

**Population Assignment Tests and Genetic Structure
Analysis Reveal Relative Productivity Between Hatchery
Origin and Natural Origin Steelhead Trout Within
Eagle Creek, OR.**

DRAFT

Report: FY2005 Results Summary
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INTRODUCTION

Background

Eagle Creek National Fish Hatchery (ECNFH) produces steelhead trout (*Oncorhynchus mykiss*) that are volitionally released into Eagle Creek within the Clackamas River basin (Figure 1). The hatchery program mitigates for fish losses in the Columbia River basin caused by federal dams, and provides commercial, sport, and tribal harvest (Eagle Creek Hatchery and Genetic Management Plan). Over the period from 1980-2002 the annual return of adult hatchery fish to ECNFH averaged 805 (range 251-3,671). On-station releases of one-year-old smolts averaged 176,000 (range 113,000 – 207,000) over the period 1990-2002.

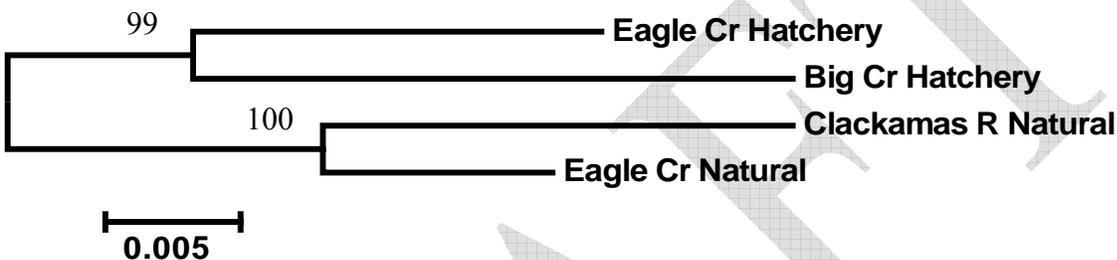
The original hatchery brood stock was comprised of native Clackamas winter steelhead stocks, mixed with earlier returning fish from Big Creek. Big Creek hatchery is located in the lower Columbia River and its broodstock is characterized by high survival rates in the hatchery. In addition, the Big Creek hatchery stock exhibits earlier return and spawn timing compared to the natural origin (NOR) steelhead in Eagle Creek. The temporal separation in return and spawn timing provides the advantage of establishing fisheries that target the earlier returning hatchery fish. Differential spawning times also minimize the opportunity for interbreeding between hatchery and natural origin fish.

Wild winter steelhead in Eagle Creek are listed as a threatened population under the Endangered Species Act (Lower Columbia River ESU, 63 FR 13347; March 19, 1998). Although most wild steelhead production in the Clackamas River system is in the North Fork of the Clackamas River, a small proportion occurs within the Eagle Creek watershed. It is believed that NOR steelhead spawn primarily in the North Fork of Eagle Creek, but some natural spawning is also thought to occur in the lower 0.3 miles of Bear Creek, the lower 2 miles of Little Eagle Creek, Delph Creek and main stem Eagle Creek downstream of the hatchery. Adult NOR steelhead trout are considered “late spawners”. Spawning begins in April and is complete by mid-June, with peak spawning activity occurring in May (ODFW 1992).

Under current protocol which began in 1992, the hatchery maintains a segregated broodstock that is collected exclusively from adults returning to the hatchery rack with identifiable ECNFH marks. Hatchery origin (HOR) steelhead trout return to the hatchery from November through April, but the overwhelming majority of steelhead return and spawn between December and mid March. Most of the NOR fish return and spawn later (May peak) than the hatchery stock at ECNFH, but some overlap occurs. This temporal isolation is thought to reduce the opportunity for genetic introgression of HOR fish into the NOR population. In another component of this study, the USFWS Columbia River Fisheries Program Office is collecting data on run timing, behavior, distribution and abundance of hatchery and wild steelhead in Eagle Creek. In addition, the USFWS Lower Columbia River Fish Health Center is collecting information on fish health and disease status of wild and hatchery fish in Eagle Creek. Together, these investigations will provide a better understanding of the ecological interactions between hatchery and wild fish and ultimately help improve our hatchery operations in the context of watershed management.

In 2000, the USFWS established contracts (FWS Agreement # H012A) with NMFS-Northwest Fisheries Science Center (NMFS) and Washington Department of Fisheries and Wildlife (WDFW) to conduct a genetic evaluation prompted by the ESA listing of Columbia River Steelhead, and a subsequent ruling stating that listed populations are jeopardized by hatcheries using out-of-basin broodstock in the same watershed. Four populations were examined: ECNFH broodstock, Clackamas River “late run”, Eagle Creek “late run” and Big

Creek Hatchery broodstock, to determine the level of genetic similarity among populations. USFWS geneticist Donald Campton presented a preliminary analysis indicating that allele frequencies were significantly different among all four populations. The two wild “late run” populations were substantially closer to one another than to the hatchery samples. The ECNFH population was most genetically similar to the Big Creek hatchery and Native Eagle Creek “late run” populations. These results suggest introgression of the Big Creek Hatchery stock into the ECNFH broodstock, resulting in relatively large genetic differences between the Native Eagle Creek late-run population and the ECNFH broodstock. The genetic evaluation further demonstrated very low levels of gene flow between ECNFH broodstock and NOR Eagle Creek fish even though both populations have the opportunity to spawn naturally in Eagle Creek below the hatchery.



(Eagle Creek NFH coordination meeting held 2/22/2001): Genetic differentiation of steelhead trout populations from Eagle Creek, Big Creek Hatchery, and Natural-origin fish from the Clackamas River. Un-rooted neighbor-joining dendrogram based on Cavalli-Sforza & Edwards (1967) chord distance calculated from allele frequencies at 15 microsatellite loci and two SNP loci. Pairwise genetic distances were calculated for all sample (n=60/population). Bootstrap probabilities, based on 10,000 replicates, provide a measure of statistical confidence for each of the indicated clusters the numbers leading to each cluster represent the percentage of times the indicated samples clustered together in the simulated, random sampling replicates.

Reproductive success of hatchery and natural origin steelhead trout in the Eagle Creek watershed

Return and spawn timing differences between HOR and NOR steelhead in Eagle Creek suggest these two groups have low rates of genetic exchange. The opportunity for gene flow between the groups is also reduced by spatial segregation of spawning locations. Previous observations indicate that late run NOR steelhead primarily spawn in the North Fork of Eagle Creek and that the majority of HOR fish that spawn in the wild, do so in the main stem of Eagle Creek below the hatchery

Several studies have documented low lifetime reproductive success of hatchery origin steelhead trout in the wild when they originate from out-of-basin multigenerational hatchery programs (see Waples 1999 for review). However, the hatchery fish in Eagle Creek could be successfully spawning in the wild and producing young that compete for resources with the listed populations in the creek (Kostow *et al.* 2003). Under this scenario, the ECNFH fish might spawn naturally in Eagle Creek and produce offspring that survive and out-migrate as smolts but do not return as adults. If this scenario does in fact occur, the natural-origin offspring parented by hatchery origin spawners would have a negative impact on the listed populations in Eagle Creek through competition and resource use.

Study Objectives

The null hypothesis: HOR and NOR steelhead contribute equally to the natural production of juvenile steelhead in Eagle Creek, will be tested using a genetic assignment test. Juvenile samples will be collected from the mainstem Eagle Creek, North Fork Eagle Creek, and ECNFH to examine spatial patterns in spawning locations and progeny rearing habitats utilized by both groups. We will conduct an analysis of population structure among the juvenile groups and a sample of returning NOR adult to determine the proportion of NOR fish that are parented by HOR fish, and if that proportion changes at different life stages. Understanding the limiting life stage for natural reproduction of hatchery fish in Eagle Creek is a crucial step to recognizing the ecological impacts and genetic consequences of the hatchery program on the listed populations in this system.

METHODS

Collection of Samples

Genetic samples were collected by the Columbia River Fisheries Program Office and Lower Columbia River Fish Health lab and provided to Abernathy Fish Technology Center for analysis. Juvenile fish collections included four groups in the Eagle Creek watershed (Figure 1). The first group was sampled from a section that begins at the confluence of Eagle Creek and North Fork Eagle creek, and extends up to the ECNFH (Upper E. C.). Group two includes a section extending from the Eagle Creek confluence with the Clackamas River, up Eagle Creek to the North Fork confluence (Lower E. C.). The third group was sampled from a section comprising the North Fork Eagle Creek (N. Fork E. C.). The last group was sampled from the raceways at ECNFH. Adult NOR returns were sampled from the lower ladder in the mainstem Eagle Creek. The fiscal year 2005 goal was to collect 50 rainbow/steelhead samples from each location; all goals were met with the exception of the adult NOR group (appendix 1). Sampling in the N. Fork E. C. was done in conjunction with normal screw trap sampling performed by the U. S. Forest Service. Because only 5 smolts were captured, the remaining samples consisted of juvenile *O. mykiss* obtained through electroshock sampling. With the exception of screw trap collections, sample sites were spread throughout each section to avoid collecting one or a few family groups. Some of the collection sites include areas approximately 200 meters below the hatchery, adjacent to Eagle Fern Park, immediately below the confluence of Eagle Creek and North Fork Eagle creeks, below the lower ladder, and near the confluence with the Clackamas River.

Summary of Samples to Be Analyzed in FY05

Location	Origin	Life History Stage	Target Sample (N)	Actual sample (N)
1.) North Fork Eagle Creek	NOR	Juvenile & Smolt	50	51*
2.) Upper E. C.	NOR	juvenile	50	50
3.) Lower E. C.	NOR	juvenile	50	50
4.) Lower Ladder	NOR	Adults	50	42
5.) Eagle Creek NFH	HOR	juvenile	50	56
Total			250	249

*Due to difficulty in obtaining smolt, this group includes only 5 smolt and 46 juvenile *O. mykiss*.

Biological data was collected by the Columbia River Fisheries Program Office and included fork length, weight, and stream section for each fish sampled (Appendix 1). Scales were taken from a representative sample of young-of-the-year fish and those deemed as age one-plus (1+) or older. Adult NOR were sampled in conjunction with radio tagging captures in the lower ladder. Fork lengths of each adult fish were measured, and scales taken for age determination. For all juvenile and adult samples, a small piece of fin tissue was removed for DNA extraction and analysis. Each fin clip was placed in a vial filled with 100% EtOH, and labeled with an individual identification number.

Microsatellite Amplification and Analysis

We used the polymerase chain reaction (PCR) to amplify microsatellite nuclear DNA loci with the following 16 locus primers: $\mu Omy1011UW$ (Spies *et al.* 2005), $\mu Ssa407$ and $\mu Ssa408$ (Cairney *et al.* 2000), $\mu One13$ and $\mu One14$ (Scribner *et al.* 1996), $\mu Ocl1$ (Condrey & Bentzen 1998), $\mu Ogo4$ and $\mu Ogo3$ (Olsen *et al.* 1998), $\mu Ots4$, $\mu Ots100$, $\mu Ots3$ and $\mu Ots1$ (Banks *et al.* 1999), $\mu Oki23$ (Smith *et al.* 1998), $\mu Omy7iNRA$ (K. Gharbi, and R. Guyomard, Unpublished), $\mu Omy77$ (Morris *et al.* 1996), and $\mu Ssa289$ (McConnell 1995). DNA was extracted by boiling fin tissue in a resin solution (Chelex 100, Sigma Chemical Co.). PCR conditions and scoring of polymorphisms followed the methods of Ardren *et al.* (1999). We used an ABI 3100 multi-channel sequencer (Applied Biosystems, Inc.) to generate electropherograms for DNA fragment analysis and allele scoring. Multilocus genotypes were compiled over all loci, for each individual fish.

Allele frequencies, number of alleles, observed and expected heterozygosities, and index of inbreeding (F_{is}) were generated using the software GDA (Lewis and Zaykin 2001). The number of private alleles per locus (alleles absent in all but one group) was calculated using the program CONVERT (Glaubitz 2003). Tests for departures from Hardy-Weinberg equilibrium (HWE) expectations (i.e. random mating) were calculated using Genepop (Raymond and Rousset 1995). Statistical significance (α) for tests of HWE within each population was adjusted for the number of simultaneous tests k (α/k for $\alpha = 0.05$) by the sequential Bonferroni correction (Rice 1989). The program FSTAT v2.9.3.2 (Goudet 1995) was used to calculate F_{ST} (θ of Weir and Cockerham 1984); indicating the proportion of total variation attributed to differences among groups, and allelic richness; a weighted estimate of the number of alleles per group, scaled to the smallest sample size. Statistical significance for allelic richness between the HOR and all NOR groups was assessed using a permutation scheme (5000 replicates) in which whole samples were allocated at random among groups (keeping the number of samples in each group constant), where the P -value of the test is the proportion of randomized data sets giving a larger value than the observed.

A pairwise genetic distance matrix of Cavalli-Sforza and Edwards (1967) chord distances (CSE) was generated using the software program PHYLIP version 3.5C (Felsenstein 1992). The NEIGHBOR application in PHYLIP was used to generate an unrooted neighbor joining (NJ) dendrogram of genetic distance, and the program MEGA2 was used to graphically display the dendrogram. We used the SEQBOOT and CONSENSE programs in PHYLIP to estimate the consistency of the dendrogram topology via 1000 replicate dendrograms generated by bootstrap resampling of the data.

Factorial correspondence (FC) analysis of individual multilocus scores was conducted using GENETIX version 4.05 (Belkhir *et al.* 2004). In this method, the unique variance is separated from the common variance with the assumption that the inter-correlations among the

original variables are generated from latent common factors (McGarigal 2000). The relationship within a multidimensional cloud of data (orthogonal system of axes) can be described by the factors (axes) with the most variance. Correlations among groups were evaluated in four comparisons: ECNFH vs. NOR adults, ECNFH vs. upper Eagle Creek, ECNFH vs. lower Eagle Creek, and ECNFH vs. N. Fork Eagle Creek.

The null hypothesis (H_0 : no difference in allelic frequencies among groups) was tested with a Monte Carlo chi-square (X^2) test of homogeneity using the program CHIRXC (Zaykin and Pudovkin 1993). The following seven comparisons were made: ECNFH vs. Adult NOR (baseline groups), N. Fork E. C. vs. ECNFH, N. Fork E. C. vs. Adult NOR, Upper E. C. vs. ECNFH, Upper E. C. vs. Adult NOR, Lower E. C. vs. ECNFH, and Lower E. C. vs. Adult NOR. The null hypothesis of homogeneity is evaluated in CHIRXC by creating simulated random samples of the original dataset and calculating a X^2 value for each pseudo-sample assuming allele frequencies are equal among the groups being compared. The proportion of the simulations with X^2 values exceeding the observed value for the actual dataset is the significance probability of the test. This approach circumvents the well-known problem of low expected numbers in X^2 tests. Statistical significance (α) was adjusted for the number of simultaneous tests (k) in each comparison (α/k for $\alpha = 0.05$) by the sequential Bonferroni correction (Rice 1989)

Assigning individuals as HOR or NOR progeny

Likelihood based procedures implemented by the program *WHICHRUN* version 4.1 (Banks and Eichert 1999) were used to assign individual fish to either natural-origin or hatchery-origin. The probability of an individual belonging to each of the ecotypes was calculated as:

$$\log_{10} \left(\prod_{l=1}^n p_{ij}^2 \text{ for } i = j, \text{ and } 2p_i p_j \text{ for } i \neq j \right) \quad (1)$$

where n denotes the number of loci, i and j denote two alleles at the l th locus, and p_i and p_j denote the frequencies of the i th and j th allele of the l th locus in the ecotype. This basic approach uses the multilocus genotype of an individual and the HOR and NOR allele frequency baselines to assign a probability of an individual belonging to one of the two groups. If an allele observed in an individual is not present in the baseline files, a frequency of $1/(2n+1)$, where n is the number of samples in the baseline, is applied to the baseline for that allele. *WHICHRUN* then assigns the individual to the ecotype with the highest probability of producing its multilocus genotype.

Log of the odds ratio (LOD) were used to assess the stringency of individual assignments. The LOD scores were calculated as:

$$\text{LOD}_{\text{for HOR}} = \log_{10} \left(\frac{\prod_{l=1}^n \text{HOR}_l}{\prod_{l=1}^n \text{NOR}_l} \right) \quad (2)$$

where n is the number of loci, and HOR_l and NOR_l are the probabilities of the individual's genotype at locus l occurring as the result of random mating within hatchery or natural

populations based on the allele frequency baselines. The LOD scores provide a convenient way of assessing the confidence of an assignment, for example a LOD score for HOR of 2.0 (equation 2) indicates the fish is 100 times more likely to be a HOR than a NOR. A similar population assignment approach was used to identify HOR and NOR steelhead in Forks Creek, WA (McLean *et al.* 2003).

Assessing the overall power of correctly assigning individual fish to hatchery or natural-origin parents: testing the baseline

In this study, the juvenile collections from throughout Eagle Creek watershed (not including ECNFH) were treated as fish of unknown origin. The HOR smolts from ECNFH and the NOR adults collected at the lower ladder were treated as known HOR and NOR groups respectively in the baseline. A baseline of “known” HOR and NOR allele frequencies must first be established to evaluate the power of the test: that is, power to differentiate between “origin” type. In other words, these allele frequencies will serve as standards in assignment tests, and individuals in the “unknown” group will be assigned to origin of source (i.e. NOR spawned or HOR spawned) based on similarity to one or the other of the population allele frequencies in the baseline.

The overall power of the 16 loci to correctly assign individuals to origin was assessed by a jack-knifing procedure that involved deleting an individual fish from the baseline data set, recalculating the allele frequencies for the source population (NOR or HOR) of that individual, and then recalculating a LOD score for that omitted fish and assigning it to one of the two populations in the baseline. This procedure was completed sequentially for each of the fish in the baseline. The percentage of fish in the baseline assigned correctly as HOR or NOR based on their multilocus genotypes represented the statistical power of the analysis. Equation 2 was used for calculating these jackknife LOD scores: fish with LOD scores >0 or <0 were provisionally assigned as HOR or NOR, respectively.

RESULTS

Descriptive Statistics

The Eagle Creek steelhead dataset had a wide range of variability and allelic polymorphism. Numbers of alleles ranged from 18 at $\mu Ssa408$ in the lower E. C. group, to 4 at $\mu Ssa289$ in the ECNFH group (mean = 16 over loci and groups). Observed heterozygosity ranged from 0.96 at $\mu Omy1011$ in the lower E. C. group, to 0.46 at $\mu Ssa289$ in the lower E. C. group (mean = 0.76 over loci and groups). The number of private alleles ranged from 2 in the lower E. C. group to 16 in the N. Fork E. C. group (Table 1). Departures from expected genotypic proportions within groups (HWE) were observed at $\mu Oki23$ in the upper E. C. group, $\mu Ocl1$ in the lower E. C. group, $\mu Ocl1$ and $\mu Ogo3$ in the ECNFH group, and at $\mu Ocl1$, $\mu Ogo3$, $\mu Ogo4$ and $\mu Oki23$ in the N. Fork E. C. group. There was no indication of heterozygote deficits in any of the departures; a deficit could mean the presence of “null” allele or large allele dropout. However the departures may be explained by several factors including rare heterozygotes, or the presence of sibling groups. Note that the group with the most departures (N. Fork E. C.) also had the highest number of private alleles (Table 1), and at least one full sibling group of 5 fish (data not shown).

Variation in the proportion and distribution of alleles among sample groups can be depicted by the number of private alleles and allelic richness between groups. The N. Fork E. C. group had 16 private alleles compared to only 3 in the ECNFH group (Table 1), and a

comparison of all NOR groups against the HOR fish shows a significant difference in allelic richness ($P < 0.01$; Figure 2).

Population Genetic Structure Analysis

Our results show significant population structure among the 5 groups of steelhead evaluated in Eagle Creek. The F_{ST} values ranged from 0 to 0.025 among loci, and the over all estimate of 0.013 (95% CI 0.009-0.016) provides small but significant genetic differentiation among groups (Table 1). Results of Monte Carlo chi-square (X^2) tests of homogeneity for H_0 : no difference in allelic frequencies among groups, were highly conclusive. Among the adult NOR and HOR groups (assignment test baseline), significant heterogeneity was observed at 7 of 16 loci, and over all loci. Among ECNFH fish and the N. Fork E. C. juveniles, significant heterogeneity was observed at 10 of 16 loci and over all loci. Both the lower and upper E. C. sample groups exhibited highly significant results (14 of 16 loci each) when allele frequencies were compared to the ECNFH group (Table 2). Tests of homogeneity between the adult NOR group and each juvenile group produced markedly different results; only $\mu Ots100$ in the ECNFH/lower E. C. comparison was significant (Table 2). All other NOR/NOR homogeneity test results were not statistically significant.

Observed genetic structure is corroborated by the relationship of genetic distances among the ECNFH and NOR groups demonstrated in the topology of a NJ dendrogram (Figure 3). The greatest similarity is seen within a cluster consisting of juvenile samples from upper and lower E. C. and the NOR Eagle Creek late-run adults. This collective cluster is most different from the ECNFH group. The N. Fork E. C. group is located on an intermediate branch between the ECNFH group and the cluster of other NOR groups. These results are further substantiated by an FC analysis, providing a visual display of maximum variability (Figure 4). The variation among the upper E. C. group and ECNFH group, and the lower E. C. group and ECNFH group is well defined in both cases, and the separation of samples is nearly discrete. The plot of ECNFH and adult NOR groups appears to have less variation but maintains a similarly well defined separation of groups. The plot of N. Fork E. C. fish and ECNFH fish shows the least amount of variation among groups, and a clean separation of groups is less evident.

Assignment tests

The overall power of the 16 loci to correctly assign individuals to group of origin was assessed by a jack-knife resampling method. The ECNFH fish in the baseline assigned to their group of origin with 85.7% accuracy ($LOD > 0$), while the adult NOR in the baseline assigned to their group of origin with 78.6% accuracy ($LOD < 0$). However, to achieve 95% confidence in that assignment, a sampled HOR had to reach $LOD_{HOR} > 1.28$ (71.4% of the baseline), and a sampled NOR fish had to reach $LOD_{HOR} < -1.66$ (57.1% of the baseline; Table 3). The 95% confidence LOD range reflects the proportion of mis-assigned individuals and the overlapping distribution of LOD scores for HOR and NOR in the baseline (Figure 5). In fact, because of misidentifications (i.e. when a known HOR earned a $LOD < 0$, or known NOR earned a $LOD > 0$), positive identification to group of origin could only be achieved with $LOD_{HOR} > 1.98$ and $LOD_{HOR} < -2.11$.

Following the baseline test, we evaluated the unknown sample groups. The probability of an individual belonging to one of the two baseline groups was calculated based on genotypic similarity, and determined the assigned origin of each fish. The lower E. C. group garnered the greatest number of NOR assignments (92%), although only 76% of those could be made with

95% confidence; this surpassed the NOR baseline assignment success (Table 3). However, only 4% of the lower E. C. group assigned to HOR with 95% confidence. The N. Fork E. C. group assigned the least proportion of NOR (64.7%), and only 39.2% of those could be made with 95% confidence. Additionally, 21.6% of the N. Fork E. C. group assigned to HOR with 95% confidence (Table 3). These assignment results can be seen graphically for the three juvenile groups of fish, using the method of Hendry et al. (2002; Figure 6). In this plot, fish that fall on the line have an equal probability of being NOR or HOR (LOD score = 0). Consequently, we have little confidence in the assignments for fish that are relatively close to the line; those that have log probability scores along the two axes that differ by less than 1.0. Recall that a LOD of 1 means that an assignment is 10 times more likely (correct) than the alternative. Note that individuals in the N. Fork E. C. group plot above the equal probability line (area of HOR probability) in greater number than either the lower or upper E. C. groups.

DISCUSSION

Previous studies have confirmed that the ECNFH broodstock is introgressed by out-of-basin Big Creek steelhead, which are a relatively early returning stock. Native Clackamas River and Eagle Creek stock are typically considered late-run fish. The temporal separation in run and spawn time between HOR and NOR is known to be a factor in low levels of gene flow and large genetic differences between the two groups. Hatchery broodstock are collected exclusively from HOR fish returning to the hatchery, a practice that allows for harvest of early returning fish in Eagle Creek, and one which is also instituted to help maintain the discreteness of listed NOR fish and the temporal separation of the stocks. However, there is concern that hatchery fish spawning in the wild before reaching the hatchery rack could be impacting the survival of listed NOR through introgression of the wild stock and competition with NOR young. In this study we have addressed the principal, overarching question; do hatchery and natural-origin steelhead contribute equally to the production of natural origin steelhead trout in Eagle Creek, if so what is the limiting life stage, and how are they distributed within the watershed?

The results of our population genetic structure analysis, particularly the homogeneity tests suggest that HOR fish have contributed very little to the production of progeny in the mainstem Eagle Creek. The juveniles collected from locations within the lower and upper reaches of the basin are decidedly genetically different from the ECNFH stock. Conversely, we observed a high level of gene flow between the juvenile samples from lower and upper Eagle Creek and the NOR adults collected at the lower ladder. Although there is evidence that N. Fork E. C. juveniles are genetically different from the ECNFH group, the difference is far less pronounced. The dendrogram topology, PC analysis and assignment test results suggest that HOR fish may contribute to production of progeny in the North Fork of Eagle Creek. However, the N. Fork E. C. group was comprised of primarily *O. mykiss* juveniles of undetermined life history. It is not clear what component of the sample are resident fish and/or potential cutthroat trout (*O. clarkii*) hybrids, or to what extent such occurrences influence the overall results, particularly the assignment tests (also see number of private alleles; Table 1). In either case, the level of HOR production in the North Fork Eagle Creek is likely minimal considering there is no evidence of restricted gene flow among N. Fork E. C. juveniles and the sample of NOR adults (chi-square analysis).

The results of assignment tests are in agreement with the results of genetic structure analysis, but are more ambiguous. This is not surprising given that the original brood stock for

the ECNFH included a significant proportion of native Clackamas River and Eagle Creek stock. The broodstock origin notwithstanding, assignment of juveniles to the NOR type was substantial in the upper and lower E. C. groups, with few HOR assignments, while the N. Fork E. C. group assigned to the NOR type with moderate success. In fact, at the 95% confidence level, the NOR assignments among the lower and upper E. C. groups were nearly two-fold greater than those observed in the N. Fork E. C. group.

One explanation for the results we observed in our FY05 genetic analyses is that there is little or no wild spawning of HOR occurring in the mainstem of Eagle Creek because HOR fish reach the hatchery before biological or environmental queues to spawn are “switched on”. However, HOR fish that stray into the North Fork of Eagle Creek and remain would not be intercepted at the hatchery and may at some point attempt to spawn. Because of the temporal separation of the NOR and HOR winter steelhead in Eagle Creek it is reasonable to infer that most HOR spawning in the wild will spawn with other HOR fish. If HOR adults are indeed spawning in the wild and producing viable progeny, it appears the effect is not realized in the adult population. The adult NOR were observed to be genetically different from the ECNFH group, but genetically similar to all three juvenile sample groups collected within Eagle Creek. The early return of HOR fish may contribute to lower fitness among wild spawned HOR progeny. For example, mal-adapted incubation periods or emergence time may contribute to low survival in the first year.

In contrast to these results, Kostow *et al.* (2003) observed a substantial contribution to natural smolt production from HOR summer steelhead in the Clackamas River Basin. However, introduced summer steelhead and co-occurring winter steelhead have different life histories (i.e. run timing vs. spawn timing) that may contribute to higher levels of introgression among the two steelhead populations. Similar to our results, McLean *et al.* (2004) observed differential reproductive success among sympatric groups of HOR and NOR steelhead, with evidence of relatively poor natural production by HOR adults. In that study, assignment success of unknown fish was as high as 82%, and natural production of smolts by HOR females was only 4.4-7.0 % that of NOR females.

The segregation of hatchery broodstock through collection of exclusively HOR adults appears to be a functional and successful method of maintaining both temporal segregation of relatively discrete NOR and HOR stocks within Eagle Creek, and the reduction of genetic introgression through wild spawning of HOR adults. However, a further understanding of temporal trends or changes in reproductive success of Eagle Creek steelhead will provide additional information on the differential production and survival of NOR and HOR and an accurate view of how NOR and HOR fish influence each other through time. For this reason, we suggest that these study objectives be implemented each year for a 4 year generation interval. In the future, adding a fourth section above the fish barrier, upstream of the hatchery, will present another opportunity for genetic analysis and characterization of *O. mykiss* within the Eagle Creek basin. Wild fish are collected in this section every year for fish health analysis by the Lower Columbia Fish Health Center personnel. Fin clips from many of these samples, not used for disease investigation, can be used for genetic analysis. Delph and Bear Creeks can also be explored for potential fish production. Depending on what is found through electrofishing and snorkel surveys, these areas may also be added for genetic analysis.

Starting in 2006, the Columbia River Fisheries Program Office will initiate sampling to estimate the population abundance of juvenile and adult steelhead in Eagle Creek. Complimentary population abundance estimates and results of population assignment tests and

genetic structure analyses will yield additional information on productivity of wild and hatchery steelhead in Eagle Creek.

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Table 1. Descriptive statistics for the 2005 Eagle Creek Steelhead population structure analysis. Results are presented by locus for each sample group. Column headings are defined as follows: **n** is the number of individuals, **A** is the number of alleles, **AR** is the allelic richness (the # of alleles measured independent of sample size), **AP** is the number of private alleles, **H_E** is Nei's (1978) unbiased estimate of expected heterozygosity, **H_O** is the observed heterozygosity, **F_{is}** is the index of inbreeding [indicates direction of Hardy-Weinberg (HW) departure], and **θ** is the unbiased estimate of Wright's F_{ST} (Weir and Cockerham 1984) - indicating the proportion of total variation attributed to differences among groups. The confidence interval for F_{ST} indicates significance based on bootstrap resampling of allele frequencies. Significance for simultaneous tests has been adjusted ($\alpha = 0.05$ adjusted by α/k) according to Rice (1989). Bold values with the symbol (*) indicate statistical significant HWE departures.

Locus	Upper E. C. - NOR juveniles							Lower E. C. - NOR juveniles						
	n	A	AR	AP	H _E	H _O	F _{is}	n	A	AR	AP	H _E	H _O	F _{is}
<i>μOcl1</i>	50	13	12.6	0	0.8394	0.7800	0.0714	48	13	12.6	0	0.7893	0.7708	0.0236*
<i>μOgo3</i>	50	7	5.8	0	0.6067	0.6400	-0.0555	50	7	6.6	0	0.5857	0.6200	-0.0593
<i>μOgo4</i>	50	8	8.0	0	0.8036	0.7800	0.0297	50	10	9.8	1	0.7646	0.7400	0.0326
<i>μOki23</i>	50	14	13.2	0	0.8723	0.7000	0.1992*	50	14	13.4	0	0.8216	0.8200	0.0020
<i>μOmy1011</i>	50	14	13.4	0	0.8752	0.8600	0.0175	50	14	13.2	0	0.8871	0.9600	-0.0831
<i>μOmy77</i>	50	11	10.8	0	0.8808	0.8200	0.0697	50	13	12.4	0	0.8747	0.8800	-0.0061
<i>μOmy7i</i>	50	10	9.9	0	0.7705	0.8400	-0.0912	48	12	11.4	0	0.7754	0.7292	0.0603
<i>μOne13</i>	50	16	15.1	0	0.8283	0.8200	0.0101	50	17	16.2	2	0.8422	0.8600	-0.0213
<i>μOne14</i>	50	10	9.6	1	0.8036	0.8600	-0.0709	50	9	8.8	0	0.8206	0.7800	0.0500
<i>μOts1</i>	50	10	9.8	0	0.7253	0.6600	0.0908	50	12	11.2	1	0.7356	0.5400	0.2678
<i>μOts100</i>	49	12	11.6	1	0.8521	0.7959	0.0666	49	12	11.5	1	0.8761	0.8776	-0.0017
<i>μOts3</i>	50	7	7.0	0	0.6776	0.6800	-0.0036	49	8	7.8	0	0.6590	0.6939	-0.0536
<i>μOts4</i>	50	7	6.9	0	0.5194	0.5000	0.0377	50	8	7.9	0	0.6489	0.6600	-0.0173
<i>μSsa289</i>	50	5	4.8	0	0.5574	0.5200	0.0677	50	5	4.6	0	0.5438	0.4600	0.1555
<i>μSsa407</i>	50	11	10.9	0	0.8305	0.7600	0.0857	50	12	11.4	1	0.8174	0.8600	-0.0527
<i>μSsa408</i>	49	17	16.4	0	0.9106	0.9388	-0.0313	50	18	17.4	1	0.9287	0.9400	-0.0123
<i>Total</i>	50	11	--	2	0.7721	0.7472	--	50	12	--	7	0.7732	0.7620	--

Locus	ECNFH - HOR smolts							Adult NOR – lower ladder						
	n	A	AR	AP	H _E	H _O	F _{is}	n	A	AR	AP	H _E	H _O	F _{is}
<i>μOcl1</i>	53	14	13.4	0	0.9003	0.8490	0.0574*	41	13	13.0	0	0.8648	0.8049	0.0701
<i>μOgo3</i>	56	5	4.9	0	0.6569	0.6071	0.0763*	42	4	4.0	0	0.5448	0.5000	0.0831
<i>μOgo4</i>	56	8	7.7	0	0.8211	0.7500	0.0873	42	7	7.0	0	0.7851	0.8095	-0.0314
<i>μOki23</i>	56	12	11.8	0	0.8869	0.8214	0.0744	42	14	13.9	1	0.8827	0.8810	0.0020
<i>μOmy1011</i>	56	14	13.2	0	0.8653	0.8750	-0.0113	42	12	12.0	0	0.8703	0.7857	0.0983
<i>μOmy77</i>	56	11	10.4	1	0.8592	0.8750	-0.0185	42	10	10.0	0	0.8402	0.8810	-0.0491
<i>μOmy7i</i>	56	9	8.6	0	0.7616	0.9107	-0.1980	42	12	11.9	0	0.8264	0.8571	-0.0376
<i>μOne13</i>	56	14	13.3	0	0.8481	0.8393	0.0105	42	14	13.9	0	0.8494	0.7857	0.0758
<i>μOne14</i>	56	8	7.7	0	0.7983	0.7679	0.0384	42	10	9.9	0	0.7711	0.7857	-0.0192
<i>μOts1</i>	56	10	9.6	0	0.7600	0.7321	0.0369	42	12	11.9	1	0.7674	0.7857	-0.0242
<i>μOts100</i>	51	12	11.8	1	0.9111	0.9020	0.0101	42	14	13.9	2	0.8798	0.8095	0.0808
<i>μOts3</i>	56	6	5.7	0	0.6308	0.5714	0.0949	42	7	7.0	0	0.6486	0.6667	-0.0282
<i>μOts4</i>	56	6	5.5	0	0.5851	0.5714	0.0236	42	7	7.0	0	0.5993	0.5714	0.0470
<i>μSsa289</i>	56	4	4.0	0	0.6572	0.7143	-0.0878	42	5	5.0	0	0.6474	0.6429	0.0072
<i>μSsa407</i>	56	13	12.6	1	0.8842	0.8929	-0.0099	42	12	12.0	0	0.7978	0.7857	0.0153
<i>μSsa408</i>	55	13	12.5	0	0.8400	0.8364	0.0044	42	16	15.9	0	0.9231	0.9048	0.0201
<i>Total</i>	56	10	--	3	0.7916	0.7822	--	42	11	--	4	0.7811	0.7661	--

Locus	North Fork EC - juveniles							Mean						
	n	A	AR	AP	H _E	H _O	F _{is}	n	A	AR	H _E	H _O	F _{is}	F _{st} (θ)
<i>μOcl1</i>	51	14	13.4	3	0.8865	0.8372	0.0562*	48.6	13.4	13.8	0.8550	0.8017	0.0629	0.0240
<i>μOgo3</i>	51	7	6.7	0	0.6408	0.5116	0.2034*	49.8	5.8	6.0	0.6012	0.5754	0.0433	0.0250
<i>μOgo4</i>	51	9	8.8	0	0.8183	0.8604	-0.0521*	49.8	8.4	8.9	0.7965	0.7845	0.0152	0.0090
<i>μOki23</i>	51	13	12.1	1	0.8424	0.7674	0.0900*	49.8	13.4	12.6	0.8617	0.8013	0.0707	0.0070
<i>μOmy1011</i>	51	17	15.8	1	0.8755	0.8837	-0.0095	49.8	14.2	15.0	0.8738	0.8765	-0.0032	0.0110
<i>μOmy77</i>	51	14	13.3	1	0.8451	0.7907	0.0651	49.8	11.8	12.0	0.8610	0.8520	0.0106	0.0100
<i>μOmy7i</i>	50	11	10.7	0	0.7822	0.7209	0.0792	49.2	10.8	10.5	0.7835	0.8154	-0.0412	0.0070
<i>μOne13</i>	50	16	15.4	0	0.8854	0.9302	-0.0513	49.6	15.4	15.4	0.8502	0.8410	0.0109	0.0240
<i>μOne14</i>	50	8	7.3	3	0.7057	0.6905	0.0218	49.6	9	9.6	0.7824	0.7787	0.0048	0.0050
<i>μOts1</i>	51	12	11.6	1	0.8235	0.7907	0.0403	49.8	11.2	11.0	0.7642	0.6926	0.0946	0.0070
<i>μOts100</i>	51	12	11.9	0	0.8979	0.8372	0.0684	48.4	12.4	13.6	0.8832	0.8495	0.0385	0.0080
<i>μOts3</i>	51	9	8.3	2	0.6347	0.5581	0.1220	49.6	7.4	7.5	0.6492	0.6361	0.0204	0.0000
<i>μOts4</i>	51	9	8.7	1	0.6908	0.6512	0.0581	49.8	7.4	6.9	0.6131	0.5900	0.0381	0.0140
<i>μSsa289</i>	51	6	5.8	1	0.6443	0.6512	-0.0107	49.8	5	4.9	0.6084	0.5968	0.0192	0.0150
<i>μSsa407</i>	51	13	12.6	1	0.8490	0.8372	0.0140	49.8	12.2	13.2	0.8356	0.8283	0.0088	0.0130
<i>μSsa408</i>	50	16	15.7	1	0.9312	0.9524	-0.0231	49.2	16	15.8	0.9064	0.9160	-0.0107	0.0230
Over All	51	12	--	16	0.7971	0.7669	--	49.5	11	--	0.7828	0.7648	--	0.0130
L95% CI	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0090
U95% CI	--	--	--	--	--	--	--	--	--	--	--	--	--	0.0160

Table 2. Monte Carlo chi-square tests of homogeneity (Zaykin and Pudovkin 1993). The procedure tests the null hypothesis - H_0 : no difference in allele frequencies among HOR and NOR groups. Bootstrap probabilities (P) were derived from 50,000 simulated random samples. Significant homogeneity is shown in bold type: $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ ***). Statistical significance (α) has been adjusted for the number of simultaneous tests k (α/k for $\alpha = 0.05$) by the sequential Bonferroni correction (Rice 1989). See methods section for descriptions of Eagle Creek steelhead groups.

A.) Adult NOR vs. ECNFH				B.) N. Fork E.C. vs. ECNFH				C.) N. Fork E.C. vs. Adult NOR			
Locus	df	χ^2	P - value	Locus	df	χ^2	P - value	Locus	df	χ^2	P - value
<i>$\mu Omy1011$</i>	14	43.73	***	<i>$\mu Ssa408$</i>	15	41.62	***	<i>$\mu Omy1011$</i>	17	28.93	0.0149
<i>$\mu One13$</i>	15	45.8	***	<i>$\mu One13$</i>	16	40.21	***	<i>$\mu Ocl1$</i>	16	27.64	0.0166
<i>$\mu Ogo3$</i>	4	22.09	***	<i>$\mu Ocl1$</i>	16	41.04	***	<i>$\mu Ogo3$</i>	6	12.16	0.0351
<i>$\mu Ssa407$</i>	14	36.65	***	<i>$\mu Ogo3$</i>	6	22.33	***	<i>$\mu Ssa408$</i>	17	26.85	0.0414
<i>$\mu Ocl1$</i>	13	35.39	***	<i>$\mu Ssa407$</i>	15	36.73	***	<i>$\mu Ots100$</i>	14	21.74	0.0613
<i>$\mu Omy77$</i>	12	31.62	***	<i>$\mu Omy77$</i>	14	32.01	***	<i>$\mu One14$</i>	12	15.90	0.1114
<i>$\mu Ssa408$</i>	15	32.68	**	<i>$\mu Ssa289$</i>	5	17.50	**	<i>$\mu Omy77$</i>	13	16.72	0.1814
<i>$\mu Omy7i$</i>	12	25.05	**	<i>$\mu Ots4$</i>	8	21.02	**	<i>$\mu Ssa289$</i>	5	7.24	0.1839
<i>$\mu Ogo4$</i>	8	18.68	0.0105	<i>$\mu Omy7i$</i>	11	24.81	**	<i>$\mu Ogo4$</i>	8	10.61	0.2140
<i>$\mu Ots100$</i>	14	24.48	0.0244	<i>$\mu Oki23$</i>	14	27.08	**	<i>$\mu Oki23$</i>	15	18.00	0.2232
<i>$\mu One14$</i>	9	17.15	0.0291	<i>$\mu Omy1011$</i>	17	30.15	0.0106	<i>$\mu Ssa407$</i>	13	15.52	0.2518
<i>$\mu Oki23$</i>	14	20.74	0.0771	<i>$\mu One14$</i>	10	19.18	0.0123	<i>$\mu Ots3$</i>	9	10.53	0.2848
<i>$\mu Ots4$</i>	6	9.26	0.1482	<i>$\mu Ots1$</i>	12	19.72	0.0488	<i>$\mu Ots1$</i>	13	14.36	0.3350
<i>$\mu Ots1$</i>	11	10.97	0.4614	<i>$\mu Ots3$</i>	8	11.91	0.1153	<i>$\mu Ots4$</i>	8	9.07	0.3367
<i>$\mu Ssa289$</i>	4	3.41	0.5290	<i>$\mu Ots100$</i>	12	17.61	0.1193	<i>$\mu Omy7i$</i>	11	11.00	0.4641
<i>$\mu Ots3$</i>	6	2.69	0.8979	<i>$\mu Ogo4$</i>	8	7.96	0.4522	<i>$\mu One13$</i>	16	15.79	0.4905

D.) Lower E.C. vs. ECNFH				E.) Lower E.C. vs. Adult NOR			
Locus	df	χ^2	P - value	Locus	df	χ^2	P - value
<i>μOmy1011</i>	17	54.32		<i>μOts100</i>	15	30.84	**
<i>μOcl1</i>	13	61.24	***	<i>μOmy1011</i>	15	26.21	0.0181
<i>μSsa408</i>	17	53.08	***	<i>μOgo4</i>	9	16.27	0.0422
<i>μOts100</i>	14	47.55	***	<i>μSsa407</i>	14	21.77	0.0475
<i>μOgo4</i>	9	34.39	***	<i>μOki23</i>	15	23.19	0.0505
<i>μSsa407</i>	15	45.88	***	<i>μSsa289</i>	4	8.72	0.0570
<i>μOne13</i>	18	59.61	***	<i>μOne14</i>	9	14.21	0.0970
<i>μOts1</i>	11	32.53	***	<i>μOne13</i>	18	24.19	0.1069
<i>μOgo3</i>	6	22.23	***	<i>μOts1</i>	13	17.57	0.1370
<i>μOmy77</i>	13	32.60	***	<i>μOcl1</i>	13	15.76	0.2583
<i>μOts4</i>	7	21.12	***	<i>μOmy77</i>	12	13.02	0.3585
<i>μOmy7i</i>	11	25.83	**	<i>μSsa408</i>	17	17.86	0.4052
<i>μOki23</i>	13	29.05	**	<i>μOts3</i>	8	8.19	0.4439
<i>μSsa289</i>	4	14.06	**	<i>μOts4</i>	7	6.79	0.4675
<i>μOne14</i>	9	16.52	0.0392	<i>μOgo3</i>	6	5.89	0.4734
<i>μOts3</i>	8	11.67	0.1237	<i>μOmy7i</i>	12	11.20	0.5418
F.) Upper E.C. vs. ECNFH				G.) Upper E.C. vs. Adult NOR			
Locus	df	χ^2	P - value	Locus	df	χ^2	P - value
<i>μOmy1011</i>	16	46.57	***	<i>μOgo4</i>	8	20.78	0.0048
<i>μOcl1</i>	13	54.77	***	<i>μSsa407</i>	12	20.20	0.0454
<i>μSsa408</i>	16	70.28	***	<i>μSsa289</i>	4	8.84	0.0593
<i>μOts100</i>	14	40.30	***	<i>μSsa408</i>	16	23.61	0.0816
<i>μOne13</i>	17	58.50	***	<i>μOts1</i>	12	16.58	0.1266
<i>μOmy77</i>	13	45.48	***	<i>μOts100</i>	15	19.64	0.1415
<i>μSsa407</i>	14	38.08	***	<i>μOmy77</i>	10	13.88	0.1658
<i>μOts4</i>	6	22.78	***	<i>μOmy1011</i>	15	19.18	0.1737
<i>μOmy7i</i>	10	28.85	***	<i>μOts3</i>	8	10.61	0.1987
<i>μOgo3</i>	5	21.08	***	<i>μOcl1</i>	13	16.58	0.2078
<i>μOgo4</i>	8	25.73	***	<i>μOne14</i>	10	12.20	0.2594
<i>μSsa289</i>	4	16.57	**	<i>μOgo3</i>	5	6.25	0.2938
<i>μOts1</i>	10	24.58	**	<i>μOts4</i>	6	6.21	0.4154
<i>μOne14</i>	10	23.67	**	<i>μOki23</i>	15	14.27	0.5312
<i>μOts3</i>	7	12.77	0.0500	<i>μOmy7i</i>	11	9.62	0.5974
<i>μOki23</i>	14	19.24	0.1243	<i>μOne13</i>	16	10.19	0.9247

Table 3. Assignment tests. The results of the jackknife-resampling assignment procedure shown in the “baseline” columns establish the power of assignment, where ECNFH smolts represent HOR genotypes, and adult returns sampled at the Eagle Creek lower ladder represent NOR genotypes in the baseline. The ECNFH group was treated as the critical population in the procedure. Columns under “unknown” show the results of assignment to parental origin for individuals from each Eagle Creek juvenile sample. Assignment to origin (HOR or NOR) was based on genotypic similarity to one or the other baseline population. The symbol (*) indicates the LOD at which 95% confidence of correct assignment is observed, (!) indicates the number of individuals with at least one allele not found in the baseline, and (^C) indicates the designated critical population in the WHICHRUN procedure. Correct Assignments are shown in bold Italics.

	Baseline Groups		Unknown Groups		
	ECNFH ^C (n = 56)	NOR adults (n = 42)	N. Fork EC (n = 51)	Upper E.C. (n = 50)	Lower E.C. (n = 50)
"WHICHRUN" Statistic ¹					
<u>NOR</u>					
Assigned (#)	8	33	33	44	46
% Assigned (LOD < 0)	13.6	78.6	64.7	88.0	92.0
% Assigned (LOD < -1.66)*	--	57.1	39.2	72.0	76.0
unique alleles (!)	--	--	22	18	28
<u>HOR</u>					
Assigned (#)	48	9	18	6	4
% Assigned (LOD > 0)	85.7	21.4	35.3	12.0	8.0
% Assigned (LOD > 1.28)*	71.4	--	21.6	2.0	4.0
unique alleles (!)	--	--	5	4	1

¹ Banks and Eichert, 1999. WHICHRUN (version 3.2)

Figure 1. Map of relevant tributaries, points of interest, and genetic sampling locations of Eagle Creek in the Clackamas Basin.

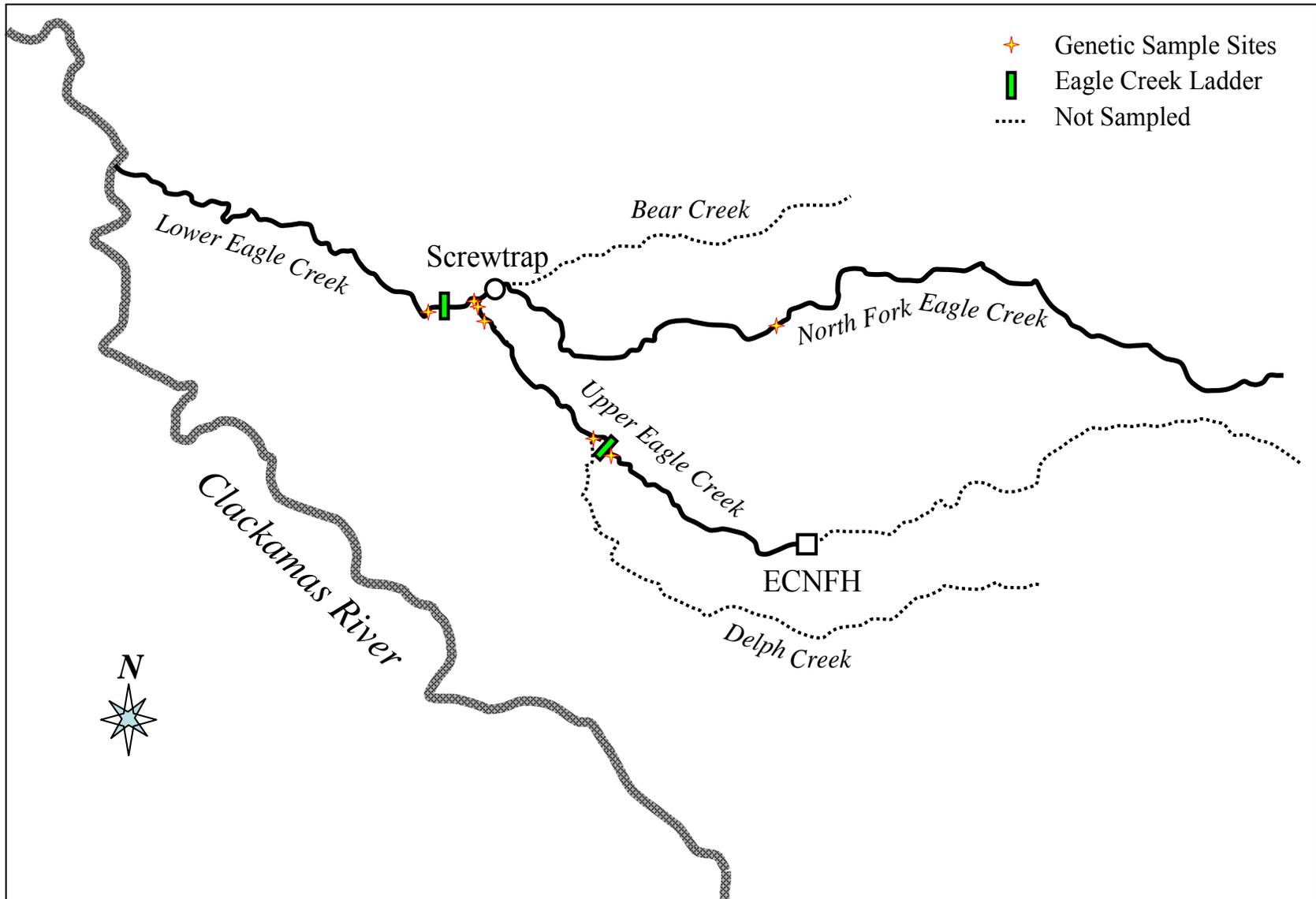


Figure 2. Comparison of allelic richness among natural-origin (HOR) smolts from Eagle Creek NFH and all NOR groups, including adult returns and juveniles from throughout the watershed. Allelic richness values were scaled to a common sample size using a rarefaction procedure in the program FSTAT (Goudet 1995). Randomization of samples groups (5000 replicates) indicates statistical significance in allelic richness between HOR and all other groups ($P = 0.008$).

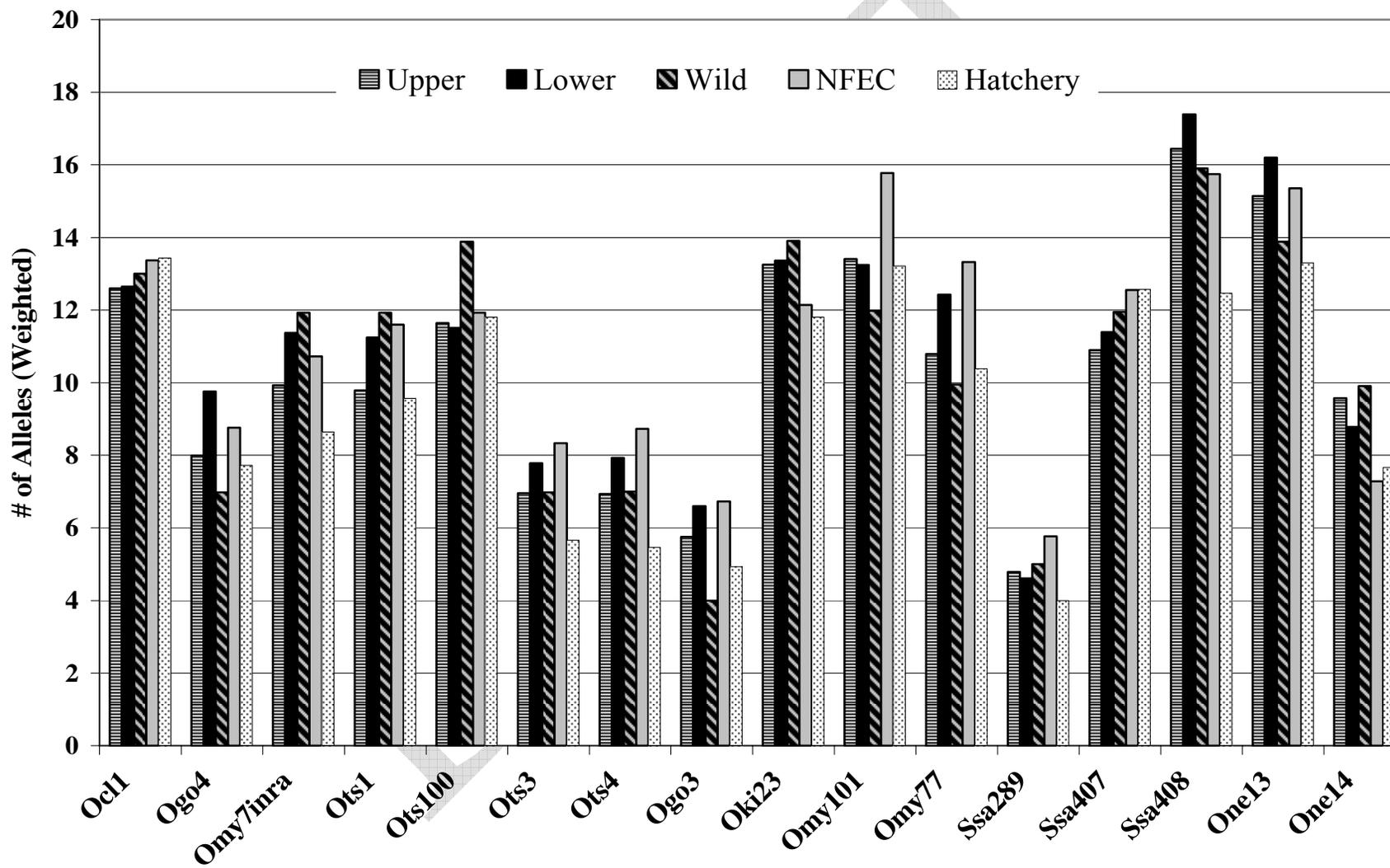


Figure 3. Un-rooted neighbor-joining phylogram based on Cavalli-Sforza & Edwards (1967) chord distance calculated from allele frequencies at 16 microsatellite loci. Pairwise genetic distances were calculated for NOR adults sampled at the lower ladder on Eagle Creek, smolts from the ECNFH, and for juveniles trapped within the upper, lower, and north fork sections of Eagle Creek. Bootstrap probabilities, based on 1000 replicates, provide a measure of statistical confidence at each branch in the topology; numbers at branch nodes and between clusters represent the percentage of times a topology occurred in simulated, random sampling replicates.

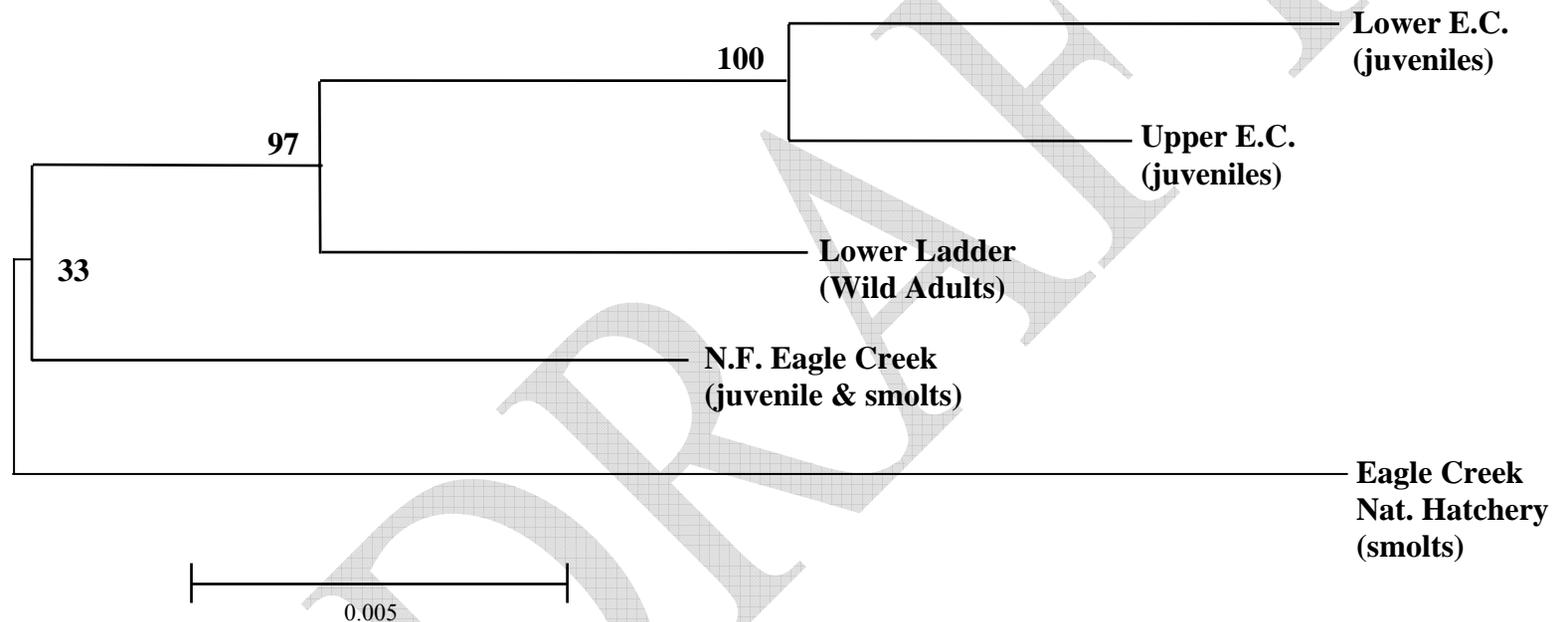
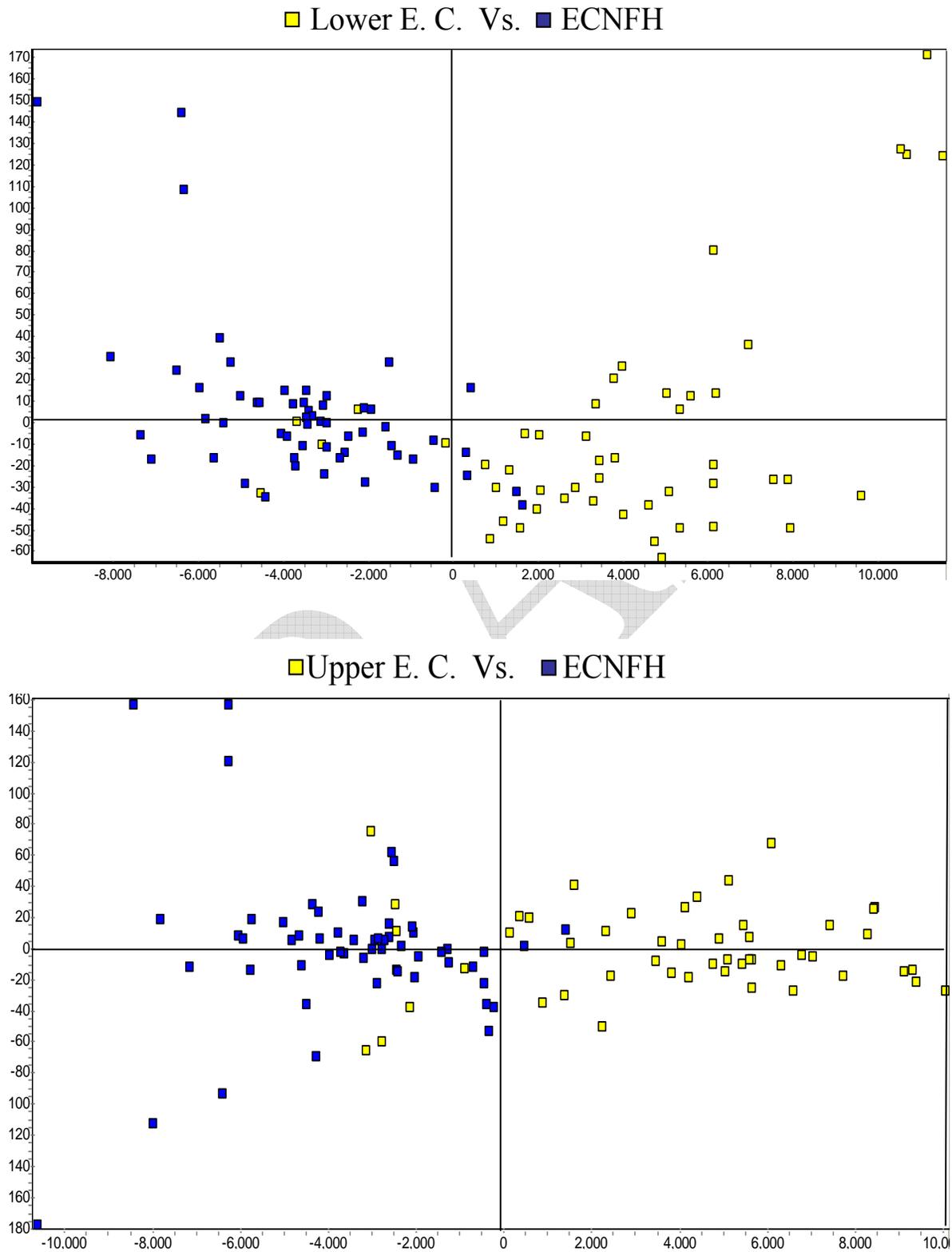
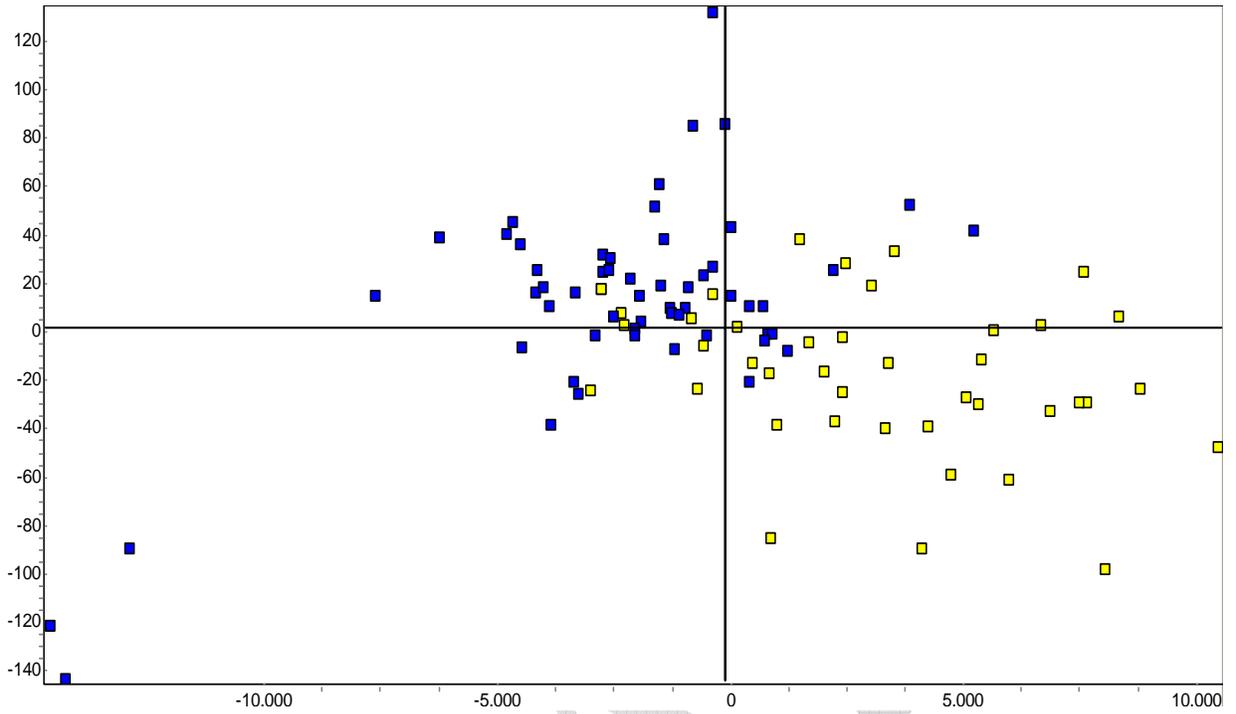


Figure 4. Factorial correspondence analysis plots for Eagle Creek steelhead groups.



■ NOR adults Vs. ■ ECNFH



■ N. Fork E. C. Vs. ■ ECNFH

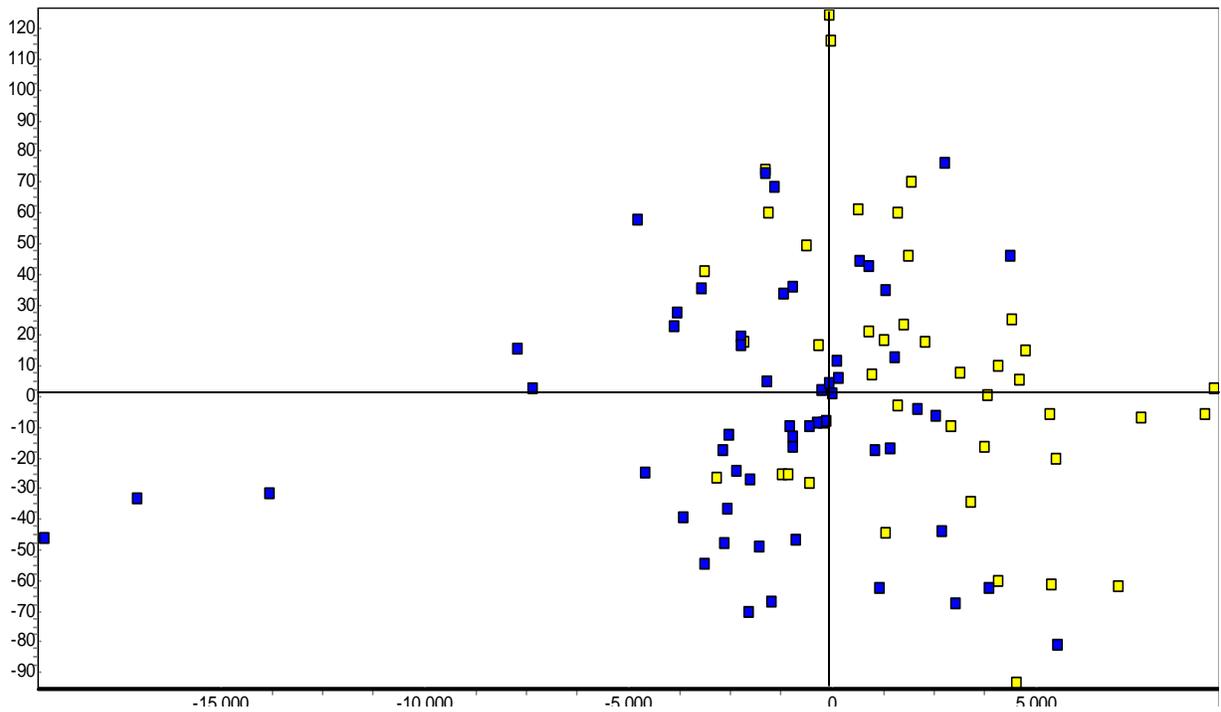


Figure 5. Distribution of LOD scores from the baseline jack-knife procedure. The baseline file consists of known hatchery juvenile steelhead and known wild adult returning steelhead. In an iterative procedure, one individual at a time is deleted from the baseline, allele frequencies in the baseline are then recalculated, and a LOD score is calculated for that individual. A LOD > 0 indicates the fish's genotype is more similar to the ECNFH (critical group). Likelihood of disposition-of-origin (i.e. HOR or NOR) increases logarithmically with LOD; for example LOD = 1 means an individual is 10 times more likely to be of hatchery origin, LOD = 2 means 100 times, etc.. A LOD < 0 indicates the fish's genotype was more similar to the adult NOR baseline. All individuals that scored above LOD = 1.97 were of hatchery origin, and all individuals that scored below LOD = -2.11 were of natural origin. Dotted lines correspond to the 95% confidence level; LOD < -1.66 for NOR, and LOD > 1.28 for HOR.

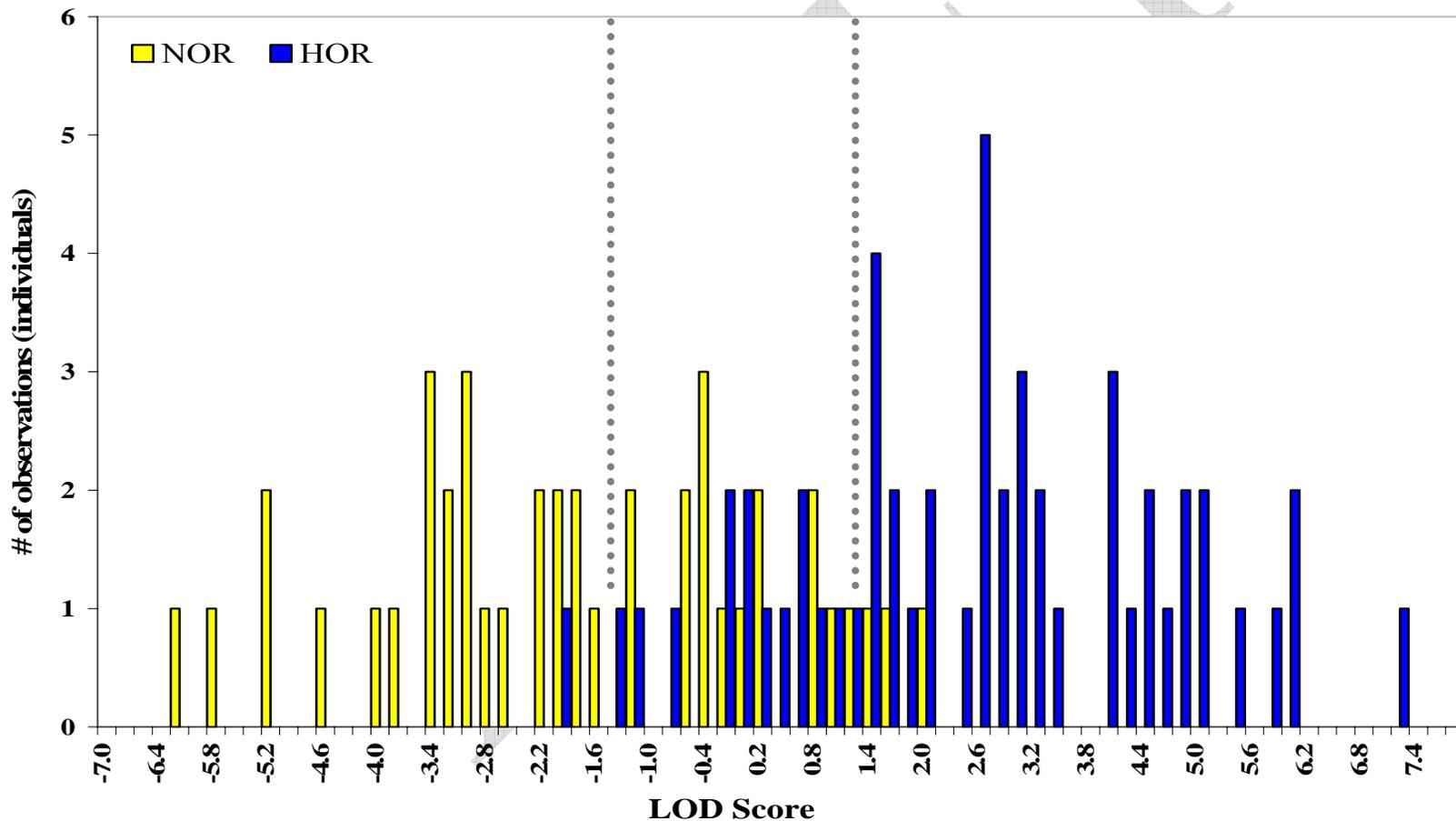
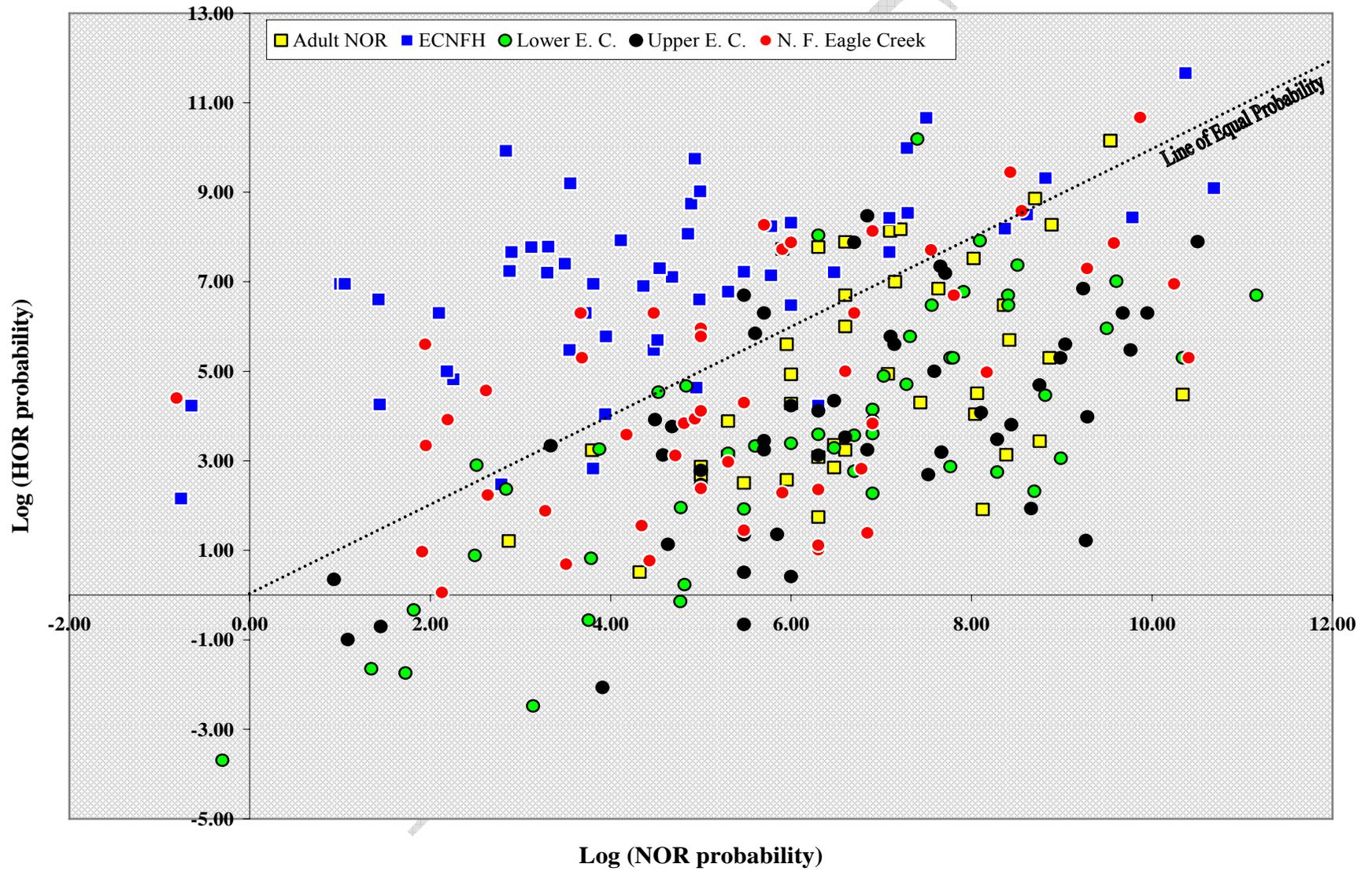


Figure 6. Population assignment probability plot of all groups and individuals. The trend-line delineates where an individual is equally likely to be of NOR origin as HOR origin.



Appendix 1. Biological data for Eagle Creek steelhead trout sampled for the 2005 population genetic evaluation. EC is Eagle Creek, NFEC is North Fork Eagle Creek, ECNFH is Eagle Creek National Fish Hatchery, “Between ECNFH & NFEC” is the UPPER section, “Lower Ladder” is NOR adults, and “Between NFEC& EC mouth” is the LOWER section. The last column is the LOD score generated in WHICHRUN, and used to assign individuals to origin (Table 3, Figure 5, Figure 6).

Location	Date	*Species	Sample ID	Length (mm)	Weight (kg)	LOD
N. Fork E. C.	27-Apr-05	WST	132056	161	37.4	-2.80
N. Fork E. C.	27-Apr-05	WST	132057	92	8.5	-0.40
N. Fork E. C.	27-Apr-05	WST	132058	137	24.9	-0.52
N. Fork E. C.	27-Apr-05	WST	132059	118	18.8	2.71
N. Fork E. C.	27-Apr-05	WST	132060	152	32.6	1.75
N. Fork E. C.	27-Apr-05	WST	132061	158	35.6	0.80
N. Fork E. C.	27-Apr-05	WST	132062	84	6.4	1.82
N. Fork E. C.	27-Apr-05	WST	132063	86	6.9	-1.99
N. Fork E. C.	27-Apr-05	WST	132064	84	6.3	-0.60
N. Fork E. C.	27-Apr-05	WST	132065	165	42.3	-0.94
N. Fork E. C.	27-Apr-05	WST	132067	114	13.1	1.39
N. Fork E. C.	27-Apr-05	WST	132068	84	6.8	-1.71
N. Fork E. C.	3-May-05	WST	132069	91	7.8	-1.60
N. Fork E. C.	3-May-05	WST	132071	107	11	1.72
N. Fork E. C.	3-May-05	WST	132072	97	8.4	0.16
N. Fork E. C.	5-May-05	WST	132075	102	10.1	-3.19
N. Fork E. C.	5-May-05	WST	132076	89	7.2	-1.00
N. Fork E. C.	5-May-05	WST	132077	112	14.8	-0.97
N. Fork E. C.	5-May-05	WST	132078	108	12.7	-0.85
N. Fork E. C.	5-May-05	WST	132079	114	15.8	1.54
N. Fork E. C.	10-May-05	WST	132082	108	12.5	-1.22
N. Fork E. C.	10-May-05	WST	132083	115	15.1	-1.09
N. Fork E. C.	10-May-05	WST	132084	114	12.2	0.95
N. Fork E. C.	10-May-05	WST	132085	107	13.6	-1.89
N. Fork E. C.	10-May-05	WST	132086	107	12.6	1.95
N. Fork E. C.	16-May-05	WST	132088	105	11.9	-1.39
N. Fork E. C.	16-May-05	WST	132089	111	13.6	-5.12
N. Fork E. C.	16-May-05	WST	132090	122	18.4	1.02
N. Fork E. C.	16-May-05	WST	132091	101	10.9	1.24
N. Fork E. C.	16-May-05	WST	132092	102	11.6	-2.24
N. Fork E. C.	16-May-05	WST	132093	106	13.1	-3.30
N. Fork E. C.	16-May-05	WST	132094	99	10.8	-3.07

N. Fork E. C.	16-May-05	WST	132095	104	11.6	-5.48
N. Fork E. C.	16-May-05	WST	132096	84	6.3	-3.88
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382001	120	18.6	-2.79
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382002	132	25.1	0.03
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382003	125	22.2	-0.97
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382004	140	29.4	-3.63
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382005	111	16.1	-2.82
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382006	115	15.6	-1.57
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382008	66	4.1	1.80
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382009	113	15.4	5.21
N. Fork E. C.	15-Aug-05	<i>O. mykiss</i>	382010	115	18.6	-3.67
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381001	115	21.5	-3.93
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381002	132	24.8	-4.05
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381003	119	17.1	-5.21
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381005	82	6	2.59
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381006	63	2.7	0.74
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381007	66	3	3.70
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381008	39	0.6	-2.07
N. Fork E. C.	2-Aug-05	<i>O. mykiss</i>	381009	35	0.4	-5.09
Upper E. C.	22-Sep-04	WST	130005	60	3.0	-4.29
Upper E. C.	22-Sep-04	WST	130007	45	1.3	-4.63
Upper E. C.	22-Sep-04	WST	130009	46	1.0	-3.60
Upper E. C.	22-Sep-04	WST	130011	52	1.5	-0.58
Upper E. C.	22-Sep-04	WST	130013	46	1.1	-3.08
Upper E. C.	22-Sep-04	WST	130015	95	8.1	-2.10
Upper E. C.	22-Sep-04	WST	130017	72	5.2	-0.52
Upper E. C.	22-Sep-04	WST	130022	77	6.1	-4.47
Upper E. C.	22-Sep-04	WST	130024	72	4.8	-2.61
Upper E. C.	22-Sep-04	WST	130027	61	2.3	-2.49
Upper E. C.	22-Sep-04	WST	130029	137	27.4	-0.58
Upper E. C.	22-Sep-04	WST	130032	54	1.9	-2.16
Upper E. C.	22-Sep-04	WST	130034	65	2.9	-0.31
Upper E. C.	22-Sep-04	WST	130036	49	1.4	-4.47
Upper E. C.	22-Sep-04	WST	130043	47	1.5	-3.08
Upper E. C.	22-Sep-04	WST	130046	71	4.9	-2.39
Upper E. C.	22-Sep-04	WST	130050	58	2.3	-2.42
Upper E. C.	28-Sep-04	WST	130052	67	3.2	-0.91
Upper E. C.	28-Sep-04	WST	130055	91	10.4	0.00
Upper E. C.	28-Sep-04	WST	130062	81	6.6	-6.16
Upper E. C.	28-Sep-04	WST	130064	63	3.1	-3.60
Upper E. C.	28-Sep-04	WST	130066	63	3.8	-4.80

Upper E. C.	28-Sep-04	WST	130068	50	1.7	-3.43
Upper E. C.	28-Sep-04	WST	130070	61	2.6	-1.93
Upper E. C.	10-Nov-04	WST	131001	70	4.5	-3.50
Upper E. C.	10-Nov-04	WST	131006	62	2.4	-3.38
Upper E. C.	10-Nov-04	WST	131008	75	5.1	-5.30
Upper E. C.	10-Nov-04	WST	131010	73	5.2	0.27
Upper E. C.	10-Nov-04	WST	131013	58	2.4	-6.73
Upper E. C.	10-Nov-04	WST	131015	72	4	-2.21
Upper E. C.	10-Nov-04	WST	131017	103	13.7	0.54
Upper E. C.	10-Nov-04	WST	131019	72	5	-1.45
Upper E. C.	10-Nov-04	WST	131021	55	1.9	-4.13
Upper E. C.	10-Nov-04	WST	131023	105	14.5	1.22
Upper E. C.	10-Nov-04	WST	131025	68	3.6	-5.98
Upper E. C.	10-Nov-04	WST	131028	50	1.6	-4.83
Upper E. C.	10-Nov-04	WST	131031	58	1.8	-4.06
Upper E. C.	10-Nov-04	WST	131033	69	3.8	-1.33
Upper E. C.	10-Nov-04	WST	131035	80	5.9	-4.03
Upper E. C.	10-Nov-04	WST	131037	55	2.2	-2.08
Upper E. C.	10-Nov-04	WST	131039	65	4.1	-2.59
Upper E. C.	10-Nov-04	WST	131041	70	3.6	-3.77
Upper E. C.	10-Nov-04	WST	131043	68	3.9	1.62
Upper E. C.	10-Nov-04	WST	131045	61	3.2	-2.37
Upper E. C.	10-Nov-04	WST	131048	53	2.1	-4.90
Upper E. C.	10-Nov-04	WST	131051	75	5.9	1.20
Upper E. C.	10-Nov-04	WST	131054	72	4.6	-2.18
Upper E. C.	10-Nov-04	WST	131057	77	5.3	-8.05
Upper E. C.	10-Nov-04	WST	131059	60	3	-1.57
Upper E. C.	10-Nov-04	WST	131061	57	2.3	-5.67
Lower E. C.	28-Sep-04	WST	130082	58	2.0	-2.44
Lower E. C.	28-Sep-04	WST	130083	60	3.1	-3.00
Lower E. C.	28-Sep-04	WST	130084	55	2.2	-2.14
Lower E. C.	28-Sep-04	WST	130085	59	2.4	-6.37
Lower E. C.	28-Sep-04	WST	130086	51	1.6	-1.55
Lower E. C.	28-Sep-04	WST	130087	48	1.1	-5.53
Lower E. C.	28-Sep-04	WST	130088	51	1.9	-0.61
Lower E. C.	28-Sep-04	WST	130090	59	2.7	-2.96
Lower E. C.	28-Sep-04	WST	130093	61	2.8	-4.43
Lower E. C.	28-Sep-04	WST	130095	65	3.5	-0.47
Lower E. C.	28-Sep-04	WST	130096	84	8.1	2.79
Lower E. C.	28-Sep-04	WST	130097	77	5.1	-1.14
Lower E. C.	28-Sep-04	WST	130098	59	2.2	-3.11

Lower E. C.	28-Sep-04	WST	130099	56	2.4	-2.15
Lower E. C.	28-Sep-04	WST	130100	52	1.6	-3.13
Lower E. C.	10-Nov-04	WST	131062	63	3.6	-3.47
Lower E. C.	10-Nov-04	WST	131063	80	6.8	-5.62
Lower E. C.	10-Nov-04	WST	131065	62	3.3	-3.54
Lower E. C.	10-Nov-04	WST	131066	75	5	-2.82
Lower E. C.	10-Nov-04	WST	131069	142	32.6	-0.17
Lower E. C.	10-Nov-04	WST	131070	114	17.9	-2.06
Lower E. C.	10-Nov-04	WST	131071	122	22.4	0.00
Lower E. C.	10-Nov-04	WST	131078	71	4.5	-2.59
Lower E. C.	10-Nov-04	WST	131079	76	6.3	-0.17
Lower E. C.	10-Nov-04	WST	131080	59	2.6	-5.08
Lower E. C.	10-Nov-04	WST	131081	83	6.9	-3.39
Lower E. C.	10-Nov-04	WST	131089	90	9	1.71
Lower E. C.	10-Nov-04	WST	131090	75	5.5	-1.61
Lower E. C.	10-Nov-04	WST	131091	55	2.4	-3.28
Lower E. C.	10-Nov-04	WST	131093	85	8	-1.14
Lower E. C.	10-Nov-04	WST	131098	63	3	-3.92
Lower E. C.	10-Nov-04	WST	131100	57	2.5	-2.58
Lower E. C.	10-Nov-04	WST	132001	83	7.1	-4.64
Lower E. C.	10-Nov-04	WST	132002	70	4.3	-2.75
Lower E. C.	10-Nov-04	WST	132005	80	6.7	-3.54
Lower E. C.	10-Nov-04	WST	132006	70	4.6	-4.91
Lower E. C.	10-Nov-04	WST	132007	60	3.1	-1.94
Lower E. C.	10-Nov-04	WST	132008	68	4	-5.94
Lower E. C.	10-Nov-04	WST	132009	80	6.6	-2.61
Lower E. C.	10-Nov-04	WST	132010	80	6.4	-2.27
Lower E. C.	10-Nov-04	WST	132011	65	3.6	-4.36
Lower E. C.	10-Nov-04	WST	132012	55	2.1	-4.59
Lower E. C.	10-Nov-04	WST	132013	70	3.6	-1.74
Lower E. C.	10-Nov-04	WST	132014	90	7.7	-1.14
Lower E. C.	15-Aug-05	<i>O. mykiss</i>	382011	53	1.8	0.39
Lower E. C.	15-Aug-05	<i>O. mykiss</i>	382012	135	26.2	-2.46
Lower E. C.	15-Aug-05	<i>O. mykiss</i>	382013	113	15.5	-4.89
Lower E. C.	15-Aug-05	<i>O. mykiss</i>	382014	143	33.9	-3.01
Lower E. C.	2-Aug-05	<i>O. mykiss</i>	381010	44	0.8	-2.67
Lower E. C.	2-Aug-05	<i>O. mykiss</i>	381011	38	0.5	-4.32
Adult NOR	25-Feb-05	WST	132016	580	--	1.04
Adult NOR	1-Mar-05	WST	132017	610	--	-1.93
Adult NOR	2-Mar-05	WST	132018	740	--	-2.13
Adult NOR	2-Mar-05	WST	132019	730	--	-2.37

Adult NOR	2-Mar-05	WST	132020	720	--	-0.56
Adult NOR	3-Mar-05	WST	132021	830	--	-3.37
Adult NOR	3-Mar-05	WST	132022	810	--	1.84
Adult NOR	3-Mar-05	WST	132023	680	--	-0.51
Adult NOR	4-Mar-05	WST	132024	660	--	-1.67
Adult NOR	8-Mar-05	WST	132025	650	--	1.27
Adult NOR	8-Mar-05	WST	132026	660	--	-2.11
Adult NOR	15-Mar-05	WST	132027	680	--	0.61
Adult NOR	17-Mar-05	WST	132028	670	--	0.61
Adult NOR	17-Mar-05	WST	132029	680	--	-0.47
Adult NOR	18-Mar-05	WST	132030	700	--	-0.35
Adult NOR	18-Mar-05	WST	132031	620	--	-0.62
Adult NOR	18-Mar-05	WST	132032	810	--	1.50
Adult NOR	18-Mar-05	WST	132033	690	--	-0.78
Adult NOR	18-Mar-05	WST	132034	650	--	-1.22
Adult NOR	18-Mar-05	WST	132035	760	--	0.05
Adult NOR	18-Mar-05	WST	132036	780	--	0.96
Adult NOR	18-Mar-05	WST	132037	860	--	-2.92
Adult NOR	18-Mar-05	WST	132038	770	--	-1.83
Adult NOR	18-Mar-05	WST	132039	690	--	-5.87
Adult NOR	18-Mar-05	WST	132040	700	--	-2.23
Adult NOR	18-Mar-05	WST	132041	760	--	0.15
Adult NOR	12-Apr-05	WST	132042	690	--	-5.25
Adult NOR	5-Apr-05	WST	132043	730	--	-3.12
Adult NOR	7-Apr-05	WST	132044	810	--	-5.32
Adult NOR	12-Apr-05	WST	132045	890	--	-4.62
Adult NOR	13-Apr-05	WST	132046	700	--	-6.22
Adult NOR	13-Apr-05	WST	132047	710	--	-0.16
Adult NOR	19-Apr-05	WST	132048	710	--	-3.80
Adult NOR	19-Apr-05	WST	132049	660	--	-3.56
Adult NOR	21-Apr-05	WST	132050	670	--	-2.72
Adult NOR	21-Apr-05	WST	132051	660	--	-3.56
Adult NOR	21-Apr-05	WST	132052	670	--	-3.36
Adult NOR	26-Apr-05	WST	132053	800	--	-1.31
Adult NOR	26-Apr-05	WST	132054	710	--	-4.02
Adult NOR	26-Apr-05	WST	132055	730	--	-3.14
Adult NOR	5-May-05	WST	132074	760	--	-3.58
Adult NOR	10-May-05	WST	132081	840	--	-3.14
ECNFH	2-Aug-05	HST	381012	76	5.4	4.65
ECNFH	2-Aug-05	HST	381013	76	5.4	3.91
ECNFH	2-Aug-05	HST	381014	86	7.6	-1.35

ECNFH	2-Aug-05	HST	381015	79	6.5	5.97
ECNFH	2-Aug-05	HST	381016	71	4.3	0.79
ECNFH	2-Aug-05	HST	381017	74	4.9	2.98
ECNFH	2-Aug-05	HST	381018	87	8.5	-0.32
ECNFH	2-Aug-05	HST	381019	76	5.2	-0.12
ECNFH	2-Aug-05	HST	381020	73	5.2	2.54
ECNFH	2-Aug-05	HST	381021	74	4.9	0.49
ECNFH	2-Aug-05	HST	381022	92	10.3	3.21
ECNFH	2-Aug-05	HST	381023	73	4.6	1.29
ECNFH	2-Aug-05	HST	381024	85	8.7	1.70
ECNFH	2-Aug-05	HST	381025	79	6.6	2.22
ECNFH	2-Aug-05	HST	381026	82	7.2	4.22
ECNFH	2-Aug-05	HST	381027	72	5.4	1.33
ECNFH	2-Aug-05	HST	381028	65	3.4	1.81
ECNFH	2-Aug-05	HST	381029	79	6.4	-1.60
ECNFH	2-Aug-05	HST	381030	79	5.8	0.32
ECNFH	2-Aug-05	HST	381031	92	9.9	2.43
ECNFH	2-Aug-05	HST	381032	80	6.3	1.98
ECNFH	2-Aug-05	HST	381033	80	6.8	2.47
ECNFH	2-Aug-05	HST	381034	83	6.7	2.56
ECNFH	2-Aug-05	HST	381035	91	10.1	4.48
ECNFH	2-Aug-05	HST	381036	88	9.2	2.70
ECNFH	2-Aug-05	HST	381037	83	7.9	0.98
ECNFH	2-Aug-05	HST	381038	74	4.8	2.76
ECNFH	2-Aug-05	HST	381039	77	5.5	3.16
ECNFH	2-Aug-05	HST	381040	80	5.6	-2.08
ECNFH	2-Aug-05	HST	381041	74	5.3	-0.98
ECNFH	2-Aug-05	HST	381042	82	5.3	2.91
ECNFH	2-Aug-05	HST	381043	80	6.3	1.25
ECNFH	2-Aug-05	HST	381044	82	5.8	1.13
ECNFH	2-Aug-05	HST	381045	69	5.4	0.57
ECNFH	2-Aug-05	HST	381046	94	10.8	5.92
ECNFH	2-Aug-05	HST	381047	83	7.2	3.91
ECNFH	2-Aug-05	HST	381048	87	9	5.22
ECNFH	2-Aug-05	HST	381049	82	6.8	1.57
ECNFH	2-Aug-05	HST	381050	86	8.1	1.59
ECNFH	2-Aug-05	HST	381051	75	5	2.51
ECNFH	2-Aug-05	HST	381052	76	5.4	-0.32
ECNFH	2-Aug-05	HST	381053	84	7	3.85
ECNFH	2-Aug-05	HST	381054	76	5.7	-0.18
ECNFH	2-Aug-05	HST	381055	82	6.8	1.34

ECNFH	2-Aug-05	HST	381056	80	6.6	3.81
ECNFH	2-Aug-05	HST	381057	72	4.3	7.09
ECNFH	2-Aug-05	HST	381058	80	6.4	4.89
ECNFH	2-Aug-05	HST	381059	88	8.2	3.13
ECNFH	2-Aug-05	HST	381060	86	7.4	4.76
ECNFH	2-Aug-05	HST	381061	79	6.2	2.81
ECNFH	2-Aug-05	HST	381062	68	3.2	0.11
ECNFH	2-Aug-05	HST	381063	65	3.5	4.03
ECNFH	2-Aug-05	HST	381064	63	3.5	4.81
ECNFH	2-Aug-05	HST	381065	64	3.5	5.64
ECNFH	2-Aug-05	HST	381066	65	3.5	3.94
ECNFH	2-Aug-05	HST	381067	60	2.5	4.36

* HST designates hatchery origin steelhead, WST designates winter steelhead spawned in the wild, and *O. mykiss* refers to juvenile rainbow trout, either anadromous or resident (life history unknown).