident *O. mykiss* are probably contributing to the anadromous population.

Ballard Locks steelhead showed the closest genetic relationship with wild Green River steelhead samples. Although this may be expected based on historical connection of Cedar and Green rivers, as well as previous genetic data from parr of both rivers, it also suggests the possibility that some fish appearing at the Ballard fish trap may not have been destined for the Cedar River. Without being able to sample adults on Cedar River spawning grounds, we can not definitively address the potential of 'wanderers' at the trap. We will try to evaluate this further through genetic and biological data in the future.

Smolt assignment tests did show that the majority of smolts were likely produced by the steelhead population. Assuming most smolts were two years old, their parents would have been 2001 spawners. Steelhead run-size in 2000-2001 was estimated at less than

50 fish. An estimated total abundance of Cedar River steelhead smolts in 2003 based on lower river trapping data will be available soon from WDFW Science Division staff.

Various test results indicate that resident adults are derived from native anadromous populations, and we think it is likely that gene flow occurs between the two life-history types, although we haven't explicitly tested for this yet. If interbreeding does occur, allelic distributions suggest that random mating is not the pattern. Assortative mating, perhaps based on size as shown in some salmonid studies (e.g. Hawkins 1997), and via temporal and spatial segregation, may be occurring, which would lead to and maintain genetically differentiated sub-populations. With the current low abundance of steelhead, we might expect the resident population's genetic trajectory to diverge more widely. The analysis of 2004 samples will improve our evaluation of resident and anadromous population relationships.

There were ample signals from genetic analyses of the putative cutthroat sample (e.g. large Hardy-Weinberg and linkage disequilibrium values) that it contained fish of more than one population or from population interbreeding. Finding hybrids in this sample was expected but we were surprised by the presence of genetic *O. mykiss* that, in the field, had enough phenotypic similarity to cutthroat that they were identified as such. It is possible that these fish had some genetic *O. clarki* influence from the past that we could not detect with the loci or the number of loci used. We want to analyze mitochondrial DNA markers and perhaps more nuclear DNA species markers in these fish and other hybrid candidates in upcoming lab analyses.

Although we had a small sample size for "pure" cutthroat, we found that at least five microsatellite DNA loci (*Ogo-3*, *Omm-1138*, *One-18*, *Ots-3m*, *Ots-103*) showed highly divergent allelic compositions between the two species, thus serving to some extent as species markers. Using the nuclear DNA species markers allowed us to assess whether fish were likely first generation hybrids or "back-crosses", i.e. hybrid by pure species matings. First generation and back-cross hybrids occurred in the putative cutthroat sample, the potential (phenotypic) hybrids samples and in phenotypic *O. mykiss*. The level of hybridization between *O. mykiss* and cutthroat found in all lower river resident fish sampled (at least 15% of these contained genes from both species) seemed fairly high although we have yet to compare this to any data from other studies. Our sampling strategy was not targeted at estimating actual hybridization level, but understanding this phenomenon may be important as it could have a significant survival effect on an *O. mykiss* population.

A tentative conclusive from our first year's work is that conservation of Cedar River resident *O. mykiss* populations is an important component of restoring the native steelhead resource. Both upper and lower basin populations appear to share the genetic legacy of local steelhead despite physical and biological perturbations to the watershed. Their situation bears a remarkable resemblance to adfluvial *O. mykiss* in the Bull Run subbasin (Sandy River, OR), which is Portland's water supply (Kostow 2003). Additional samples and extended analyses expected in 2004-2005 should significantly improve our data set and enable us to formulate management recommendations.

Figure 1. Multidimensional scaling plot of genetic distances among *O. mykiss* and *O. clarki* samples from Cedar River, Green River, Ballard Locks and Puyallup Hatchery. Abbreviations are as follows: Omy = *O. mykiss*; cutts = *O. clarki*; Keta HB = Keta Creek Hatchery wild broodstock; Hatch = hatchery; 01, 02, etc. = year of sample, except that 97 for Ballard Locks sample includes 97, 98, 99 and 03.

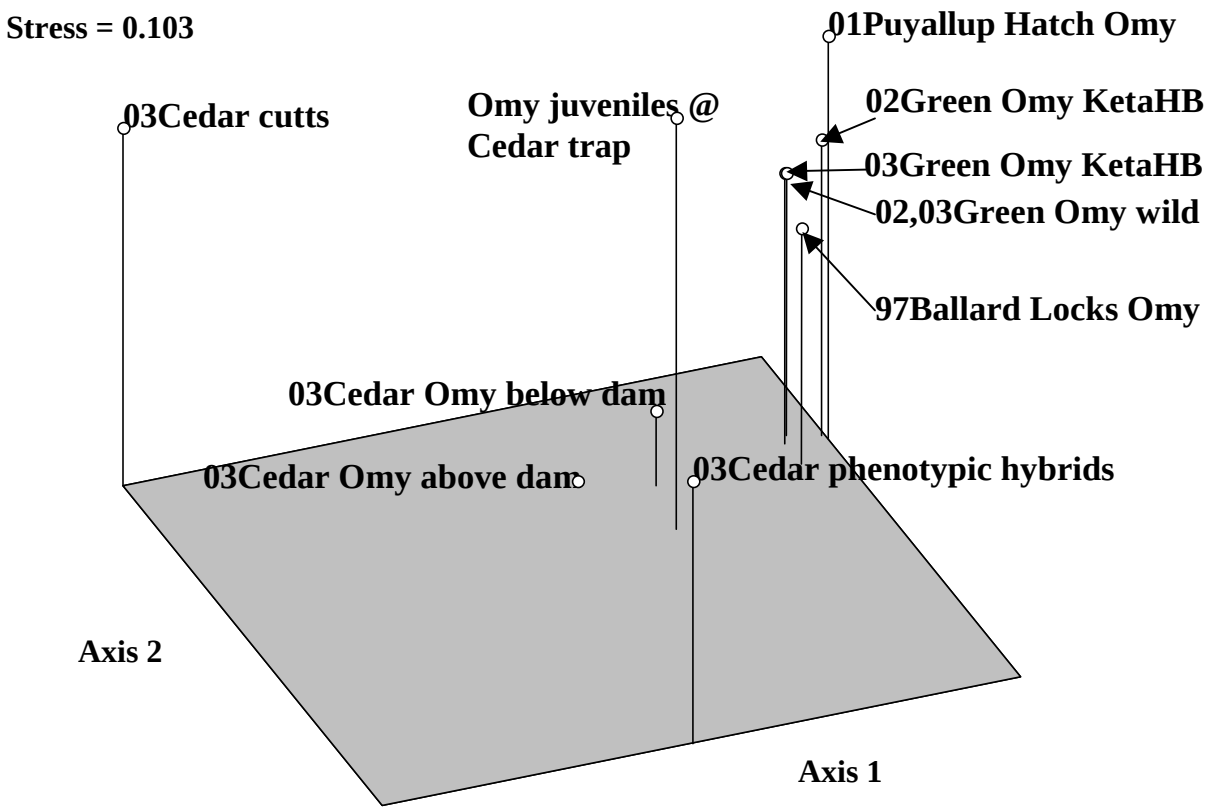

Figure 2. Principle components analysis of samples. Position of number next to the sample name is the position of the sample in the PCA plot. Abbreviations follow Figure 1.

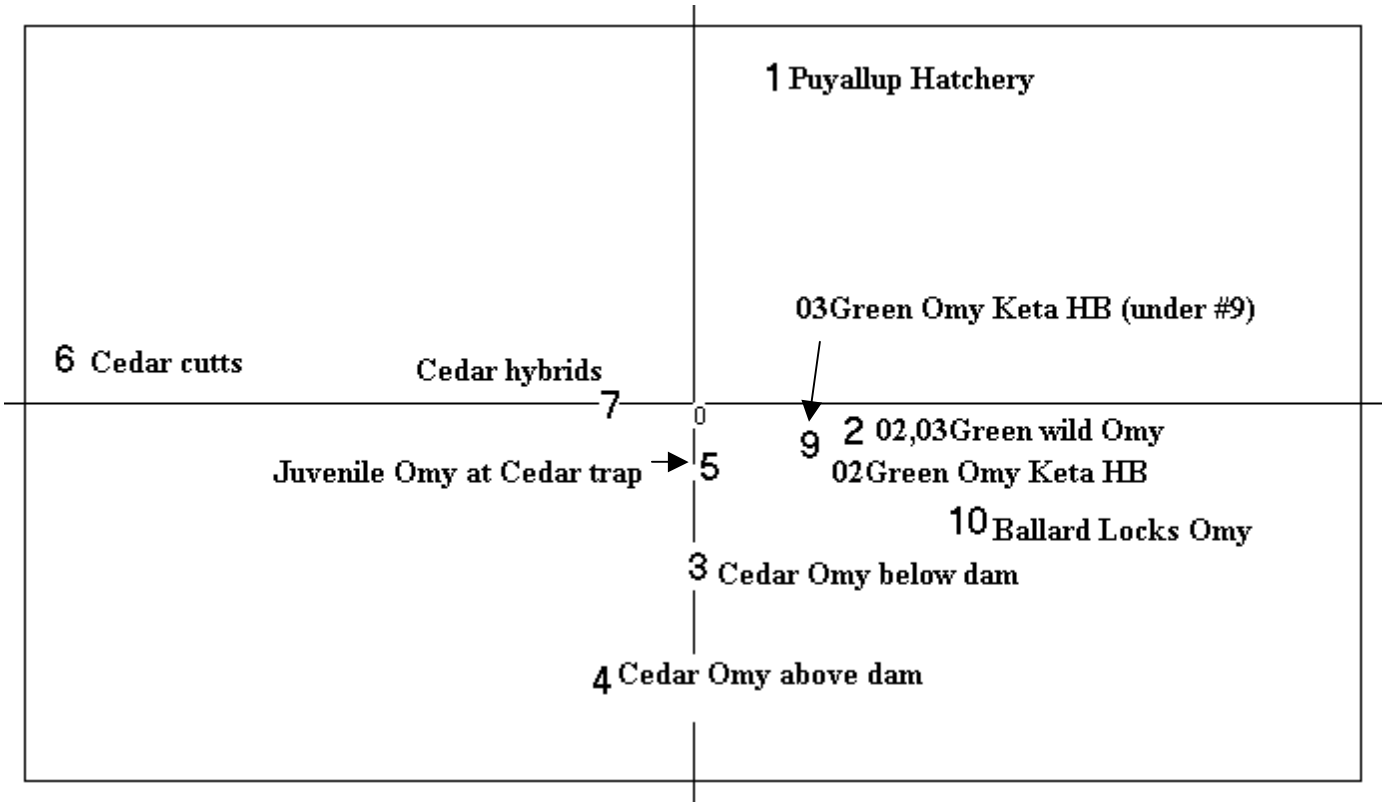

Figure 3. Factoral correspondence plot of individuals from Genetix. Circles were drawn around most individuals within a sample and circles were labeled since colors were difficult to discern. Abbreviations follow Figure 1.

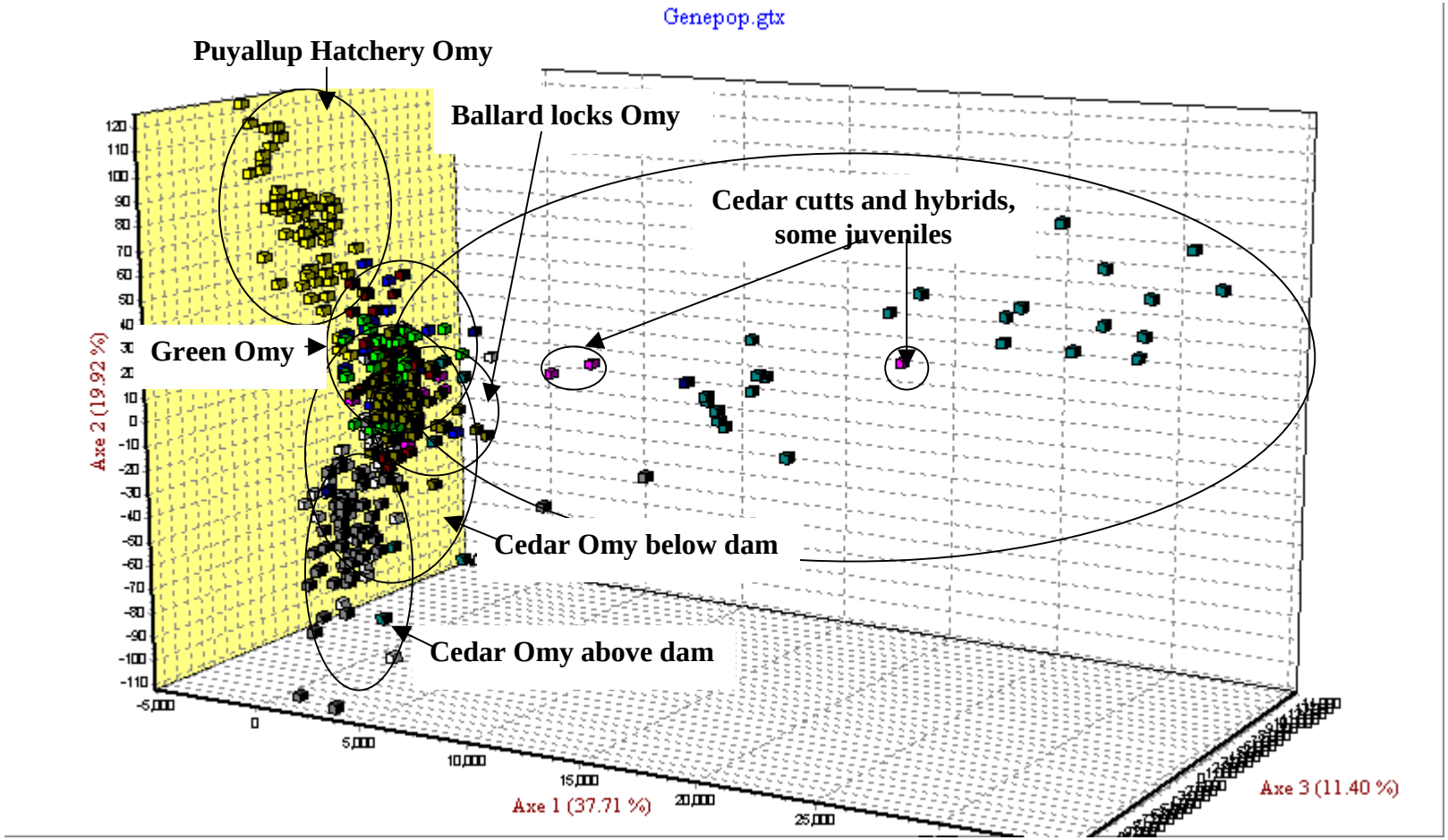

Figure 4. Factoral correspondence analysis plot of Cedar River and Green River samples in comparison to hatchery stocks and other *O. mykiss* (Omy) samples. The program repeated the colors for populations - not all cubes of the same color are for the same collection.

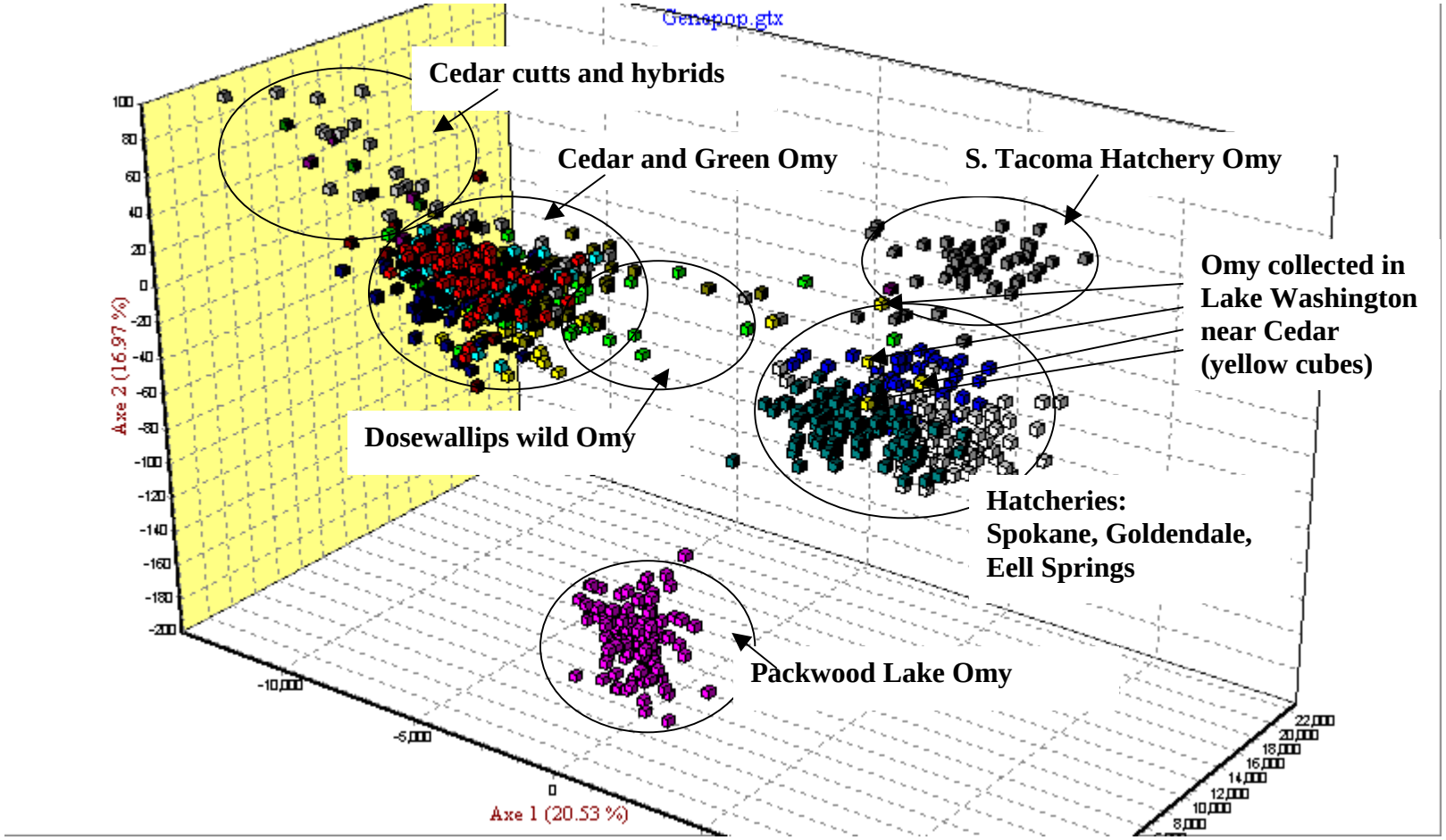

Table 1. Information for multiplexes and loci: number of alleles in this study, size range (in basepairs), observed heterozygosity (Hobs), repeat unit size (in basepairs), and *P*-value for deviation from Hardy-Weinberg equilibrium (HWE). Loci were tested for excesses of homozygotes using GENEPOP3.3 (Raymond and Rousset, 1995) with 100 batches and 2000 iterations. Values out of equilibrium are underlined. 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 $MgCl_2$, 0.05 units of *Taq* polymerase and 1X Promega PCR buffer. Number of PCR cycles for *O. mykiss* is under "cycles" with number of cycles for *O. clarki* following in parentheses. Dye concentration for *O. mykiss* is under "conc Omy" and for *O. clarki* under "conc Ocl". Two loci were dropped: *Omm-77* for Hardy-Weinberg disequilibrium (HWE), and *Omm-1070* for linkage with *Sco-103* (Link).

Multiplex	Anneal T	cycles	Locus 1	conc Omy [uM]	conc Ocl [uM]	Dye	#alleles	range	Hobs	repeat	HWE P	SE
Omy-B2	55	26 (32)	One-102	0.05	0.08	6fam	23	182-269	0.883	4	0	0
			One-114	0.05	0.1	vic	25	177-322	0.896	4	0.1076	0.017
			Ots-100	0.04	0.09	ned	24	168-224	0.860	2	0.0676	0.0106
Omy-C2	55	28 (32)	One-108	0.02	0.04	6fam	13	148-337	0.897	4	0.0048	0.0016
			Ots-103	0.015	0.025	vic	29	56-119	0.218	4	0.034	0.0017
			One-101	0.02	0.04	ned	7	119-230	0.521	4	0.0034	0.0007
Omy-D2	49	25 (29)	Ots-1	0.03	0.07	6fam	27	158-284	0.813	2	0	0
			Omy-77	0.03	0.03	vic	(HWE)	97-147	0.801			
			Ots-3M	0.02	0.02	ned	22	128-203	0.677	2	0.1814	0.0146
Omy-E2	62	26 (32)	Omm-1130	0.05	0.06	6fam	33	190-391	0.913	4	0.0932	0.0148
			Omm-1070	0.025	0.04	vic	(Link)	164-291	0.843			
			Omy-1011	0.045	0.06	ned	27	134-249	0.918	4	0	0
Omy-F2	52	25 (35)	Omy-1001	0.03	0.04	6fam	25	160-242	0.878	2	0.0176	0.0051
			Omm-1128	0.025	NA	vic	32	211-381	0.942	4	0.1131	0.02
			One-18	0.02	0.025	ned	9	170-186	0.766	2	0.2267	0.0104
			Oki-10	0.02	0.04	vic	16	97-160	0.628	2	0.0573	0.0067
			Ogo-3	0.06	0.06	ned	11	182-213	0.589	2	0.025	0.0074
Ocl-E2	50	35 (32)	Sco-110	0.12	0.08	6fam	26	150-263	0.827	2	0	0
			One-2	0.06	0.025	ned	45	193-293	0.909	2	0	0
Ocl-F2	50	35 (32)	Omm-1138	0.05	0.03	6fam	7	142-159	0.669	2	0.0002	0.0001
			Sco-103	0.1	0.035	vic	26	199-303	0.911	4	0	0
			Omy-325	0.08	0.04	ned	39	98-180	0.891	2	0	0

Table 2. Sample statistics from genetic analyses. If samples were pooled, two or more identification codes are presented in the code column. *O. mykiss* samples are shown as "Omy". N = the total number of individuals sampled. The number of sampled individuals that amplified at eight or more loci is shown under "worked > 8 loci", these were the fish employed in analyses. Observed and expected heterozygosity ('Hobs' and 'Hexp') were averaged over all loci using MSA (Dieringer and Schlotterer, 2003). Gene diversity (Gene Div) is expected heterozygosity corrected for sample size, calculated using FSTAT2.9.3 (Goudet 2001). Allelic richness (Richness) is number of alleles corrected for sample size, calculated using FSTAT2.9.3. The number of genotypic disequilibria (Dis) was calculated using GENEPOP 3.3 (Raymond and Rousset, 1995). Cedar River phenotypic hybrids (03BJh sample) was too small to calculate diversity, disequilibria or allelic richness (NA). Hardy-Weinberg equilibrium (HWE) was calculated using GENEPOP3.3, and the *P*(probability)-values (HWE *P*), followed by their standard errors (SE), indicate whether samples deviated significantly from Hardy-Weinberg expectations with an excess of homozygotes, significant values are underlined.

Sample (Year, location, identity)	Code	N	worked >8 loci	Hobs	Hexp	Gene Div	Richness	Dis	HWE *P*	SE
01Puyallup Hat. Omy (steelhead)	01GB	71	68	0.764	0.787	0.796	8.20	13	0	0
03Cedar River Cutthroat (below dam)	03BJ	35	34	0.692	0.848	0.861	9.94	8	0	0
03Cedar River phenotypic hybrids	03BJh	12	9	0.727	0.812	NA	NA	NA	0.0008	0.0003
03Cedar River Omy above dam	03BF	50	50	0.748	0.778	0.786	7.88	2	0.0002	0.0002
03Cedar River Omy below dam	03BD	53	48	0.750	0.790	0.803	8.71	0	0	0
03Cedar River smolts @ trap	03BH	20	18	0.738	0.796	0.791	7.90	1	0.0037	0.0012
02Green River Omy (Keta Hat. brdstk.)	02BI	40	38	0.733	0.764	0.780	8.17	4	0.0247	0.0082
03Green River Omy (Keta Hat. brdstk.)	03BK	43	42	0.736	0.773	0.791	8.52	1	0.0007	0.0004
02,03Green River Omy (steelhead)	02BJ+03BU = 03BUU	38	37	0.736	0.774	0.784	8.22	1	0	0
97-03Ballard Locks Omy (steelhead)	97ZY+98ZZ+99ZX+03AV = 97ZZZ	57	56	0.672	0.717	0.762	7.74	1	0	0
			400	0.730	0.784	0.795	8.360	4.222		

Table 3. Estimates of allelic associations within individuals within samples, "F_{IS}", for each sample. Under each sample is the F_{IS} value at each locus (values significant before Bonferroni correction are underlined; values in bold type were significant after correction), and last line shows the *P*-value for the F_{IS} value calculated over all loci in each sample. F_{IS} values were calculated using FSTAT2.9.3 (Goudet 2001) with 10,000 iterations. Abbreviations used: Hat. =hatchery; stlhd=steelhead; pheno.=phenotypic; brdstk=broodstock.

	01Puyallup Hat. stlhd.	03Cedar cutthroat	03Cedar pheno. hybrids	03Cedar above dam	03Cedar below dam	03Cedar smolts @ trap	02Green (Keta Hat. brdstk)	03Green (Keta Hat. brdstk)	02,03Green River	97-03Ballard locks steelhead
One-102	0.093	0.083	-0.098	0.068	0.153	-0.018	0.054	-0.06	0.116	0.091
One-114	-0.054	0.091	0.066	-0.005	0.047	-0.078	-0.118	0.074	0.172	-0.012
Ots-100	0.021	0.101	-0.133	-0.036	-0.002	-0.051	0.019	0.064	0.085	0.027
One-101	0.1	0.3	-0.391	-0.059	-0.065	-0.05	0.154	0.308	-0.129	0.259
One-108	0.029	0.07	-0.053	0.023	0.149	-0.047	0.069	0.112	-0.065	-0.028
Ots-103	0.136	0.359	0.439	-0.133	-0.072	0.36	-0.019	-0.014	-0.015	0.167
Ots-1	0.038	0.226	0.293	**0.317**	0.153	-0.054	0.259	0.158	0.219	0.132
Ots-3M	0.015	-0.048	0.091	-0.087	0.162	-0.055	0.139	0.168	-0.222	-0.019
Omm-1130	-0.07	0.155	-0.047	0.017	0.067	0.207	0.03	0.011	0.084	-0.022
Omy-1011	-0.017	**0.475**	0.084	0.003	0.131	0.091	-0.015	0.098	0.001	0.036
Oki-10	0.044	0.173	0.391	0.172	-0.004	-0.034	-0.158	-0.091	0.018	0.225
Omm-1128	0.026	0.064	0.153	-0.055	0.027	-0.064	0.011	-0.033	-0.022	-0.004
Omy-1001	0.109	0.06	-0.2	-0.016	-0.014	-0.067	-0.042	0.119	0.094	0.057
One-18	0.02	0.261	0.016	-0.018	-0.098	-0.117	0.055	-0.074	0.044	0.03
Ogo-3	0.018	**0.269**	0.032	0.101	-0.048	0.107	0.008	0.001	0.001	NA
One-2	0.045	**0.266**	0.111	0.047	0.041	0.167	-0.038	0.045	0.1	0
Sco-110	0.141	0.083	0.655	0.097	0.109	0.264	0.116	-0.012	0.15	0.085
Omm-1138	0.022	0.308	0.325	0.153	0.091	0.046	0.282	-0.006	0.121	0.064
Omy-325	-0.003	**0.478**	0.667	0.038	0.01	0.356	-0.002	0.071	0.006	**0.203**
Sco-103	0.02	0.054	-0.12	0.062	0.061	0.13	0.046	0.051	0.059	0.171
F_{IS} over all	0.03	0.172	0.103	0.037	0.058	0.051	0.043	0.054	0.052	0.075
F_{IS} P value	0.0062	0	0.0014	0.0035	0	0.0185	0.003	0.0002	0.0006	0

Table 4. Table of pairwise genotypic comparisons among *O. mykiss* (Omy) and *O. clark*i (cutthroat) study samples from Cedar and Green rivers, Ballard Locks, and Puyallup Hatchery. Lower matrix presents *P*(probability)-values for Chi-square tests of genotypic distributions summed over all loci using GENEPOP3.3 (Raymond and Rousset, 1995) with 300 batches and 2000 iterations. Where *P*-values are shown as "H. s.", the Chi-square value was infinity and the *P*-value was undefined – these comparisons were all highly significant. Upper matrix indicates the number of loci with significantly different genotypic distributions after Bonferroni corrections for multiple tests.

	03Cedar cutthroat	01Puyallup Hatchery	03Cedar above dam	03Cedar below dam	03Cedar smolts	02Green Keta	03Green Keta	02,03 Green	Ballard steelhead
03Cedar Cutthroat	-	19	14	13	7	18	14	16	18
01Puyallup Hat.	H. s.	-	18	15	11	10	14	11	18
03Cedar Omy above	H. s.	H. s.	-	0	8	12	10	10	13
03Cedar Omy below	H. s.	H. s.	0.00096	-	1	3	1	3	2
03Cedar smolts	H. s.	H. s.	H. s.	0	-	1	1	1	1
02Green (Keta brdstk)	H. s.	H. s.	H. s.	H. s.	0	-	0	1	2
03Green (Keta brdstk)	H. s.	H. s.	H. s.	0	0	0.01553	-	0	0
02,03 Green steelhead	H. s.	H. s.	H. s.	0	0	0.0875	0.37282	-	1
Ballard Locks steelhead	H. s.	H. s.	H. s.	H. s.	0	0.00019	0	0.00001	-

Table 5. Results from STRUCTURE 2.1 analysis with two populations hypothesized. The percentage of ancestry in each hypothetical population is indicated for four sample groups.

	Pop 1	Pop 2	N
Cedar *O. clarki*	0.349	0.651	35
Green *O. mykiss*	0.994	0.006	118
Cedar *O. mykiss*	0.949	0.051	98
Cedar hybrids	0.791	0.209	9

Table 6. Hybrid analysis results including phenotype (*O. clarki* = cutt, *O. mykiss* = rbow, and hybrid = hyb), microsatellite DNA ancestry estimated using STRUCTURE 2.1, and nuclear DNA marker (RFLP and SSR) genotypes. Individual identification code (see Table 1) shown in Sample column. Under "msat ancestry" is the estimated percentage of *O. mykiss* and *O. clarki* ancestry. Under RFLP are the patterns identified by Baker et al. (2002) for ITS, GnRH, and p53-7. Under SSR are patterns identified by Ostberg and Rodriguez (2002) for Occ-16, Omm-28 and Omm-35. 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 two studies cited above. Individuals with pure genotypes matching phenotype are shown with *, phenotypic *O. clarki* with pure *O. mykiss* genotypes in bold type, phenotypic hybrids with pure *O. mykiss* genotypes are underlined. Microsatellite ancestry over 75% in either *O. mykiss* or *O. clarki* is identified by shading (green highlighting) in the cell.

		msat ancestry		RFLP Pattern, JB			SSR Pattern, CO		
Sample	**Phenotype**	**rbow**	**cutt**	**ITS**	**GnRH**	**p53-7**	**OCC-16**	**OMM28**	**OMM-35**
03BH0013*	cutt	0.001	0.999	BB	BB	BB	BB	BB	
03BJ0005	cutt	0.001	0.999	BB	AB	BB	BB	BB	
03BJ0035*	cutt	0.001	0.999	BB	BB	BB	BB	BB	
03BJ0065	cutt	0.001	0.999	BB	AB	BB	BB	BB	AA
03BJ0067	cutt	0.001	0.999	BB	AA	BB'	BB	BB	
03BJ0069*	cutt	0.001	0.999	BB	BB	BB'	BB	BB	
03BJ0024*	cutt	0.002	0.998	BB		BB	BB	BB	
03BJ0032	cutt	0.002	0.998	BB	AB	BB'	BB	BB	
03BJ0034*	cutt	0.002	0.998	BB	BB	B'B'	BB	BB	
03BJ0075*	cutt	0.002	0.998	BB	BB	B'B'	BB		
03BJ0077*	cutt	0.002	0.998	BB		BB'	BB	BB	
03BJ0010*	cutt	0.003	0.997	BB	BB	BB	BB	BB	
03BJ0026*	cutt	0.005	0.995	BB	BB	BB	AB	BB	
03BJ0006	cutt	0.015	0.985	AB	AB	BB'	AB	AB	
03BJ0011	cutt	0.016	0.984	AB	AB	BB'	AB	AB	AB
03BJ0004	cutt	0.025	0.975	AB	AB	BB'	AB	AB	
03BJ0002	cutt	0.036	0.964	AB	AB	BB'	AB		AA
03BJ0068	cutt	0.077	0.923	AB	BB	AB'	AB	AB	AA
03BJ0028	cutt	0.089	0.911				AB		
03BJ0003	cutt	0.092	0.908	AB	AB	AB'	AB	AB	AA
03BJ0066	cutt	0.173	0.827	BB	AA'	AB	AB	AB	AA
03BH0005	cutt	0.273	0.727	AB	AB	AB	AB	AB	AA
03BJ0076	cutt	0.302	0.698	AB	AB	AB'	AB	AB	AA
03BJ0072	cutt	0.399	0.601	AB	AB	BB'	AB	AB	AB
03BJ0007	cutt	0.765	0.235	AA	AA	AB'	AA	AA	AA
03BJ0009	cutt	0.984	0.016	AA	AA	AB'	AA	?A	AA
03BJ0074	cutt	0.991	0.009	AA	AA'	AA		AA	AA

Table 6. cont.

		msat ancestry		RFLP Pattern, JB			SSR Pattern, CO		
Sample	**Phenotype**	**rbow**	**cutt**	**ITS**	**GnRH**	**p53-7**	**OCC-16**	**OMM28**	**OMM-35**
03BJ0071	cutt	0.995	0.005	AA		AA	AA	AA	AA
03BJ0022	cutt	0.996	0.004	AA	AA	AA	AA	AA	AA
03BJ0025	cutt	0.997	0.003	AA	AA	AA	AA	AA	AA
03BJ0008	cutt	0.998	0.002	AA	AA	AB'	AA	AA	AA
03BJ0064	cutt	0.998	0.002	AA	AA	AA	AA	AA	AA
03BJ0070	cutt	0.998	0.002	AA	AB	AA	AA	AA	AA
03BJ0027	cutt	0.999	0.001	AA	AA	AA	AA		AA
03BH0022	hyb	0.144	0.856	AA	AA	AA	AA	AA	AA
03BJ0001	hyb	0.173	0.827	AB	AB	AB'	BB	AB	AA
03BJ0073	hyb	0.829	0.171	AA	AB	AB'	AA	AA	AA
03BJ0060	hyb	0.994	0.006	AA	AA	AA	AA	AA	AA
03BJ0021	hyb	0.995	0.005	AB	AA	AA	AA	AA	AA
03BJ0031	hyb	0.995	0.005	AA	AA	AB'	AA	AA	AA
03BJ0023	hyb	0.997	0.003	AA	AA	AA	AA	AA	AA
03BJ0029	hyb	0.997	0.003	AA	AA	AB'	AA	AA	AA
03BJ0013	hyb	0.998	0.002	AA	AA	AA	AA	AA	AA
03BJ0061	hyb	0.998	0.002	AA	AA	AA	AA	AA	AA
03BD0038	rbow	0.357	0.643	AB	AB	AA	AB	AB	AA
03BF0034	rbow	0.43	0.57	AB	AB	AB'	AB	AB	AA
03BD0010	rbow	0.668	0.332	AB	A'A'	AA	AB	AB	AA
03BU0001*	rbow	0.85	0.15	AA	AA	AA	AA	AA	AA
03BK0013*	rbow	0.975	0.025	AA	AA	AA	AA	AA	AA
03BK0007*	rbow	0.987	0.013	AA	AA	AA	AA	AA	AA
02BI0004	rbow	0.991	0.009	AA	AA	AB'	AA	AA	AA
03BF0013	rbow	0.992	0.008	AB	AA	AA	AA		AB
03BK0008	rbow	0.993	0.007	AA	AA	AA	AB	AA	AA
02BI0010*	rbow	0.996	0.004	AA	AA'	AA	AA	AA	AA
03BK0002*	rbow	0.996	0.004	AA	AA	AA		AA	AA
03BK0012*	rbow	0.996	0.004	AA	AA	AA	AA	AA	AA
03BK0003	rbow	0.997	0.003	AA	AA	AA	AB	AA	AA
03BK0005	rbow	0.997	0.003	AA	AA	AA	AB	AA	AA
02BI0001*	rbow	0.998	0.002	AA	AA	AA	AA	AA	AA
02BI0002*	rbow	0.998	0.002	AA	AA	AA	AA	AA	AA
02BI0003	rbow	0.998	0.002	AA	AA'	AA	AA		AB
02BI0007	rbow	0.998	0.002	AA	AA	AA	AA		AB
02BI0008*	rbow	0.998	0.002	AA	AA		AA	AA	AA
02BI0009	rbow	0.998	0.002	AA	AA	AB'	AA		AA
03BK0014	rbow	0.998	0.002	AA	AA	AA	AA	AA	AB

Table 7. WHICHRUN results for assignment tests. The first test ("Smolts") examined relationships of Cedar River smolts to Cedar River *O. mykiss* and *O. clarki* (cutthroat minus genotypic *O. mykiss*) and Ballard Locks steelhead. The second test ("Ballard steelhead") included all *O. mykiss* samples and *O. clarki* in the baseline. In the third test ("Ballard, not in baseline) Ballard steelhead were assigned without the Ballard sample in the baseline. The fourth test ("Cedar and Ballard") included Cedar River *O. mykiss* and *O. clarki* and Ballard Locks steelhead in the baseline and calculated assignments for all fish. The fifth test ("Below Landsburg Dam") assigned *O. mykiss* (Below Dam Omy) and the below-dam phenotypic *O. clarki* that were genotypic *O. mykiss* ("Cedar Ocl phen, Omy geno"), treating these fish as unknowns (not included in baseline) for assignments.

Smolts

	Below Landsburg Dam	Above Landsburg Dam	Ballard steelhead	cutthroat
Individuals	4	0	12	4

Ballard steelhead

	Below Landsburg Dam	Above Landsburg Dam	Ballard steelhead	cutthroat	Green River
Individuals	2	1	33	0	20

Ballard, not in baseline

	Below Landsburg Dam	Above Landsburg Dam	cutthroat	Green River
Individuals	5	4	0	47

Cedar and Ballard

	Below Landsburg Dam	Above Landsburg Dam	Ballard steelhead	cutthroat
Below Landsburg Dam	11	19	18	0
Above Landsburg Dam	13	33	2	2
Ballard Locks steelhead	5	1	49	0
cutthroat	0	0	0	22

Below Landsburg Dam

	Green River	Above Landsburg Dam	Ballard steelhead	Puyallup Hat	Spokane Hat
Below Dam Omy	23	20	5	0	0
Cedar Ocl phen, Omy geno	4	3	2	1	0

Table 8a. Fork-Length and age of *O. mykiss* in Cedar River (below and above Landsburg Dam) and Ballard Locks samples used for genetic data analysis.

Sample Population	**Ave. Length cm (N)**	**Ave. Age (range)**
Steelhead- Ballard Locks	72.7 (53)	see Table 8b (3 – 6)
Below-dam *O. mykiss*	35.7 (53)	3.6 (2 – 8)
Above-dam *O. mykiss*	24.3 (50)	2.0 (1 – 5)
Smolts	18.7 (17)	n/a

Table 8b. 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

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Appendix I. Twenty microsatellite DNA loci allelic data for 10 samples (codes in Table 2). Allele sizes are indicated in basepairs above columns and allele counts are in rows.

One-102	182	185	188	192	196	200	204	208	212	216	221	225	229	233	237	241	245	249	253	257	261	269	
01GB	0	0	0	12	9	14	0	27	5	3	3	9	20	9	1	1	0	4	2	5	6	0	130
02BI	0	0	1	2	6	0	1	26	9	1	1	2	12	5	5	0	0	0	0	1	2	0	74
03BD	0	0	2	9	11	3	3	7	7	2	1	1	8	1	1	0	0	0	0	1	3	0	60
03BF	0	0	3	7	24	0	3	8	16	3	3	3	15	1	0	1	0	0	0	1	0	0	88
03BH	0	0	1	2	5	0	2	3	4	5	0	2	4	0	3	0	0	0	0	1	0	0	32
03BJ	0	0	4	12	14	0	2	6	8	4	0	2	5	5	1	0	1	1	0	0	1	0	66
03BJh	1	0	0	1	3	1	0	2	3	1	0	0	3	0	0	0	0	0	0	0	0	1	16
03BK	0	0	0	7	7	1	1	13	11	2	0	3	15	2	5	0	1	1	0	3	2	2	76
03BUU	0	0	0	3	7	2	4	15	13	2	0	2	14	2	3	0	0	0	0	2	1	0	70
97ZZZ	0	2	1	2	21	3	2	27	16	1	1	4	17	2	3	1	0	0	0	2	0	1	106
Total	1	2	12	57	107	24	18	134	92	24	9	28	113	27	22	3	2	6	2	16	15	4	718

One-114	177	185	189	193	197	201	205	209	213	217	221	225	229	233	236	240	244	248	252	256	276	285	289	292	
01GB	0	2	15	12	17	22	15	7	11	1	1	6	0	10	1	1	0	0	5	0	1	2	1	0	130
02BI	0	0	7	13	1	6	20	5	3	6	0	5	2	3	2	0	0	0	0	1	0	0	0	0	74
03BD	0	0	2	8	5	3	18	2	5	7	1	2	1	1	3	2	1	2	3	0	0	0	0	0	66
03BF	0	0	1	4	10	9	18	3	0	1	1	3	2	0	3	3	10	5	10	0	0	0	0	3	86
03BH	0	0	5	7	1	0	8	5	0	2	1	1	0	0	0	0	1	1	0	0	0	0	0	0	32
03BJ	1	3	4	12	9	12	8	3	3	3	3	1	0	2	1	1	0	0	1	1	0	0	0	0	68
03BJh	0	1	0	3	2	0	2	2	0	1	2	0	1	2	1	0	0	0	0	1	0	0	0	0	18
03BK	1	0	12	15	1	4	16	4	7	3	5	4	2	1	2	0	0	1	0	2	0	0	0	0	80
03BUU	0	2	6	11	4	10	13	6	9	2	1	3	1	1	1	0	0	0	0	0	0	0	0	0	70
97ZZZ	0	1	5	28	11	5	15	12	3	5	8	1	1	2	4	0	1	0	1	1	0	0	0	0	104
Total	2	9	57	113	61	71	133	49	41	31	23	26	10	22	18	7	13	9	20	6	1	2	1	3	728

Ots-100	168	173	175	177	179	181	183	185	187	189	191	195	197	199	201	203	205	207	209	211	213	215	222	224	
01GB	50	17	0	8	0	4	0	2	20	3	11	3	0	0	2	0	3	0	1	0	6	0	0	0	130
02BI	24	6	0	9	0	2	0	2	1	4	7	0	1	4	9	0	1	0	3	0	3	0	0	0	76
03BD	18	3	0	3	0	1	0	3	4	0	9	2	0	10	2	0	1	5	2	1	0	2	0	0	66
03BF	25	3	1	4	0	0	0	2	3	0	7	3	0	18	8	3	1	1	0	1	0	6	0	0	86
03BH	6	5	0	0	0	3	0	1	2	0	4	0	0	1	6	0	0	0	1	0	3	0	0	0	32
03BJ	10	2	5	6	1	0	10	1	0	0	8	1	0	3	2	1	1	3	0	3	0	0	8	5	70
03BJh	7	5	1	0	0	0	0	0	1	0	2	0	1	0	0	0	0	1	0	0	0	0	0	0	18
03BK	20	8	0	9	0	6	0	3	4	0	12	1	1	4	8	0	1	0	2	0	3	0	0	0	82
03BUU	18	10	0	7	0	1	0	1	9	1	8	1	0	5	5	0	0	0	1	0	3	0	0	0	70
97ZZZ	32	12	0	4	0	4	0	7	5	0	16	0	0	7	10	0	2	0	1	0	2	0	0	0	102
Total	210	71	7	50	1	21	10	22	49	8	84	11	3	52	52	4	10	10	11	5	20	8	8	5	732

One-101	119	123	127	131	136	140	153	157	162	170	174	226	230	
01GB	53	0	54	1	0	0	0	0	0	0	0	0	0	108
02BI	46	0	20	0	0	0	0	0	0	0	0	0	0	66
03BD	51	0	31	0	0	0	0	2	0	2	4	0	0	90
03BF	44	1	42	0	0	0	0	0	1	0	6	0	0	94
03BH	21	0	9	0	0	0	0	0	0	0	0	0	0	30
03BJ	15	1	34	0	1	5	1	1	0	0	2	1	1	62
03BJh	3	0	6	0	0	0	0	0	0	0	1	0	0	10
03BK	34	0	34	0	0	0	0	0	0	0	0	0	0	68
03BUU	34	0	25	2	0	0	1	0	0	0	0	0	0	62
97ZZZ	81	0	30	0	0	0	1	0	0	0	0	0	0	112
Total	382	2	285	3	1	5	3	3	1	2	13	1	1	702

One-108	148	152	156	161	164	169	173	177	181	185	189	193	197	201	205	209	213	217	221	225	229	233	237	241	244	249	261	329	337	
01GB	0	0	0	3	20	0	0	0	4	12	4	16	10	14	10	4	0	8	0	0	1	0	2	0	6	0	0	0	0	114
02BI	0	0	0	0	4	0	0	1	4	4	11	13	9	7	2	11	1	0	0	0	0	0	1	0	0	0	0	0	0	68
03BD	0	0	1	0	0	1	0	2	2	8	6	14	23	11	1	2	4	0	2	1	0	0	1	5	2	0	0	0	0	86
03BF	1	0	2	1	0	6	0	1	13	13	14	9	23	8	3	3	0	0	0	0	0	0	0	1	0	0	0	0	0	98
03BH	0	1	0	0	0	0	0	1	3	5	5	1	6	1	0	5	1	0	1	0	0	0	0	0	0	0	0	0	0	30
03BJ	10	14	7	1	5	2	3	0	4	2	4	1	3	3	1	2	0	0	0	1	0	0	1	0	0	1	0	1	0	66
03BJh	1	0	0	0	0	0	0	0	0	2	0	2	2	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	1	12
03BK	0	0	0	0	3	0	0	6	4	10	13	5	5	8	6	7	0	3	0	0	1	2	1	0	1	0	1	0	0	76
03BUU	0	0	0	0	5	0	0	1	5	9	13	6	14	8	0	3	0	1	2	0	0	0	0	0	3	0	0	0	0	70
97ZZZ	0	0	0	0	0	0	0	11	12	8	27	15	18	5	5	9	1	0	0	0	0	1	0	0	0	0	0	0	0	112
Total	12	15	10	5	37	9	3	23	51	73	97	82	113	66	28	47	7	12	5	3	2	3	6	7	12	1	1	1	1	732

Ots-103	56	60	74	78	82	86	119	
01GB	0	0	1	7	119	1	2	130
02BI	0	0	0	2	69	0	1	72
03BD	2	2	0	6	84	0	0	94
03BF	1	2	0	12	85	0	0	100
03BH	0	1	0	2	29	0	0	32
03BJ	3	36	0	2	25	0	0	66
03BJh	0	3	0	0	14	1	0	18
03BK	0	0	0	2	74	0	0	76
03BUU	0	0	0	2	66	0	0	68
97ZZZ	0	0	1	8	99	0	4	112
Total	6	44	2	43	664	2	7	768

Ots-1	158	162	164	166	168	170	177	179	181	183	237	241	243	245	247	249	258	260	262	264	266	268	272	276	278	284	
01GB	0	0	0	35	1	4	5	14	0	3	0	5	18	18	4	6	0	0	0	1	0	0	0	0	0	0	114
02BI	0	1	0	25	0	5	7	1	0	0	0	6	6	13	6	2	0	0	0	0	0	0	0	0	0	0	72
03BD	1	2	2	29	5	7	5	1	0	0	0	5	6	7	18	2	0	0	0	0	0	0	0	0	0	0	90
03BF	0	1	6	29	13	4	0	0	0	0	0	7	1	9	23	0	1	0	1	0	0	0	0	1	0	0	96
03BH	0	0	0	11	0	2	1	4	0	0	0	1	2	0	4	0	0	0	0	0	0	0	0	1	0	0	26
03BJ	0	2	3	16	2	3	0	0	0	0	2	2	0	3	1	0	0	15	2	2	1	1	4	9	1	1	70
03BJh	1	0	0	4	0	4	0	3	0	0	0	1	0	0	2	0	0	1	0	0	0	0	0	0	0	0	16
03BK	0	0	0	38	0	5	2	0	1	0	0	4	7	7	9	1	0	0	0	0	0	0	0	0	0	0	74
03BUU	0	0	0	29	0	6	3	2	0	2	0	4	0	3	11	0	0	0	0	0	0	0	0	0	0	0	60
97ZZZ	0	2	0	34	0	7	9	0	1	3	0	13	3	15	9	2	0	0	0	0	0	0	0	0	0	0	98
Total	2	8	11	250	21	47	32	25	2	8	2	48	43	75	87	13	1	16	3	3	1	1	4	11	1	1	716

Ots-3M	128	132	134	136	138	140	145	147	152	156	160	164	166	168	172	174	176	178	182	190	194	203	
01GB	0	0	11	38	16	39	0	0	8	3	0	0	1	0	0	0	0	0	0	0	0	0	116
02BI	0	0	1	36	23	7	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	72
03BD	2	1	6	50	21	5	0	1	2	0	0	0	0	1	1	0	0	0	0	0	0	0	90
03BF	1	1	1	46	44	3	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	98
03BH	0	0	0	15	7	3	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	28
03BJ	4	0	0	14	9	9	1	1	1	0	1	2	0	1	7	8	5	2	1	0	1	1	68
03BJh	0	0	1	8	5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
03BK	1	0	0	37	32	5	0	2	5	0	0	0	0	0	0	0	0	0	0	0	0	0	82
03BUU	0	0	1	35	20	2	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	62
97ZZZ	3	0	3	51	30	6	2	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Total	11	2	24	330	207	80	5	7	29	3	1	3	1	2	8	8	5	2	1	1	1	1	732

Omm-1130	190	202	204	206	208	210	212	214	216	218	220	222	224	228	232	236	240	244	248	252	256	260	264	268	272	276	280	284	292	296
01GB	0	0	12	0	1	0	4	0	7	0	14	0	9	2	30	6	2	13	7	2	3	4	4	8	0	0	1	0	0	0
02BI	0	0	0	0	2	0	3	0	6	0	1	0	2	2	17	3	4	5	7	3	3	2	2	2	1	1	1	0	0	0
03BD	0	0	0	0	0	0	3	0	6	0	6	0	2	0	15	8	14	11	7	0	10	2	1	0	2	1	0	0	1	1
03BF	0	0	0	1	0	1	3	0	3	0	3	0	0	2	12	10	23	10	13	5	4	0	3	0	2	2	0	0	0	3
03BH	0	0	0	0	0	0	0	0	1	0	0	0	0	5	3	10	1	4	1	1	1	2	0	1	0	0	1	0	0	1
03BJ	1	6	0	13	0	1	0	7	1	4	0	2	1	0	6	5	4	0	3	0	4	1	0	2	0	0	0	0	0	1
03BJh	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	3	1	2	0	0	0	0	1	2	0	1	0	0	0	0
03BK	0	0	3	0	0	0	4	0	2	0	3	0	0	3	9	7	6	6	11	5	5	1	5	1	1	0	0	0	0	0
03BUU	0	0	0	0	0	0	4	0	7	0	0	0	0	4	11	6	3	4	7	6	4	2	3	3	1	0	0	1	0	0
97ZZZ	0	0	0	0	0	0	5	0	12	0	2	0	2	3	20	13	3	7	14	3	9	4	1	1	5	0	0	0	0	0
Total	1	6	15	15	3	2	27	7	46	4	29	2	17	22	124	71	61	62	70	25	43	18	20	20	12	5	3	1	1	6

Omy-1011	134	138	143	147	151	155	159	163	167	171	175	179	183	185	187	191	195	199	203	206	214	218	221	226	230	241	249	
01GB	0	14	0	11	5	13	11	0	9	3	4	14	6	0	31	7	0	1	0	0	4	0	1	0	0	0	0	134
02BI	0	9	1	6	5	2	0	0	4	8	5	1	3	0	6	4	1	0	3	0	3	0	9	0	0	0	0	70
03BD	0	16	0	6	2	0	8	2	4	10	1	2	0	0	19	4	2	0	1	0	6	0	6	0	0	0	1	90
03BF	2	25	0	6	2	0	13	2	3	5	0	3	2	0	8	4	1	0	3	0	16	0	5	0	0	0	0	100
03BH	0	6	2	4	1	2	0	0	2	0	0	1	1	0	1	2	4	0	0	0	5	0	1	0	0	0	0	32
03BJ	0	6	0	1	0	0	3	5	3	2	0	1	3	1	3	0	2	8	1	1	1	1	4	3	1	1	1	52
03BJh	0	1	0	1	1	0	2	0	1	1	2	0	0	0	3	1	0	0	0	0	2	0	1	0	0	0	0	16
03BK	0	14	2	3	4	7	2	0	3	3	1	8	1	0	7	3	2	1	1	0	5	0	4	1	0	0	0	72
03BUU	0	6	1	5	4	1	2	1	7	3	4	7	3	0	8	4	4	0	1	0	2	0	2	1	0	0	0	66
97ZZZ	0	15	1	12	5	0	6	0	9	5	2	1	0	0	13	5	6	0	6	0	7	0	12	1	0	0	0	106
Total	2	112	7	55	29	25	47	10	45	40	19	38	19	1	99	34	22	10	16	1	51	1	45	6	1	1	2	738

Oki-10	97	99	101	103	105	109	113	117	121	125	129	137	142	151	156	160	
01GB	7	55	6	16	0	2	12	0	0	19	8	3	0	1	0	1	130
02BI	2	48	1	4	1	14	1	0	0	2	0	0	0	0	1	2	76
03BD	8	42	14	0	0	16	0	6	0	0	0	0	0	0	0	0	86
03BF	12	46	18	0	0	14	0	6	1	1	0	0	0	0	0	0	98
03BH	1	23	1	0	0	2	0	2	0	1	0	0	0	0	0	2	32
03BJ	0	15	1	0	2	27	3	7	5	5	1	1	0	0	0	1	68
03BJh	0	10	2	0	0	2	0	2	0	0	0	0	0	0	0	0	16
03BK	5	54	4	1	0	11	1	2	0	2	2	1	0	0	1	0	84
03BUU	3	43	1	2	1	13	1	3	0	3	0	1	1	0	1	1	74
97ZZZ	0	78	4	1	0	19	0	2	0	4	0	0	0	0	0	2	110
Total	38	414	52	24	4	120	18	30	6	37	11	6	1	1	3	9	774

Omm-1128	211	215	219	223	227	231	235	239	243	247	250	255	259	263	267	271	275	279	282	286	290	295	300	304	308	312	316	320	325	329
01GB	0	0	4	7	6	11	3	5	4	3	2	22	3	5	3	1	3	1	7	3	7	17	4	1	1	2	0	0	0	1
02BI	0	0	0	2	3	2	4	2	1	5	1	4	1	2	6	1	0	1	5	8	6	14	4	1	2	0	1	0	0	0
03BD	0	1	0	5	5	3	6	3	1	2	1	3	1	4	0	4	3	3	3	5	5	9	5	0	8	0	0	0	0	0
03BF	0	1	0	0	6	10	2	5	0	2	0	2	4	13	2	0	8	1	6	9	3	3	3	2	13	0	0	1	0	0
03BH	0	1	0	1	2	1	0	0	0	0	1	0	2	2	0	0	1	1	2	5	4	3	4	0	1	0	0	0	0	1
03BJ	4	12	1	0	0	1	1	0	6	2	0	0	0	2	1	1	3	1	4	4	1	3	2	0	3	1	1	7	1	0
03BJh	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2	1	0	1	0	2	1	0	0	0	0	0	0	1
03BK	0	0	1	10	8	4	2	4	1	1	2	0	5	4	5	0	1	5	3	3	3	8	2	2	1	1	0	0	0	0
03BUU	0	0	1	3	4	5	7	2	3	1	1	2	1	5	1	2	1	2	3	6	4	9	3	3	4	0	0	0	0	0
97ZZZ	0	0	0	1	12	7	5	6	1	1	0	3	7	2	4	6	0	12	2	10	2	11	4	3	3	1	1	0	0	1
Total	4	16	7	29	46	44	30	28	17	17	8	36	24	39	23	15	22	28	35	54	35	79	32	12	36	5	3	8	1	4

Omy-1001	160	175	177	179	181	183	185	187	191	193	195	198	199	202	204	206	208	210	212	216	220	222	224	238	242	
01GB	0	4	0	29	2	10	3	10	24	32	1	0	3	0	0	3	1	0	0	0	0	0	3	3	0	128
02BI	0	3	0	11	0	9	3	9	6	4	13	0	6	1	1	1	1	2	0	0	5	0	1	0	0	76
03BD	0	4	0	17	0	7	0	19	4	2	13	4	7	1	1	0	1	0	0	0	0	1	1	0	0	82
03BF	2	10	0	18	0	8	0	20	4	0	10	10	8	1	0	0	0	0	0	0	3	0	4	0	0	98
03BH	0	0	0	4	0	6	0	5	4	0	6	0	1	2	0	0	0	0	0	1	2	0	1	0	0	32
03BJ	0	3	2	1	0	7	0	12	10	0	8	5	2	1	8	4	0	0	1	0	2	0	0	0	0	66
03BJh	0	0	0	1	0	3	0	2	4	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	12
03BK	0	6	0	17	0	9	3	9	4	3	8	0	8	1	2	1	0	1	0	0	4	0	2	0	0	78
03BUU	0	3	0	16	0	11	1	11	11	0	12	0	5	0	0	3	0	1	0	0	0	0	0	0	0	74
97ZZZ	0	11	0	26	0	12	2	12	8	2	14	0	6	1	1	0	0	2	0	2	7	0	0	0	2	108
Total	2	44	2	140	2	82	12	109	79	43	85	19	47	9	13	12	3	6	1	3	23	1	12	3	2	754

One-18	170	172	174	176	178	180	182	184	186	
01GB	9	1	66	11	6	31	5	0	1	130
02BI	8	1	25	22	1	7	9	0	1	74
03BD	10	0	20	23	6	7	11	0	5	82
03BF	15	0	20	33	13	2	10	1	6	100
03BH	5	0	15	5	0	3	4	0	0	32
03BJ	4	0	9	10	14	2	25	0	2	66
03BJh	2	0	7	1	0	1	3	0	0	14
03BK	8	0	20	16	2	15	16	0	1	78
03BUU	11	0	20	19	5	9	4	1	1	70
97ZZZ	10	0	45	26	8	7	10	0	0	106
Total	82	2	247	166	55	84	97	2	17	752

One-2	193	200	206	210	212	216	218	220	222	224	225	227	228	230	232	234	236	238	239	241	244	246	248	250	251	253	255	257	259	261
01GB	0	0	0	0	0	0	1	0	9	0	0	0	5	9	5	13	3	0	0	1	1	4	5	5	0	5	2	0	1	0
02BI	0	0	0	0	0	0	0	0	10	0	0	0	1	5	3	4	0	1	4	0	2	1	0	5	0	1	0	2	0	0
03BD	0	0	14	0	4	0	1	0	4	0	0	0	0	3	0	6	3	0	0	2	1	2	2	1	1	0	0	2	3	0
03BF	0	2	22	0	9	0	0	0	1	0	0	0	0	3	0	4	0	0	0	6	0	8	1	1	0	0	0	1	2	0
03BH	0	0	0	0	0	0	0	0	5	0	0	0	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0
03BJ	1	1	3	2	2	0	0	3	6	3	2	1	1	0	0	4	1	2	0	1	0	0	1	0	0	0	0	0	0	0
03BJh	0	0	3	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	2	1	0	0	0	0	0	0
03BK	0	0	0	0	0	0	0	0	3	0	0	0	0	6	2	9	1	0	0	0	0	2	5	4	3	0	0	3	0	1
03BUU	0	0	0	0	0	0	0	0	6	0	0	0	0	7	1	4	0	1	0	1	1	5	4	1	0	1	3	0	1	0
97ZZZ	0	0	0	0	1	0	0	0	8	0	0	0	0	6	2	3	0	0	0	0	0	1	1	3	0	0	0	0	0	0
Total	1	3	42	2	16	1	2	3	53	3	2	1	7	41	13	48	9	4	4	14	5	23	21	21	4	7	5	8	7	1

One-2 cont	263	265	267	269	271	273	275	276	279	282	284	286	288	290	293	
01GB	0	2	9	23	0	0	0	7	0	0	1	1	0	0	6	118
02BI	3	2	7	15	0	0	0	0	0	1	1	0	0	0	0	68
03BD	1	1	7	21	6	1	0	0	0	0	0	0	0	0	0	86
03BF	1	0	3	21	0	0	1	0	0	0	0	0	0	0	0	86
03BH	0	0	5	5	1	0	0	0	1	0	0	0	0	1	0	22
03BJ	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0	40
03BJh	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	14
03BK	3	1	5	12	7	0	1	0	1	2	0	1	0	0	0	72
03BUU	3	1	7	10	2	0	0	0	0	1	0	1	1	0	0	62
97ZZZ	0	0	2	6	0	1	0	0	0	0	0	0	0	0	0	34
Total	12	7	47	118	16	3	2	7	2	4	2	3	1	1	6	602

Sco-110	150	154	162	166	170	174	178	180	182	184	186	194	198	201	209	213	217	220	225	228	232	240	244	248	256	263	
01GB	65	11	0	0	0	0	0	6	0	0	0	0	1	0	1	0	26	7	0	5	0	0	10	0	0	0	132
02BI	25	6	0	0	0	0	0	2	0	0	0	8	0	0	2	2	14	4	1	0	0	0	8	0	0	0	72
03BD	24	13	0	0	1	0	1	2	2	0	0	0	14	1	3	1	6	0	3	0	1	8	6	0	1	1	88
03BF	18	17	0	0	2	0	2	4	1	0	0	0	19	2	3	1	3	0	9	0	0	9	5	0	5	0	100
03BH	6	7	0	0	0	1	0	2	0	0	0	0	0	0	0	0	4	0	0	2	0	2	4	0	0	0	28
03BJ	8	3	7	3	3	11	6	0	2	1	2	0	2	5	3	0	6	0	0	0	0	3	0	0	1	0	66
03BJh	3	2	0	0	0	1	0	1	0	0	0	0	1	0	0	0	2	0	0	0	0	2	0	0	0	0	12
03BK	31	9	0	0	0	0	0	2	0	0	0	8	2	0	2	2	9	2	5	0	0	8	4	0	0	0	84
03BUU	31	8	0	0	0	0	0	0	0	0	0	4	4	0	0	1	5	0	3	1	0	8	4	0	0	1	70
97ZZZ	39	17	0	0	0	0	0	0	0	0	0	3	2	2	0	0	5	1	0	1	0	4	3	1	0	0	78
Total	250	93	7	3	6	13	9	19	5	1	2	23	45	10	14	7	80	14	21	9	1	44	44	1	7	2	730

Omm1138	142	144	146	148	151	155	159	
01GB	24	25	31	49	3	0	0	132
02BI	9	17	7	37	6	0	0	76
03BD	8	14	10	56	5	0	1	94
03BF	8	11	5	71	0	0	3	98
03BH	0	9	2	13	5	1	0	30
03BJ	2	4	2	18	3	5	34	68
03BJh	2	3	0	8	2	0	1	16
03BK	15	13	6	33	13	0	0	80
03BUU	12	15	5	32	6	0	0	70
97ZZZ	6	13	8	58	13	0	0	98
Total	86	124	76	375	56	6	39	762

Omy325	93	94	95	96	97	98	99	101	102	103	105	106	107	109	112	113	116	118	120	122	126	128	130	132	135	137	139	141	146	149
01GB	0	0	0	0	16	0	4	10	0	0	0	1	19	2	12	10	6	8	1	0	5	0	0	0	0	2	2	0	10	0
02BI	0	0	1	0	13	0	1	6	0	1	0	0	21	0	8	1	2	0	0	0	0	1	0	2	0	2	0	0	0	0
03BD	0	2	0	2	13	1	6	5	1	4	2	2	19	1	2	4	4	1	2	0	3	2	0	1	1	2	0	7	1	0
03BF	0	1	2	0	18	0	4	1	0	12	0	0	25	1	2	10	3	0	0	0	1	0	0	0	0	1	2	6	0	0
03BH	0	0	0	0	1	0	3	0	0	2	0	0	6	0	3	0	0	0	1	0	1	2	0	0	0	0	0	1	0	1
03BJ	2	0	0	0	7	0	0	3	0	1	0	0	6	0	1	2	4	1	0	2	1	0	3	0	0	0	2	2	0	0
03BJh	2	0	0	0	0	0	0	1	0	0	0	0	6	0	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0
03BK	0	0	5	2	17	0	0	10	0	3	0	0	6	1	4	2	5	0	1	0	2	0	2	4	1	2	0	4	0	0
03BUU	0	0	2	0	11	0	1	4	0	1	0	0	13	3	11	1	2	0	0	0	0	0	1	2	3	0	0	2	0	1
97ZZZ	0	3	8	7	24	0	2	3	0	6	0	1	25	4	3	1	4	0	3	0	0	1	0	2	0	0	0	6	0	0
Total	4	6	18	11	120	1	21	43	1	30	2	4	146	12	46	32	30	11	10	2	13	6	6	11	5	9	6	28	11	2

Omy325 cont	151	153	155	157	159	165	170	172	180	
01GB	1	10	13	0	0	0	0	0	0	132
02BI	4	2	8	1	0	0	0	0	0	74
03BD	0	1	3	0	0	0	0	0	0	92
03BF	0	0	2	0	0	0	1	0	0	92
03BH	0	1	0	0	0	0	0	0	0	22
03BJ	0	0	1	0	3	1	2	2	2	48
03BJh	0	0	1	0	0	0	0	0	0	14
03BK	0	1	8	0	0	0	0	0	0	80
03BUU	2	0	12	0	0	0	0	0	0	72
97ZZZ	1	1	3	0	0	0	0	0	0	108
Total	8	16	51	1	3	1	3	2	2	734

Sco-103	199	204	208	211	215	219	223	227	231	235	239	244	248	251	255	260	263	268	272	276	280	284	288	292	296	303	
01GB	4	1	6	6	0	3	4	1	6	10	19	16	5	12	24	4	3	3	0	0	0	3	0	0	2	0	132
02BI	1	0	4	2	0	4	6	4	8	4	5	12	4	4	3	3	6	1	0	1	1	0	0	1	0	0	74
03BD	2	0	0	3	3	10	13	5	5	0	5	14	6	2	1	3	9	2	2	0	0	0	1	2	0	2	90
03BF	2	0	1	10	3	3	8	4	5	1	2	14	9	3	1	4	19	4	0	0	2	0	0	1	0	0	96
03BH	0	1	0	1	0	2	4	2	0	3	1	5	0	3	3	0	5	0	0	0	0	0	0	0	0	0	30
03BJ	1	0	5	7	12	13	6	2	0	1	2	6	6	2	1	0	4	1	0	1	0	0	0	0	0	0	70
03BJh	0	0	0	0	0	0	3	2	1	2	4	0	0	0	0	2	1	0	0	1	0	0	0	0	0	0	16
03BK	7	0	1	5	1	5	8	3	8	0	6	14	7	3	4	0	5	1	0	0	0	0	0	0	0	0	78
03BUU	1	0	2	9	2	1	9	2	11	2	5	11	5	2	4	0	3	0	0	0	1	0	0	0	0	0	70
97ZZZ	2	1	0	1	6	1	20	2	10	5	8	25	5	2	3	3	8	1	0	0	0	0	1	1	0	1	106
Total	20	3	19	44	27	42	81	27	54	28	57	117	47	33	44	19	63	13	2	3	4	3	2	5	2	3	762

Ogo-3	182	186	189	191	193	195	197	199	201	203	213	Total
01GB	8	10	0	55	3	27	0	15	12	0	0	130
02BI	1	2	0	41	0	18	0	8	4	0	0	74
03BD	6	4	0	43	0	27	3	4	3	0	0	90
03BF	8	8	0	25	0	33	1	3	8	0	0	86
03BH	3	0	0	17	0	11	0	0	1	0	0	32
03BJ	36	4	0	14	0	9	0	2	1	1	1	68
03BJh	3	0	0	6	0	4	0	0	1	0	0	14
03BK	2	1	0	42	0	17	1	4	1	0	0	68
03BUU	5	2	1	32	1	15	0	9	1	0	0	66
97ZZZ	0	0	0	6	0	0	0	0	0	0	0	6
Total	72	31	1	281	4	161	5	45	32	1	1	634