Ecological and Genetic Interactions between Hatchery and Wild Steelhead in Eagle Creek, Oregon

Final Report

Jeff Hogle and Ken Lujan collecting genetic and fish health samples in Eagle Creek, Oregon.
Photo Credit: Maureen Kavanagh

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Table of Contents

1.0 Executive Summary 9
2.0 Introduction 10
3.0 Geographic Area 12
4.0 Background Information on Winter Steelhead in the Clackamas River & Eagle Creek 13
5.0 Methods 14
5.1 Adult Return Timing, Distribution, and Abundance 14
5.1.1 Lower Ladder Trap Operations 2005-2007 14
5.1.2 Radio-Telemetry 2005-2007 15
5.1.3 Spawning Ground Surveys 16
5.1.4 Mark-Recapture Population Estimates 2007-2008 16
5.1.5 Length, Sex, and Age Class Structure 17
5.2 Smolt Migration Monitoring and Abundance 17
5.2.1 Radio-Tagging and Tracking of Steelhead Smolts 2005-2008 17
5.2.2 Smolt Trap Operation and Abundance Estimates 2006 and 2008 18
5.3 Distribution, Summer Rearing Density, and Habitat Selection of Juvenile Fish 19
5.3.1 Habitat Surveys 2007 19
5.3.2 Snorkel Surveys and Population Estimates 2007 20
5.4 Disease Profile 2005-2007 21
5.5 Genetic Analysis 22
5.5.1 Genetic Sample Collections 2005-2007 22
5.5.2 Microsatellite Amplification and Analysis 23
5.5.3 Species ID: hybrid screening 25
6.0 Results 25
6.1 Adult Steelhead Return Timing, Distribution and Abundance 25
6.1.1 Lower Ladder Trap Operations 2005-2007 25
6.1.2 Radio-Telemetry 2005-2007 26
6.1.3 Spawning Ground Surveys 27
6.1.4 Mark-Recapture Population Estimates 2007-2008 28
6.1.5 Length, Sex and Age Class Structure 28
6.2 Smolt Migration Monitoring and Abundance 28
6.2.1 Radio Tagging and Tracking of Steelhead Smolts 2005-2008 28
6.2.2 Smolt Trap Operations and Abundance Estimates 2006 and 2008 31
6.3 Distribution, Summer Rearing Density,and Habitat Selection of Juvenile Fish 32
6.3.1 Habitat Surveys 2007 32
6.3.2 Snorkel Surveys and Population Estimates 2007 32
6.3.3 Fish Distribution and Density 2007 32
6.3.4 Wild Fish Displacement by Residual Hatchery Winter Steelhead 33
6.4 Disease Profile 2005-2007 33
6.5 Genetic Analysis 34
6.5.1 Descriptive Statistics 2005-2007 34
6.5.2 Population Genetic Structure Analysis 35
6.5.3 Baseline Assignment Tests 36
7.0 Discussion 36
7.1 Adult Return Timing, Distribution, and Abundance 36
7.2 Smolt Migration Monitoring and Abundance 39
7.3 Distribution, Summer Rearing Density, and Mesohabitat Selection of Juvenile Fish 41
7.4 Disease Profile 43
7.5 Genetic Analysis 46
8.0 Recommendations 47
9.0 Tables 51
10.0 Figures 69
Literature Cited 106

Appendix 1: List of loci amplified for the Eagle Creek analysis (2005-2007). The symbol (*) designates a locus that is standardized in the Stephen Phelps Allele Nomenclature (SPAN) standardization effort. The annealing temperatures and MgCl2 concentrations are optimized PCR conditions for the conservation genetics program at Abernathy Fish Technology Center 116

Appendix 2: Results from wild fish health survey conducted on juvenile salmon and steelhead in Eagle Creek from 2004-2007. Unless otherwise noted, all fish were tested for the following fish pathogens: infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), infectious salmon anemia virus (ISAV), *Aeromonas salmonicida* (AS, furunculosis), *Yersinia ruckeri* (YR, enteric redmouth disease), *Flavobacterium columnare* (CD, columnaris), *Flavobacterium psychrophilum* (CWD, coldwater disease), *Renibacterium salmoninarum*, (RS, bacterial kidney disease), and *Myxobolus cerebralis* (MC. whirling disease). Larger fish were screened for RS using the enzyme-linked immunosorbent assay (ELISA); a positive finding of RS denotes that ELISA results were confirmed by a second test, the polymerase chain reaction assay (PCR). All methods for detecting and identifying fish pathogens were based on standardized procedures from the AFS Fish Health Blue Book (USFWS and AFS-FHS 2007) and the National Wild Fish Health Survey – Laboratory Procedures Manual (Puzach 2006). 117

Appendix 3: Results from fish health survey conducted on hatchery adults returning to Eagle Creek NFH and hatchery smolts prior to release from the hatchery in 2005-2007. Fish were tested for the following fish pathogens: infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), infectious salmon anemia virus (ISAV), *Aeromonas salmonicida* (AS, furunculosis), *Yersinia ruckeri* (YR, enteric redmouth disease), *Flavobacterium columnare* (CD, columnaris), *Flavobacterium psychrophilum* (CWD, coldwater disease), *Renibacterium salmoninarum*, (RS, bacterial kidney disease), and *Myxobolus cerebralis* (MC. whirling disease).All methods for detecting and identifying fish pathogens were based on standardized procedures from the AFS Fish Health Blue Book (USFWS and AFS-FHS 2007). For detection of RS in the hatchery fish, both the enzyme-linked immunosorbent assay (ELISA) and direct fluorescent antibody test (DFAT) is used for the adults and the DFAT alone is used for the juvenile fish. 121

List of Tables

Table 1: Number of hours per month the lower ladder was fished using V-trap fish weir in 2006 and 2007. 51

Table 2: Sample frame of available habitat units, number of habitat units selected for first phase snorkel surveys and number of habitat units selected for second phase calibration estimates, summer 2007..... 51

Table 3: Total numbers of genetic samples collected for each reach and sample year in Eagle and North Fork Eagle Creek. 52

Table 4: The estimated monthly catch of adult winter steelhead at the lower ladder on Eagle Creek in 2006 53

Table 5: The estimated monthly catch of adult winter steelhead at the lower ladder on Eagle Creek in 2007 53

Table 6: Last known locations of radio-tagged adult steelhead in Eagle Creek, 2005-2007..... 54

Table 7: Summary of winter steelhead spawning ground surveys conducted in Eagle Creek and North Fork Eagle Creek by Oregon Department of Fish and Wildlife and Bureau of Land Management. For a detailed description of survey reaches in Eagle Creek please see Hutchinson et al. 2008. Downstream and upstream reach boundaries for North Fork Eagle Creek are identified by river kilometer..... 55

Table 8: Population estimate for adult steelhead at the lower ladder on Eagle Creek. Confidence Intervals (95%) are reported in parentheses 56

Table 9: Age class structure and mean length of wild steelhead trapped at the lower ladder and hatchery fish sampled at Eagle Creek NFH..... 57

Table 10: Mean length, weight, and condition factor of radio-tagged hatchery smolts from low, medium, and high-density groups. Steelhead smolts that shed their tags in the hatchery raceways were excluded from analysis 58

Table 11: Mean migration time, fork length and condition factor for smolts released from Eagle Creek NFH and detected at the telemetry station at the mouth of Eagle Creek in 2003-2008. The number of fish that shed their tags in the raceways prior to release is in (italics), and these fish were excluded from all analyses. No siginficant difference in fork length or condition factor of migrant and mon-migrant steelhead smolts was detected with ANOVA..... 59

Table 12: Population estimates for smolts trapped in the rotary screw trap on Eagle Creek, 2006 and 2008. 60

Table 13: Estimated number of juvenile steelhead migrating from North Fork Eagle Creek, 1998- 2008. A 95% confidence interval is expressed as a percentage of the population estimate. Population estimates for 2006-2007, and confidence intervals for the 2008 estimates were not available at the time of this report. Peak capture dates were only available for some years. 61

Table 14: Summary of habitat unit characteristics of lower Eagle Creek, upper Eagle Creek, and North Fork Eagle Creek, summer 2007. 62

Table 15: Population estimates of juvenile fish in Lower Eagle Creek (LEC), Upper Eagle Creek (UEC), and North Fork Eagle Creek (NFEC) calculated from two phase snorkel surveys conducted during the summer of 2007. Confidence intervals (95%) are reported in parentheses. 63

Table 16: Descriptive statistics among the Eagle Creek Steelhead genetic collections for combined years 2005-2007. Column headings are defined as follows: n is the number of individuals, A is the number of alleles, AR is the allelic richness, 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} in the index of inbreeding, and θ is the unbiased estimate of Wright's F_{ST} (Weir and Cockerham 1984). Values with the symbol (*) indicate statistical significance (Rice 1989; α= 0.05 adjusted α/k by)..... 64

Table 17: The top matrix provides the pairwise Fst values for all comparisons across temporal replicate collections. Significant among-group variation is indicated in the bottom matrix, and is based on 20,000 permutations and an adjusted P-value (*0.0005) multiple tests. 67

Table 18: Proportion of correct assignments by year and group using the jackknife re-sampling procedure. A positive LOD score for HAT (ECNFH) individuals from the designated "critical" population indicated a correct assignment, while a negative LOD represented correct assignments among NOR individuals within independent comparisons with HAT. The HAT samples were pooled across years. (2005-2007)……68

List of Figures

Figure 1: Map of Eagle Creek and North Fork Eagle Creek study area. Stationary telemetry receivers were located at the mouth, on the North Fork, and at the hatchery. Smolt traps were operated in Eagle Creek (above the confluence with North Fork Eagle Creek) and in North Fork Eagle Creek. Adult winter steelhead were radio and Floy tagged at the lower ladder. 69

Figure 2: Map showing the spatial distribution of first phase snorkel units and second phase diver calibration units in Eagle Creek and North Fork Eagle Creek. 70

Figure 3: Length-frequency distribution of O. mykiss and coho salmon captured from Eagle Creek and North Fork Eagle Creek while electrofishing, summer 2007. O. mykiss age 0 and O. mykiss age 1 fish are separated by a fork length of 110mm which was verified by scale analysis. 71

Figure 4: Results from second phase snorkeler calibrations in riffle habitat for O. mykiss and coho salmon, summer 2007. The black line is the estimated regression of the "true" fish population explained by single pass diver counts. The red lines represent the 95% confidence bounds for the estimated mean. The equation for the regression line, correlation coefficient (r^2), and calibration ratio (R) are given for each species. 72

Figure 5: Results from second phase snorkeler calibrations in pool habitat for O. mykiss and coho salmon, summer 2007. The black line is the estimated regression of the "true" fish population explained by single pass diver counts. The red lines represent the 95% confidence bounds for the estimated mean. The equation for the regression line, correlation coefficient (r^2), and calibration ratio (R) are given for each species. To allow for visualization of all data some points are jittered along the x-axis. 73

Figure 6: Results from second phase snorkeler calibrations in glide habitat for O. mykiss and coho salmon, summer 2007. The black line is the estimated regression of the "true" fish population explained by single pass diver counts. The red lines represent the 95% bounds for the estimated mean. The equation for the regression line, correlation coefficient (r^2), and calibration ratio (R) are given for each species. To allow for visualization of all data some points are jittered along the x-axis. 74

Figure 7: Monthly catch of adult winter steelhead at the lower ladder on Eagle Creek 2005-2007. 75

Figure 8: Distribution and movement of radio-tagged hatchery males in 2005. Steelhead that shed tags at the lower ladder are not included on this graph. 76

Figure 9: Distribution and movement of radio-tagged hatchery females in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.. 77

Figure 10: Distribution and movement of radio-tagged hatchery steelhead of unknown sex in 2005. Steelhead that shed tags at the lower ladder are not included on this graph. 78

Figure 11: Distribution and movement of radio-tagged wild male steelhead in 2005. Steelhead that shed tags at the lower ladder are not included on this graph. 79

Figure 12: Distribution and movement of radio-tagged wild female steelhead in 2005. Steelhead that shed tags at the lower ladder are not included on this graph. 80

Figure 13: Distribution and movement of radio-tagged wild steelhead of unknown sex in 2005. Steelhead that shed tags at the lower ladder are not included on this graph. 81

Figure 14: Distribution and movement of radio-tagged hatchery males in 2006. Steelhead that shed tags at the lower ladder are not included on this graph. 82

Figure 15: Distribution and movement of radio-tagged hatchery females in 2006. Steelhead that shed tags at the lower ladder are not included on this graph. 83

Figure 16: Distribution and movement of radio-tagged wild males in 2006. Steelhead that shed tags at the lower ladder are not included on this graph. 84

Figure 17: Distribution and movement of radio-tagged wild females in 2006. Steelhead that shed tags at the lower ladder are not included on this graph. 85

Figure 18: Distribution and movement of radio-tagged hatchery males in 2007. Steelhead that shed tags at the lower ladder are not included on this graph. 86

Figure 19: Distribution and movement of radio-tagged hatchery females in 2007. Steelhead that shed tags at the lower ladder are not included on this graph. 87

Figure 20: Distribution and movement of radio-tagged wild males in 2007. Steelhead that shed tags at the lower ladder are not included on this graph. 88

Figure 21: Distribution and movement of radio-tagged wild females in 2007. Steelhead that shed tags at the lower ladder are not included on this graph. 89

Figure 22: Migration time (in days) from Eagle Creek NFH to the mouth of Eagle Creek for winter steelhead smolts released in 2003-2008. A linear regression showed no relationship between fork length and migration time for any of the years evaluated. The black lines represent the estimated regression of smolt migration for a given fork length, and the red lines represent the 95% confidence limits. The correlation coefficient (r^2) and p-value are given for each year. 90

Figure 23: Thermographs of the three study reaches from July 18th to October 2nd 2007. Snorkel surveys were conducted between July 10th and September 14th 2007. 91

Figure 24: Bubble scatter plots of O. mykiss age 0 densities from sampled habitat units of Eagle Creek and North Fork Eagle Creek, summer 2007. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 1,085 fish in the Eagle Creek plot and 135 fish in the North Fork Eagle Creek plot. Three points of reference are labeled in the Eagle Creek plot: first detection of residual hatchery winter steelhead (red line), the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line). 92

Figure 25: Bubble scatter plots of O. mykiss age 1 densities from sampled habitat units of Eagle Creek and North Fork Eagle Creek, summer 2007. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 260 fish in the Eagle Creek plot and 43 fish in the North Fork Eagle Creek plot. Three points of reference are labeled in the Eagle Creek plot: first detection of residual hatchery winter steelhead (red line), the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line). 93

Figure 26: Bubble scatter plots of coho salmon densities from sampled habitat units of Eagle Creek and North Fork Eagle Creek, summer 2007. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 457 fish in the Eagle Creek plot and 722 fish in the North Fork Eagle Creek plot.Three points of reference are labeled in the Eagle Creek plot: first detection of residual hatchery winter steelhead (red line), the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line). 94

Figure 27: Bubble scatter plots of residual hatchery winter steelhead densities from sampled habitat units of Eagle Creek, summer 2007. Residual hatchery winter steelhead were not observed in North Fork Eagle Creek. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 50 fish. The two points of reference labeled in this plot are the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line). 95

Figure 28: Box-and-whisker plots of O. Mykiss age 0 densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA detected density differences between stream reaches (χ^2 = 37.1, *P* < 0.001) and different letters above the x-axis represent significant differences (P < 0.05) in pairwise comparisons..... 96

Figure 29: Box-and-whisker plots of O. Mykiss age 1 densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA did not detected density differences between stream reaches (χ^2 =1.82, *P* = 0.40) therefore the same letter is used above the x-axis to represent no significant differences (P < 0.05) in pairwise comparisons. 97

Figure 30: Box-and-whisker plots of residual hatchery winter steelhead densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA detected density differences between stream reaches (χ^2 = 33.7, *P* < 0.001) and different letters above the x-axis represent significant differences (P < 0.05) in pairwise comparisons..... 98

Figure 31: Box-and-whisker plots of coho salmon densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA detected density differences between stream reaches (χ^2 = 30.8, *P* < 0.001) and different letters above the x-axis represent significant differences (P < 0.05) in pairwise comparisons..... 99

Figure 32: Number of sampled habitat units in Eagle Creek where fish species were observed, summer 2007. Residual hatchery winter steelhead is abbreviated R-HWST. 100

Figure 33: Distribution of pairwise among-group variation (Fst) displayed graphically for each pair of collections across years (2005-2007). Comparisons are arranged by NOR group. The symbol Δ indicates pairwise Fst comparisons between temporal replicate collections in four NOR groups (A-D), and (*) indicates all HAT pairwise comparisons with NOR-2007 collections. The dashed line marks the global F_{ST} across all collections, and the dotted lines indicate the 95% confidence interval for significance. 101

Figure 34: Un-rooted neighbor-joining tree: The phylogram topology was constructed from pairwise genetic (chord) distances (Cavalli-Sforza & Edwards 1967), and includes the 2005-2007 Eagle Creek steelhead collections evaluated in this study, and 2001 SPAN standardized data (*) contributed by Maureen Waite and Paul Moran from NOAA fisheries. Bootstrap support among 1000 replicate data sets is shown between branch nodes, indicating concordance level among loci for each branch in the topology. 102

Figure 35: Bar plot of proportional population membership from the program STRUCTURE version 2.2. The K=3 inferred population clusters are each represented by a different color (e.g. the red cluster found largely among HAT samples). Proportional membership is displayed within each of 15 collections evaluated, where the x-axis is individual fish. The upper plot displays samples (individual fish) within collections arranged by time of return/capture. The bottom plot is arranged by collection and *Q* membership among clusters. 103

Figure 36-39: Population assignment likelihood plots for all groups and replicate collections. Likelihood values are derived from the jackknife re-sampling method. Data is presented as the absolute value of the log likelihood for assignment to HAT and a given NOR group (XY coordinates); the log likelihood ratio is the LOD score (Table 18). The diagonal line delineates where an individual is equally likely to be of NOR origin as HAT origin. Comparisons are: a) UEC & HAT, b) LEC & HAT, c) NFEC & HAT, and d) adult NOR & HAT. 104

1.0 Executive Summary

The purpose of this project was to determine the ecological and genetic interactions between a hatchery stock of steelhead raised at Eagle Creek National Fish Hatchery for harvest and a U.S. Endangered Species Act threatened steelhead population in Eagle Creek, Oregon. We evaluated adult returns, hatchery smolt releases, abundance and habitat selection of juvenile fish, and genetics of hatchery and wild origin steelhead. From these evaluations we : 1) determined hatchery and wild adult return timing, distribution and abundance 2) determined migration time for juvenile hatchery steelhead from the release site to the mouth of Eagle Creek 3) determined the distribution and summer rearing densities of juvenile salmonids in Eagle Creek and North Fork Eagle Creek 4) determined the presence or absence of residual hatchery steelhead, and if present, determined what impact if any, they have on mesohabitat (riffle, pool, and glide) selection of juvenile wild salmonids 5) determined the genetic contribution of hatchery and wild fish to natural production and 6) determined the fish health and disease profile of hatchery and wild fish in Eagle Creek. During the study, adult and juvenile steelhead were radio-tagged and monitored through a combination of mobile and fixed telemetry stations, snorkel and habitat surveys were conducted on Eagle Creek and North Fork Eagle Creek, and health and genetic samples were collected from hatchery and naturally produced adult and juvenile fish. Fish health data indicated that hatchery fish at Eagle Creek posed little risk of disease transmission to wild fish. Telemetry, snorkel, and genetic data indicated that few to no hatchery steelhead occupied the North Fork Eagle Creek and we concluded that hatchery steelhead posed a low/acceptable risk to the wild population in that tributary. However telemetry data also indicated that less than 25% of radio-tagged adult hatchery fish returned to Eagle Creek National Fish Hatchery for brood stock collection. Even with a moderate to high harvest on hatchery fish, a substantial number of hatchery steelhead remained in Eagle Creek. Spawning surveys conducted by Oregon Department of Fish and Wildlife confirmed presence of hatchery fish on redds in upper Eagle Creek, most notably in February. Genetic analyses indicated that in some years natural production in upper Eagle Creek was influenced by natural spawning of hatchery steelhead. Despite overlaps in return timing, gene flow between hatchery and wild fish was minimal (i.e. hatchery fish mostly spawned with other hatchery fish); however this was recognized as a moderate risk level to ESA listed fish in Eagle Creek. From our findings, we also concluded that hatchery fish may pose an ecological risk to wild steelhead in Eagle Creek, particularly at the juvenile/smolt stage. The occurrence of non-migrating and presence of residual juvenile hatchery steelhead in upper and lower Eagle Creek was confirmed through radio-telemetry and snorkel surveys. We found that hatchery residuals made up approximately nine percent of the yearling stream population and one percent of the subyearling stream population of steelhead. Although no displacement of wild fish was documented, the abundance of hatchery residuals may still pose a risk to the wild steelhead in Eagle Creek. We recommend continued sampling during summer low flows to further evaluate the effects of juvenile hatchery steelhead residuals on wild steelhead. We also recommend the following hatchery practices at the 100,000 or lower smolt production level: spawn hatchery

steelhead no later than March 15 and preferably all takes completed by the end of February in order to retain the early spawn and run timing of hatchery fish; keep the hatchery ladder open through April to remove hatchery fish from Eagle Creek; conduct hatchery evaluation studies such as evaluating the downstream migration rate of forced vs. volitionally released juvenile fish from the hatchery to achieve quick outmigration of smolts released from the hatchery; compare productivity in North Fork Eagle Creek to that in upper Eagle Creek using spawner recruitment rates and smolt to adult recovery rates to assess the impact of naturally spawning hatchery fish on fitness and productivity of the natural population; continue periodic fish health sampling on hatchery and wild fish to monitor disease risks; and adaptively manage the program in the future, weighing the benefits and risks of alternative production programs, including continuing a segregated brood stock for harvest vs. developing an integrated brood stock for conservation and harvest vs. terminating the hatchery steelhead program.

2.0 Introduction

Eagle Creek National Fish Hatchery (NFH) spawns and rears juvenile coho salmon (*Oncorhynchus kisutch*) and juvenile steelhead trout (*O. mykiss*) that are released into Eagle Creek within the Clackamas River Basin, Oregon. The purpose of the program is to mitigate fish losses in the Columbia River Basin caused by the federal hydropower system, as well as providing commercial, sport, and tribal harvest opportunities, and to support tribal restoration programs upstream of Bonneville Dam. Eagle Creek NFH currently operates as part of the Columbia River Fisheries Development Program and is funded through the Mitchell Act - a program administered by NOAA Fisheries. On-station releases of age 1 steelhead smolts averaged 176,000 per year (range 113,000 – 207,000) over the period 1990-2007. Over this same time period, approximately 836,325 coho were annually released on-station and from 1995-2007, 822,373 were annually transferred to Yakama Nation and Nez Perce tribal programs. As a result of recommendations in a recent U.S. Fish and Wildlife Service review of the hatchery, the annual release target into Eagle Creek was reduced in spring of 2008 to 100,000 age1 steelhead smolts and 350,000 coho smolts in order to reduce ecological risks (USFWS 2007). The necessity for further reductions in hatchery production of winter steelhead at Eagle Creek NFH is pending the outcome of Mitchell Act funding and from results of this study. The focus of this report addresses ecological and genetic interactions between hatchery and wild steelhead in Eagle Creek.

Eagle Creek NFH commenced winter steelhead spawning operations in late spring 1957 using Clackamas River natural-origin winter-run steelhead as brood stock. The fish were released as either one-year-old yearlings ranging in size from 16 to 150 fish per pound or two-year-old smolts ranging from 6 to 10 fish per pound and adults returned from April through mid-June with peak returns in May. The hatchery continued spawning and releasing this native stock through 1965 when on-station rearing limitations and the call for an early season fishery in the basin necessitated the switch to an earlier returning stock. The current brood stock used for winter steelhead production is derived from a composite of native Clackamas River late run, Big Creek, and

Skamania River stocks. The Big Creek winter run brood stock is from Oregon Department of Fish and Wildlife's (ODFW) Big Creek Hatchery, located in the lower Columbia River, and is characterized by high survival rate in the hatchery, and early return- and spawn-times.

Hatchery production of steelhead at Eagle Creek NFH provides sport fishery opportunities in Eagle Creek, Clackamas and Willamette rivers. Previous estimates of harvest contribution suggests that for every fish returning to the hatchery another one to two fish is harvested in the sport fishery (USFWS 2007).

Within Eagle Creek there is also natural production of winter steelhead. Naturally produced steelhead in Eagle Creek are a population component of a Distinct Population Segment (DPS) having "threatened" status under the Endangered Species Act (ESA; Lower Columbia River DPS, 63 FR 13347; March 19, 1998).

Hatchery mitigation programs operating in watersheds where an ESA listed stock is present are of special concern. Releasing hatchery fish into natural water systems pose ecological risks that can harm naturally produced fish (Kostow 2008; Ham and Pearson 2001; McMichael et al. 1997). Non-native stock introductions have been identified as one of the causes for the decline in numbers of Clackamas River late-run winter steelhead returning to the North Fork Dam (Shibahara 1997). Following release from the hatchery, stocked salmonids can interact with wild salmonids through a variety of mechanisms, including competition for food and habitat (Jacobs 1981, Bachman 1984), predation (Cannamela 1993), spread of disease (Ratliff 1981; Goede 1986) and behavioral disturbances (Berejikian et al. 1999; McMichael et al. 1999). These ecological impacts from releases of hatchery steelhead trout on populations of wild salmonids are highest when hatchery fish do not emigrate quickly (i.e. residual steelhead) (McMichael et al. 2000, Viola and Schuck 1995; McMichael et al. 1997; McMichael et al. 1999; Brostrom 2003). Results from a 2003 study in Eagle Creek suggested that hatchery steelhead may not be emigrating quickly from Eagle Creek (Hoffman et al. 2003).

Genetic risks are another concern. The potential for genetic interactions between hatchery and wild fish can occur directly through natural spawning and interbreeding; indirectly though competition, predation, and behavior; or through a combination of the two mechanisms (Campton 1995). Several studies have documented low lifetime natural reproductive success of hatchery origin steelhead trout, particularly when they originate from out-of-basin multigenerational hatchery programs (Waples 1999; Araki et al. 2007). Naturally produced hatchery steelhead progeny may have high survival through the first year (age 0) followed by increased mortality before reaching the smolt stage (Chilcote et al. 1986), and/or subsequent low escapement rate (i.e. smolt-to-adult survival; Kostow et al. 2003). These potential genetic risks have led to recommendations for hatchery programs operating where ESA listed natural fish populations exist. The Hatchery Science Review Group (HSRG 2009a) has recommended for segregated hatchery programs that the proportions of hatchery origin fish spawning naturally should not exceed 5% of the total spawning population (pHOS <

5%). Hatchery steelhead that spawn naturally (interbreed) with wild fish represent an unknown genetic or fitness risk to the wild population in Eagle Creek.

The U.S. Fish and Wildlife Service (USFWS) Columbia River Fisheries Program Office (CRFPO), Lower Columbia River Fish Health Center and Abernathy Fish Technology Center cooperatively worked together to collect and analyze data on hatchery and natural origin steelhead in Eagle Creek as part of an overall ecological and genetic interactions monitoring and evaluation effort. The goal of this collaborative effort is to improve hatchery operations within the context of watershed and ecosystem management. Our study objectives for winter steelhead in the Eagle Creek Basin were to: 1) determine hatchery and wild adult return timing, distribution and abundance 2) determine migration time for juvenile hatchery steelhead from the release site to the mouth of Eagle Creek 3) determine the distribution and summer rearing densities of juvenile salmonids in Eagle Creek and North Fork Eagle Creek 4) determine the presence or absence of residual hatchery steelhead, and if present, determine what impact if any, they have on mesohabitat selection of juvenile wild salmonids 5) determine the genetic contribution of hatchery and wild fish to natural production and 6) determine the fish health and disease profile of hatchery and wild fish in Eagle Creek.

Additionally we include findings from our cooperators, including estimates of natural smolt production (U.S. Forest Service) and spawning ground abundance estimates (ODFW).

3.0 Geographic Area

The Clackamas River originates from the west slope of the Cascade Mountains between Mount Hood and Mount Jefferson and flows 134 km to its mouth at river kilometer 40 on the Willamette River (ODFW 1992). The watershed encompasses approximately 2,435 square kilometers and seventy five percent of this land is in federal forestlands managed by US Forest Service and Bureau of Land Management (Shibahara 1997). Eagle Creek, one of the major tributaries to the lower Clackamas River, originates in the Mount Hood National Forest and flows northwest where it enters the Clackamas River at river kilometer 27. Eagle Creek NFH is located at river kilometer 19.8, approximately 0.5 kilometers below the upper falls on Eagle Creek. The hatchery operates fish ladders located at the lower (rkm 8) and middle falls (rkm 15) to allow for fish passage and a ladder at the hatchery to collect brood stock and surplus hatchery fish. The upper falls are an impassable fish barrier. The hatchery operates a Smith-Root electric weir (Smith, Root, Inc. Vancouver, WA USA) located approximately 3 meters upstream of the hatchery ladder. This electric barrier directs fish into the hatchery ponds via the ladder.

4.0 Background Information on Winter Steelhead in the Clackamas River & Eagle Creek

In the Lower Columbia River Basin, most winter steelhead are between 4-6 years of age, 50-91cm in length and 2-8kg in weight at first spawn (Myers et al. 2003). Wild Clackamas River winter steelhead spawn predominately at four and five years of age (Chilcote 1998). Wild winter steelhead enter the Clackamas River as sexually mature fish from December through May with peak spawning in April and early May (McElhany et al. 2007). Smolt age is typically two years (yearling) and approximately 140mm, but age at downstream migration can range from one to three years (ODFW 2006 Hatchery and Genetic Management Plan). Steelhead typically spend two years in the ocean before returning to freshwater; however, populations in Oregon have an increased number of 1-year ocean winter steelhead. While most winter steelhead die after spawning, the frequency of two spawning migrations for many populations in Oregon and California is greater than for populations north of Oregon (Howell et al. 1985). Kelt survival for populations in the Clackamas is estimated at 10-11% (McElhany et al. 2007; Chilcote 2001).

Historically, 24 demographically unique populations of steelhead (18 winter run and 6 summer run) are thought to have existed in the Lower Columbia River DPS (Myers et al. 2003). Of the eighteen winter runs, three stocks return to the Clackamas River Subbasin. These include the native Clackamas River stock, an integrated hatchery stock reared at Clackamas State Fish Hatchery, and the Eagle Creek hatchery stock.

From 1905 to 1939, upstream migration was blocked at times by dams in the Clackamas River; however, winter steelhead have been reestablished and sustained in the upper drainage (ODFW 1992; Gunsolus and Eicher 1970). The 1990 to 2004 winter steelhead counts at the North Fork Dam in the Clackamas River have ranged from 189 fish in 1998 to 3,941 in 2003, with the fifteen year average of 1,526 fish. The recruit per spawner ratio (R/S) was estimated as R/S < 1.0 for brood years 1988-95, but R/S greatly increased for brood years 1996-99 ranging from 2 to 12 recruits per spawner (ODFW 2006). These fish are part of the coastal genetic group (Reisenbichler et al.1992; Schreck et al. 1986) genetically distinct from populations in upper Willamette River basin above Willamette falls.

The most productive habitat and main spawning area for winter steelhead in the Clackamas River is in the upper basin (McElhany et al. 2007). Although the majority of naturally produced steelhead in the Clackamas River system occurs in the upper Clackamas River (upstream of North Fork Dam), the lower Clackamas River and the Eagle Creek watershed is a valuable contributor to overall production in the basin. North Fork Eagle Creek (which enters Eagle Creek at rkm 10, just upstream of the lower ladder) is believed to be the primary natural spawning area in the Eagle Creek watershed (ODFW 1992).

Within the Clackamas River basin, including Eagle Creek, the natural origin steelhead occur from April through mid-June and peaks in May. Returning hatchery origin steelhead from the Eagle Creek NFH program are in Eagle Creek from November through April, with peak return activity occurring between December and mid March. Limited information exists on the age composition for wild steelhead returning to the lower Clackamas River, including Eagle Creek. The segregated return timing between the hatchery and wild adult populations is managed to allow for a fishery which targets harvestable early run hatchery fish with the intent to have minimal impact on the natural population. Similarly, differential spawning times are managed to minimize the opportunity for interbreeding (i.e. genetic introgression) between hatchery and wild steelhead in the Eagle Creek Basin (ODFW 1992). The area downstream of the North Fork Dam on the Clackamas River is managed for hatchery and wild fish, where the area upstream of the dam is managed for wild fish and no hatchery fish have been intentionally passed upstream since 1997 (Todd Alsbury, ODFW, pers. com).

5.0 Methods

5.1 Adult Return Timing, Distribution, and Abundance

5.1.1 Lower Ladder Trap Operations 2005-2007

Adult fish returning to Eagle Creek were captured using a V-trap fish weir located within the lower ladder on Eagle Creek. Located below the confluence of Eagle Creek and North Fork Eagle Creek (Figure 1), the lower ladder was closed periodically from January through June in 2005-2007 to trap adult winter steelhead. During the peak of the hatchery run (January – February), the trap was fished in the morning between 9-10 am, checked at noon, and if necessary, checked again between 2-4 pm. If fish were present in the ladder at noon, they were removed for sampling and trapping operations ceased for the day. If fish were present in the trap after 2 pm, they were removed for sampling, and the trap was either removed for the evening or left in place to capture fish overnight. Trapping duration at the lower ladder increased from March-June to compensate for the relatively low numbers of hatchery and wild fish migrating upstream. The trap was fished in the morning, checked between 2-4 pm, left to fish overnight, and checked again the next morning (Table 1).

Fish captured in the lower ladder were placed in a 110 gallon water filled tub and information on sex, fork length (cm), presence of marks, and origin (wild/hatchery) were recorded. Marks consisted of missing fins and maxillary clips. Hatchery fish were differentiated from wild fish by the lack of an adipose and/or other fins. Scale samples were collected from all wild steelhead to determine age (see section 5.1.5) and a tissue sample was collected for genetic stock identification analysis by the USFWS Abernathy Fish Technology Center (see Section 5.5).

Daily catch in the trap was summarized by month. Trapping effort (hours) was recorded in 2006 and 2007 and trap catch per hour was calculated. Average monthly catch per unit effort (CPUE) was weighted by effort (trap hours). Upper and lower confidence intervals were calculated for the bounds around the mean CPUE as described by Snedecor and Cochran (1989). Total hours by month multiplied by trap catch per hour were used to estimate total abundance of hatchery and wild steelhead by month.

95% confidence intervals were calculated from the following equation:

$$95\%CI = Mean \pm 1.96\sqrt{\frac{\text{var}}{n}} \qquad \text{Equation 1}$$

We reported the lower confidence limit as zero if the calculated value was negative as negative values were not meaningful.

5.1.2 Radio-Telemetry 2005-2007

Biologists visually evaluated the overall condition of fish at the lower ladder and only those determined to be “strong and healthy” were selected for radio-tagging (i.e. no fish with open wounds and deteriorating bodies due to the physiological demand of spawning).

Fish selected for tagging received a coded radio-transmitter (Lotek Wireless; model MCFT-3A), which was gastrically inserted through the esophagus into the stomach. Each transmitter emitted a unique code, which allowed individual fish to be detected. Transmitters were tuned to distinct frequencies within the 161-164 MHz frequency range with a 3 second burst rate and an estimated battery life of 394 days. They were 4.6 cm in length, 1.6 cm in diameter and weighed 16 grams. One hundred radio-tags were available in 2005, and 50 were available in 2006 and 2007. To reduce tag loss in 2006 and 2007, the radio-tags were wrapped in rubber bands prior to implantation in the fish (Keefer et al. 2004). Tagged fish were placed in a tub of fresh water and allowed to recover from the tagging process before being released back in the ladder or just upstream of the ladder.

Three fixed telemetry stations were set up to monitor fish movement; two on Eagle Creek and one on North Fork Eagle Creek (Figure 1). Each fixed station consisted of a four element Yagi antenna, a Lotek SRX-400 continuous data logging receiver and were powered by a 12-volt deep-cycle battery. Antennas were angled toward the stream and attached to 3 meter long metal conduit pipes secured to fence posts which were driven into the ground. Receivers were downloaded weekly with a Rugged laptop computer, and batteries were changed bi-weekly at each station. Fish were mobile tracked 2-3 times per week along North Fork and mainstem Eagle Creek. Eagle Creek flows downstream from the hatchery through a canyon making mobile tracking difficult in many areas. As a result, mobile tracking was concentrated in the stream area between the lower ladder and middle ladder. Mobile tracking took place from the end of March through the end of May to document movements downstream.

The fixed stations were removed from the field at the end of May and fish were mobile-tracked once per week until the end of June.

5.1.3 Spawning Ground Surveys

Spawning ground surveys were conducted by Oregon Department of Fish and Wildlife (ODFW) in Eagle Creek from February through June 2006 and 2007 following protocol described in Hutchinson et al. (2008). Surveys in North Fork Eagle Creek were conducted in 2005 and 2006 by the Bureau of Land Management (BLM). The data used in this report is from Hutchinson et al. (2008), and unpublished spawning ground survey reports provided by Dave Roberts (formerly with BLM).

5.1.4 Mark-Recapture Population Estimates 2007-2008

Coinciding with the radio-telemetry trapping at the lower ladder in 2007 and 2008, adult hatchery winter steelhead were tagged with individually numbered and colored Peterson disc tags (Floy Tag), inserted just below the dorsal fin. To estimate tag loss, the right opercle was marked with a small hole punch. Tag number, sex, and length of fish was recorded. Disc tagged steelhead collected during spawning operations at Eagle Creek NFH were used to estimate the population of hatchery fish at the lower fish ladder. The Chapman modification of the Lincoln-Peterson Index for calculating population density was used to account for low sample size and unbiased estimate (Van Den Avyle 1993).

$$N = \frac{(M+1)\times(C+1)}{(R+1)} - 1 \qquad \text{Equation 2}$$

Where M is the number of fish initially marked, C is the number of fish collected and examined for marks in the second sample period, and R is the number of recaptures in C. In addition, the total counted and recovered at the hatchery was enumerated each year.

95% confidence intervals were calculated using equation 1 (pg.15).

An estimate of wild fish abundance was also extrapolated from hatchery and wild fish trapping data in 2007. We used the ratio of total hatchery to trapped hatchery to estimate the total abundance of wild fish where:

$$total\ \ wild = trapped\ \ wild \times \frac{total\ \ hatchery_{mark-recapture}}{trapped\ \ hatchery} \qquad \text{Equation 3}$$

Assuming the wild fish had the same coefficient of variation as the hatchery fish estimated from mark-recapture; we calculated a standard deviation for the wild fish and used that to derive the confidence limits for the wild fish.

$$CV_{hatchery} = \frac{SD_{mark-recapture}}{total\ \ hatchery_{mark-recapture}} \qquad \text{Equation 4}$$

$$SD_{wild} = total\ \ wild \times CV_{hatchery} \qquad \text{Equation 5}$$

$$\mathrm{var}_{wild} = SD_{wild^2} \qquad \text{Equation 6}$$

$$95\%CL = total\ \ wild \pm 1.96\sqrt{\mathrm{var}_{wild}} \qquad \text{Equation 7}$$

5.1.5 Length, Sex, and Age Class Structure

To address the question of age structure, scales from wild adult steelhead migrating upstream were collected at the lower ladder and analyzed. Three to four scales per fish were taken and acetate impressions were made from the original scale cards. Scales were viewed under a microfiche reader, and the best quality scale (not regenerated, clean) was selected for aging. Two technicians independently viewed and aged the scales, and if there was a dispute in aging a third technician was assigned to view the scale. Technicians were trained and tested using collections of known origin and known age scales from coded-wire tagged fish. During spawning operations, length, sex and scale samples were collected from adult hatchery steelhead.

5.2 Smolt Migration Monitoring and Abundance

5.2.1 Radio-Tagging and Tracking of Steelhead Smolts 2005-2008

A couple weeks prior to release, hatchery smolts were randomly selected, anesthetized with MS-222 (25 ppm solution), fork length (mm) and weight (g) recorded, and surgically implanted with coded radio-transmitters (Lotek Wireless, NTC-4-2L, and 2.1 grams). Radio-tags were implanted using techniques described by Adams et al. (1998). Steelhead smolts were initially radio-tagged in 2003 and 2004 as part of a pilot study identifying the feasibility of using radio-telemetry to describe smolt movement in Eagle Creek. The data from that study is included in Table 11; however, for a detailed description of the 2003 and 2004 telemetry study please see Hoffman et al. (2003 and 2004). Juvenile steelhead radio-tagged in 2005-2007 were part of a rearing density study comparing smolt to adult survival for steelhead reared in low (7,500), medium (15,000) and high (22,500) density raceways. To account for any potential variation in smolt behavior as a side effect of the density study we stratified our radios tags as equally as possible throughout the three density groups. The transmitters had an

estimated battery life of 87 days and were 18.3mm in length, 8.3mm in diameter and weighed 2.1 grams. Seventy five fish were implanted with radio-tags in 2005, 45 in both 2006 and 2007, and 30 in 2008. Each transmitter emitted a unique signal which allowed individual fish to be identified. Transmitters were tuned to distinct frequencies within the 161-164 MHz frequency range and had a burst rate of 5 seconds. Following surgery, fish were placed in a bucket of fresh water and allowed to recover from the anesthetic before being returned to their respective raceways. Smolts were volitionally released from the hatchery during the 2005-2007 evaluations in April through May. Any fish remaining at the end of May were forced from the raceway into Eagle Creek. To facilitate smolt trapping operations (see Section 5.2.2 below), in 2008 a forced release occurred in early April. Mobile and stationary telemetry equipment used to monitor adult movement was simultaneously used to track juvenile fish. The relationship between smolt size at release and migration time from the hatchery to the mouth of Eagle Creek was analyzed using a linear regression. Analysis of variance (ANOVA) was used to compare length, weight, and condition factor of radio-tagged smolts from different density groups, and condition factors of migrant and non-migrant smolts. A smolt was considered a migrant if it was detected at the mouth receiver and a non-migrant if it was not detected at the mouth receiver. Non-migrants may consist of residual fish, shed tags, non-detected tags and/or mortalities.

5.2.2 Smolt Trap Operation and Abundance Estimates 2006 and 2008

Mainstem Eagle Creek

A five-foot rotary screw trap was used in mainstem Eagle Creek in 2006 and 2008 to capture naturally produced migrating juvenile fish. The screw trap was placed in the stream within Eagle Fern Park located above the mouth of North Fork Eagle Creek (Figure 1). We placed the trap above the North Fork confluence in order to avoid capturing and subsequently including North Fork Eagle Creek migrants in our abundance estimates for mainstem Eagle Creek. The trap was operated five days a week from March through April 2006 and six days a week from April through May 4, 2006. Trapping occurred seven days a week from April through June 15, 2008 with assistance from field crews with the U.S. Forest Service. Trapping was suspended during periods of high flows and when large numbers of hatchery fish were being released.

The trap was checked once daily in 2006 and 2-3 times daily in 2008, depending on debris load and capture rates. Fish captured in the trap were enumerated by species and origin (hatchery or natural). Smolts were distinguished from juveniles by having a silver color (or the beginnings of a silver sheen), and fading or loss of parr marks. Because it is difficult to distinguish resident rainbow trout from steelhead, especially at the juvenile stage, fish that did not show obvious signs of smolting were classified as *O. mykiss*. Wild salmonids captured in the trap were measured for fork length, marked with a unique fin clip, and released 150-200 meters upstream of the trap. O.mykiss less than 110mm in length were classified as age 0 and greater than 110mm as age 1. To

accurately estimate the number of juvenile fish migrating downstream, trap efficiency estimates were calculated on a weekly basis using the following formula:

$$N_i = \frac{n_i}{m_{recapi} / m_{reli}} \qquad \text{Equation 8}$$

Where N_i is the total number of migrants passing the trap location in week *i*, n_i is the number of unmarked fish caught in trap in week *i*, m_{recapi} is the number of marked fish recaptured in trap on week *i*, and m_{reli} is the number of marked fish released above the trap in week *i*.

95% confidence intervals were calculated from equation 1 (pg. 15).

North Fork Eagle Creek

The U.S. Forest Service (USFS) operates rotary screw traps on several tributaries in the upper and lower Clackamas River Basin, including North Fork Eagle Creek. The traps are used to estimate production of salmonid smolts and juvenile migrants in the basin. Since 1998, the North Fork Eagle Creek trap has been operated annually from mid-March to mid-June. In 2006 and 2008, the USFWS provided support to the USFS for North Fork smolt trapping. The trap is located approximately 1rkm upstream from the confluence with Eagle Creek (Figure 1), and operated seven days a week except during periods of high flows and equipment failure. For a detailed description of trap operations and data collection please see Strobel (2006).

5.3 Distribution, Summer Rearing Density, and Habitat Selection of Juvenile Fish

5.3.1 Habitat Surveys 2007

A habitat survey was completed in 2007 to determine how much and what type of mesohabitats (riffles, pools, and glides) were available in Eagle Creek and North Fork Eagle Creek. These habitat surveys were conducted between June and August of 2007 with total area and total number of mesohabitat units being enumerated in both streams. These mesohabitat units compose the sample frame for this portion of the study. Traveling upstream, a two-person survey crew classified habitat units using definitions described in Herger et al. (1996) and recorded habitat unit length and width to the nearest 0.5 meters using a laser rangefinder (Nikon Monarch Laser 800). Average and maximum depth to the nearest 0.1 meters were estimated using a measuring rod. Surveyed units were sequentially numbered for future identification by the snorkel crew. Three Hobo Water Temp Pros (Onset Computer Corporation, Bourne, MA.) were deployed to each reach (Figure 2) and programmed to record the water temperature (ºC) every four hours.

5.3.2 Snorkel Surveys and Population Estimates 2007

A two phase sampling design modified from Hankin and Reeves (1988) was conducted to estimate the density of juvenile salmonids, verify presence or absence of residual juvenile hatchery winter steelhead, and document any displacement of wild salmonids that may occur in the presence of residual hatchery winter steelhead in Eagle Creek and North Fork Eagle Creek. The surveys took place between July 10th and September 14th 2007 when the creeks were experiencing summer base flow. In the first phase of sampling, habitat units were stratified by type and chosen at random from the sample frame. Two divers then conducted single pass snorkel counts of juvenile salmonids in these units (Figure 2). Table 2 shows a detailed layout of sampling frame, sampling fractions and number of units selected for sampling. The surveys began at the mouth of Eagle Creek and proceeded upstream past Eagle Creek NFH to the upper falls. North Fork Eagle Creek was sampled from the mouth to approximately 14.8 rkm upstream. Air temperature and water temperature (ºC) were recorded daily prior to entering the stream. Snorkel surveys were only conducted on days when weather conditions permitted a high degree of underwater visibility (i.e., little to no rain on the previous day). A total of three snorkelers in two pairings (W. R. Brignon/J. S. Hogle and W. R. Brignon/T. E. Conder) took part in the surveys. Snorkel crews traveled upstream and followed the protocol described by Thurow (1994). Each snorkeler visually estimated abundance of salmonids by species, age (estimated by size), and origin (hatchery vs. wild). Winter steelhead less than 110mm were considered age 0 fish and fish greater than 110mm were considered age 1 fish (Figure 3). The 110mm length was verified as a reasonable point for age demarcation by conducting a scale analysis of samples collected from fish captured while electrofishing. Any hatchery fish residing in the stream after July 1st was considered residual and identified as being from hatchery origin by the lack of an adipose fin.

A second phase of sampling was conducted to determine accuracy and precision of the diver counts. In this phase a smaller subset of habitat units were randomly selected from the sample frame (Table 2) with the overriding condition that they were in near proximity to adequate stream access to mitigate for equipment and personnel concerns (Figure 2). The selection interval for second phase units was approximately 1/10th of the first phase units, as suggested by Dolloff et al. (1993). The upper and lower limits of selected habitat units were block netted to limit immigration and emigration. Observers conducted single pass snorkel counts using identical methodology as in the first phase of sampling. To account for individual snorkeler biases the unit was sampled by both pairs of snorkelers. We then used multiple-pass removal (Zippin 1958) or mark recapture (Engle et al. 2006) to determine the “true” abundance of fish within the selected habitat unit. The multiple-pass depletion was conducted using two Smith-Root backpack electroshockers (Model LR-24, Smith-Root Inc., Vancouver, WA.). Electroshocking passes continued until fish sampled during a pass are less than or equal to 25% of the fish sampled during the previous pass. All fish captured for each electrofishing pass were enumerated by species and age, fork lengths were recorded, and scale samples were collected from a subsample of the fish. Multiple-pass depletion electrofishing was conducted on all calibration units with one

exception of a pool unit which was considered too deep to accurately conduct electrofishing therefore; a mark-recapture was conducted as described by Engle et al. (2006) to account for snorkeler bias associated with deep pool habitats. Using equations found in Dolloff et al. (1993), calibration ratios were then calculated and applied to first phase diver counts to correct for snorkeler bias (Figures 4-6). Population estimates were then calculated and expanded to the reach scale. Eagle Creek was divided into two reaches with the line of demarcation being the confluence with North Fork Eagle Creek, which was considered its own reach. The section of Eagle Creek upstream of the North Fork Eagle Creek confluence is referred to as upper Eagle Creek and the section downstream of the confluence is referred to as lower Eagle Creek.

Data analysis

To detect differences in water temperature between the reaches, data were analyzed using ANOVA to test Ho: there is no difference in water temperatures between the reaches. A Student-Newman-Keuls multiple range test was used to determine pairwise differences between the three reaches when a difference in water temperature was detected by ANOVA (Zar 1984). A 3 x 3 contingency table was used to test for independence of habitat types by stream reach. Population estimates with 95% confidence intervals were calculated for all species in each habitat type and stream reach (Dolloff et al. 1993). Density estimates were compared between stream reaches using non-parametric ANOVA (Kruskal-Wallis test, Zar 1984). When non-parametric ANOVA rejects Ho: there is no difference in density between the stream reaches, a non-parametric analog to the Student-Newman-Keuls multiple range test was conducted as described by Dunn (1964) to detect pairwise differences between study reaches. Percentage of population estimates of wild fish that were rearing in the presence of residual hatchery winter steelhead are reported for Eagle Creek. Wild fish will be considered “displaced” if a low proportion of wild fish are found rearing in mesohabitat units where residual hatchery fish are present, relative to those habitat units where residual hatchery fish are absent. An alpha of 0.05 was used to determine significance in all statistical tests.

5.4 Disease Profile 2005-2007

The USFWS Lower Columbia River Fish Health Center conducted health surveys of the native fish in Eagle Creek from 2005-2007. Age 0 and age 1 winter steelhead and coho salmon were collected using backpack electrofishers in the upper and lower Eagle Creek during summer low flows (Appendix 2). A necropsy examination of each fish was performed to assess external and internal health status and parasite loads. Tissue samples (whole fry, kidney, spleen, gill, head and other) were assayed for pathogens of concern using standardized methodologies from the AFS Fish Health Blue Book (USFWS and AFS-FHS 2007) and the National Wild Fish Health Survey – Laboratory Procedures Manual (Puzach 2006). Unless otherwise noted, all fish were screened, using culture and microscopic procedures, for virus, bacteria, and parasites.

This included: infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), and infectious salmon anemia virus (ISAV) by culture on EPC and CHSE-214 cell lines,; *Aeromonas salmonicida* (furunculosis), *Yersinia ruckeri* (enteric redmouth disease), *Flavobacterium columnare* (columnaris), and *Flavobacterium psychrophilum* (bacterial coldwater disease) by culture on BHIA/TYES media; *Myxobolus cerebralis* (whirling disease) by pepsin-trypsin digest.The larger fish were also screened for *Renibacterium salmoninarum*, causative agent of bacterial kidney disease (BKD), using the enzyme-linked immunosorbent assay (ELISA) which measures levels of the soluble antigen produced by the bacterium. If positive by ELISA, the samples were further tested by the polymerase chain reaction assay for confirmation of bacterial DNA (as per protocols of the National Wild Fish Health Survey).

The same methods of collection and sampling were also done in 2004 when 32 wild cutthroat trout were collected from the Salmon - Huckleberry Wilderness, about four miles upstream of the Eagle Creek NFH. This area of Eagle Creek has no hatchery influence and the fish are considered to be a pure wild (unmanipulated) stock of fish.

The winter steelhead and coho originating from Eagle Creek NFH (Appendix 3) were examined for the pathogens listed above, following the inspection protocols from the AFS Fish Health Blue Book, and using the same methodologies. During spawning operations at the hatchery, tissue samples (kidney, spleen, gill, and ovarian fluid) were taken from the adults and assayed for fish pathogens. The juvenile fish were monitored monthly for signs of disease and diagnostic examinations were performed as needed to ascertain health status. Before the smolts were released into Eagle Creek, a 60 fish pre-release examination was performed to check the overall health of the population. For both the adult and juvenile fish, the sample size provided a ≥ 95% confidence of detecting the presence of a pathogen if present at a 5% level in the population.

5.5 Genetic Analysis

5.5.1 Genetic Sample Collections 2005-2007

Genetic samples were collected by field crews from the CRFPO and Lower Columbia River Fish Health Center. Temporal replicate samples were collected over three years (2005-2007), and five study groups are identified by river reach or collection: 1) Eagle Creek- NFH (HAT) sampled across raceways, 2) Lower Eagle Creek (LEC) from the Eagle Creek and Clackamas River confluence, upstream to the North Fork Eagle Creek confluence, 3) the North Fork Eagle Creek (NFEC), 4) Upper Eagle Creek (UEC) from the confluence with North Fork Eagle Creek to the hatchery, and 5) returning unmarked “wild” adult fish (“Adult NOR”) sampled at the lower ladder in the main stem Eagle Creek. All collections with the exception of HAT are putative wild steelhead trout (Table 3).

Sampling in the NFEC was completed in conjunction with regular screw trap operation by the U.S.Forest Service. Because too few smolts were encountered during collections, it was necessary to include juvenile *O. mykiss,* which were sampled using electroshock methods. With the exception of screw traps, sample collections were distributed within each section to avoid sampling bias (family groups). Biological data recorded by field crews included fork length and weight measurements taken during sample collection; scales were taken for age determination from a subset of juveniles. Adult NOR were sampled in coordination with radio tagging captures at the Eagle Creek main stem lower ladder. Fork lengths were recorded for each adult fish, and scales were taken for age determination. A small piece of fin tissue (2-3mm^2) was removed from each sampled fish, and placed in an individually labeled vial containing 100% non-denatured EtOH. Vials were sent to the Conservation Genetics Program Laboratory at Abernathy Fish Technology Center for DNA extraction and analysis.

5.5.2 Microsatellite Amplification and Analysis

DNA was extracted from samples by placing approximately 1mm^2 of tissue in 195µl of a 5% Chelex 100 solution (Sigma Chemical Co.), boiling for eight minutes, and vortexing for 30 seconds to suspend DNA. We amplified the following 16 locus primers: *µOmy1011UW, µSsa407*, *µSsa408*, *µOne13*, *µOne14*, *µOcl1, µOgo4*, *µOgo3*, *µOts4, µOts100, µOts3, µOts1, µOki23, µOmy7iNRA, µOmy77,* and *µSsa289* using the polymerase chain reaction (PCR) in the following reaction mix: 2µ DNA template, 0.1 units of Taq polymerase (Promega Corp.), 1.5µl of 10x buffer [10mM Tris-HCL (pH 9.0 @ 25°C), 50mM KCl and 0.1% Triton®X-100], 0.3µl of 10µM dNTPs, 0.75µl each of 10µM reverse and forward primers, and a final concentration of 1-2 mM MgCl2 (Appendix 1) in a 15µl reaction volume. The PCR amplifications were carried out using PTC-200 or PTC-225 Thermal Cyclers (MJ Research, Inc.) programmed with the following cycling regime: 94°C (1 min), followed by 38 cycles of 94°C (1 s) + X ^{o}C (30 s) + 72°C (30 s), where X is an annealing temperature (Appendix 1), and a final extension at 72°C (7 min). The DNA Fragment analysis was conducted using an Applied Biosystems Inc. (ABI) 3100 or 3130xl automated DNA sequencer, and microsatellite allele size (in base pair units) was evaluated with GeneMapper version 4.0 software (ABI). Individual multilocus genotypes were compiled over all loci and fish.

Descriptive statistics were calculated for each of five population groups across all collections (2005-2007) combined. Allele frequencies, number of alleles, observed and expected heterozygosities, and index of inbreeding (Fis) were generated using the program 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 2004). 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 $\alpha = 0.05$) with a sequential Bonferroni correction (Rice 1989).

For genetic population structure analyses we evaluated 15 groups identified by collection (i.e. HAT, NFEC, LEC, adult NOR, UEC) and sample year (i.e. 2005, 2006, 2007). The program FSTAT v2.9.3.2 (Goudet 1995) was used to calculate pairwise F_{ST} (θ of Weir and Cockerham 1984); indicating the proportion of total variation attributed to differences among groups, and significance was evaluated using Arlequin version 3.1 (Excoffier *et al*. 2005). Allelic richness, an index of gene diversity, was evaluated across all collections and loci. An among-group significance test of allelic richness: G1) three HAT replicate collections, G2) all NOR collections combined, was conducted using a rarefaction method (Hurlbert 1971) as implemented in the program FSTAT.

A pairwise genetic distance matrix of Cavalli-Sforza and Edwards (1967) chord distances (CSE) was generated using the program PHYLIP version 3.5C (Felsenstein 1992), and a neighbor-joining (NJ) phylogram was constructed based on pairwise genetic distances. In addition, we incorporated standardized[1] genotypic data at 10 of the original 16 loci, and repeated the genetic distance analysis to display temporal trends and NJ topology relative to Big Creek broodstock. Standardized data was collected during the initial 2001 inter-agency contracted evaluation, and was contributed by NOAA fisheries (Paul Moran and Maureen Waite, pers. comm.).

We used the program STRUCTURE version 2 (Pritchard *et al.* 2000) to estimate the membership coefficients ($\hat{Q}$), or fractional membership in *K* inferred populations or clusters for replicate samples from each of the five putative groups. We evaluated *K* ranging from 1-6 clusters using program default settings for the Monte Carlo Markov Chain procedure, with a 30,000 burn-in period and 100,000 MCMC iterations. We used the ΔK statistic, derived from the second order rate of change of the likelihood function in STRUCTURE to provide an improved estimate of the mode of true *K*; the true number of clusters (Evanno et al. 2005). The program CLUMPP (Jakobsson and Rosenberg 2007) was used to optimize alignment of 20 replicate cluster analyses for the number of inferred clusters (ΔK).

Likelihood based methods implemented by the program WHICHRUN version 4.1 (Banks and Eichert 1999) were used to assign population-of-origin in five independent comparisons evaluating each putative NOR collection (across years) vs. HAT. In each pairwise population comparison, fish were evaluated sequentially using a jackknife ("leave one out") re-sampling method, where assignment likelihood to either baseline population was calculated. The absolute value of the ratio of assignment likelihood scores (the LOD) was used to assess the stringency of individual assignments, where the HAT group was designated as the "critical" (numerator) population for scaling purposes. A LOD=0 indicates that an individual's population-of-origin is equally likely among sources (i.e. HAT or other), and likelihood increases exponentially with LOD.

[1] The SPAN steelhead (*O. mykiss*) baseline is a collection of genotypic data contributed by a consortium of multiple state and federal agencies and Tribes (see Appendix 1). The data are standardized among laboratories and shared among all collaborative parties for the benefit of steelhead trout management, conservation, and research.

5.5.3 Species ID: hybrid screening

In reaches of the Eagle Creek watershed where coastal cutthroat trout (*O. clarkii*) are present, hybridization with steelhead trout may occur. In other regional watersheds where both species co-occur, hybrid individuals have been observed with intermediate physical traits (Erik Olsen, ODFW, pers. comm.). We confirmed the species, or F1-hybrid identity of all juveniles sampled for the Eagle Creek analysis that were identified phenotypically as *O. mykiss*. We used the bi-parentally inherited, species-specific markers OCC-42 and OCC-35 (Ostberg and Rodriguez 2004). Sample DNA was amplified under the same conditions as described for all other loci, with an annealing temperature of 60°C. PCR product sizes were visualized on 3.5 agarose gels stained with ethidium bromide, and standardized with Hi-Lo^{TM} DNA Marker (Minnesota Molecular Inc.).

6.0 Results

6.1 Adult Steelhead Return Timing, Distribution and Abundance

6.1.1 Lower Ladder Trap Operations 2005-2007

In 2005, 95 fish were caught between January 20th and May 10th; however, fishing effort was not recorded. The lower ladder trap was fished 435 hours from January 4th through May 5th 2006 and 173 fish were caught. In 2007, the trap was fished 834 hours from January 8th through June 8th and 218 fish were caught. In 2008, the V-trap fish weir was removed from the ladder in late January due to malfunctions with the ladder gate.

Catch per unit effort (CPUE) was highest for hatchery fish in February and for wild fish March through May (Tables 4 and 5). Estimated abundance at the lower ladder was 1,411 (232-3069 95% CI) hatchery and 233 (80-386 95% CI) wild in 2006 and 1,885 (64-5002 95% CI) hatchery and 139 (15-349 95% CI) wild in 2007.

Temporal overlap in return timing of hatchery and wild fish was most evident in 2005 (Figure 7). Hatchery steelhead did not return to the system until late February, and the peak return timing for hatchery and wild fish was mid-March. Hatchery fish continued returning to Eagle Creek through April even though brood stock collections at the hatchery had halted. Temporal separation of the hatchery and native stock was more evident in the 2006 and 2007 returns. Hatchery steelhead migration at the lower ladder peaked between February 1-15 in 2006 and 2007, while the peak of the native run was between March 16-31 in 2006 and May 1-15 in 2007. Six Clackamas hatchery winter steelhead and two Clackamas hatchery summer steelhead were also trapped at the lower ladder. Clackamas hatchery steelhead were identified by mark; summer steelhead were adipose and right maxillary clipped and winter steelhead were adipose

and left maxillary clipped (hatchery steelhead from Eagle Creek NFH were all adipose and right ventral fin clipped).

6.1.2 Radio-Telemetry 2005-2007

A total of 105 hatchery and 60 wild adult steelhead were radio-tagged from 2005-2007 (Table 6). The overall sex ratio of males to females radio-tagged was similar for wild and hatchery fish, 1:0.96 and 1:0.91, respectively. Regurgitation of radio-tags was greatest in 2005 with 22% being shed at the lower ladder post tagging and release. Wild fish had a greater propensity for regurgitation than hatchery fish. Known tag losses in 2006 and 2007 were 11% and 16%, respectively. Two hatchery steelhead died during trapping operations at the lower ladder in 2005. One fish was in poor condition when removed from the ladder during trapping and was immediately placed in the recovery tub without attempting to radio-tag or sample. The fish died in the recovery tub and was placed back in Eagle Creek. A second fish died after being radio-tagged and placed back into the stream. No adult wild fish mortalities were observed during this study.

Hatchery Fish Distribution

For all years combined, the majority of detections and last known locations of radio-tagged hatchery fish was above the lower ladder in Eagle Creek (Table 6). Tag recoveries during brood stock collection at the hatchery averaged 22% with 2005 having the least percentage of returns (13%). Of the radio-tagged hatchery adults that did not return to the hatchery fish ladder for brood stock collection, 53% were detected at least once in the section of stream between the lower ladder and hatchery. Reported angler recoveries accounted for 5% (5 tags) of terminal locations of hatchery fish, however the actual number of angler recoveries may be much higher. Straying of hatchery steelhead into North Fork Eagle Creek was minimal. In 2005, one hatchery male was detected at the North Fork receiver on March 19th and again on April 11th. His last known location was at the mouth receiver on April 12th. In 2006, one hatchery female was detected at the North Fork receiver. This fish was radio-tagged at the lower ladder on April 12th and detected on April 16th at the North Fork receiver. The tag was last detected at the North Fork receiver on April 17th. In 2007, one hatchery male was recovered in the North Fork screw trap by USFS personnel. One Clackamas hatchery winter steelhead was radio-tagged and went into the North Fork. Individual hatchery fish movements are described in Figures 8 through 10 (2005), Figures 14 through 15 (2006), and Figures 18 through 19 (2007).

Wild Fish Distribution

Similar to radio-tagged hatchery fish, we observed the greatest number of wild fish detections in upper Eagle Creek (51%; Table 6). Wild fish detections in North Fork Eagle Creek for all years were 16%. Throughout the entire study (2005-2007), 14% of the tags were regurgitated at or near the lower ladder, and 19% of wild fish did not

resume upstream migration after tagging. In 2007, the largest percentage of downstream migration post tagging was observed. Five of 7 females and 4 of 9 males were detected at the mouth receiver within twenty four hours post tagging. Individual wild fish movements are described in Figures 11 through 13 (2005), Figures 16 through 17 (2006), and Figures 20 through 21 (2007).

6.1.3 Spawning Ground Surveys

During 2006 and 2007 the Oregon Department of Fish and Wildlife conducted spawning ground surveys in the lower Clackamas River downstream of North Fork Dam and in the lower tributaries, including Eagle Creek (Hutchinson et al. 2008). Nine surveys were conducted between 5 February and 3 June 2006 (Table 7). Expanded counts from the 2006 surveys estimate 8.1 redds/rkm from the Clackamas River confluence to 1 rkm upstream in mainstem Eagle Creek, 18 redds/rkm from N.F. Eagle Creek confluence to the middle ladder and 52.5 redds/rkm in upper Eagle Creek from the Middle Ladder (Delph Creek) to the Hatchery. In upper Eagle Creek (from the middle ladder near Delph Creek to the hatchery), 35 live fish were observed on redds. Ten were identified as hatchery steelhead, 2 as wild/unmarked fish and 23 were unknown. In 2007, five surveys were conducted on Eagle Creek, however the lower section (mouth to N.F. Confluence was not surveyed). During the 2007 surveys, twelve redds/rkm were estimated from N.F. Eagle Creek confluence to the middle ladder with an average 18 redds/rkm for upper Eagle Creek. In 2007 four live fish were observed (3 hatchery and one unknown). The peak spawning periods in 2006 and 2007 were between mid March to late April. In addition, ODFW found a spawning peak in mid February of 2006 due to early run hatchery fish.

The Bureau of Land Management conducted spawning ground surveys on North Fork Eagle Creek in 2005 and 2006. Surveys were generally conducted in March and again in May, however, some reaches were only surveyed once. For the most part, very few adult steelhead were identified on redds, although three adult wild fish were counted on redds (rkm 0.4-2.0) in 2005. The majority of redds were located between rkm 0.4-2.0 and rkm 4.4-7.2 (Table 7).

In 2007, ODFW conducted spawning ground surveys in North Fork Eagle Creek. They found 13.7 redds/rkm in the lower section and 1.8 redds/rkm in the upper sections surveyed, averaging 5.5 redds/rkm for the entire North Fork (Table 7).

Using redd /rkm information from ODFW surveys combined with total stream length, in 2007 approximately 30 redds were in lower Eagle Creek (from mouth to North Fork), 31 redds were from the North Fork to the middle ladder, and another 114 redds were from the middle ladder to the hatchery. In the North Fork of Eagle Creek approximately 81 redds were estimated. Total redd count estimated at 256.

6.1.4 Mark-Recapture Population Estimates 2007-2008

One hundred eighty adult hatchery winter steelhead were Floy disc tagged at the lower ladder from January through March 2007, and 44 were tagged between January 4 and 8, 2008. A malfunction with the valve that regulates flow into the ladder prevented further trapping and tagging of steelhead in 2008. Sixty seven tagged fish were recaptured at the hatchery during spawning operations in 2007 and 16 tags were recaptured in 2008. Tag loss was identified in four steelhead in 2007 and one steelhead in 2008. These fish had right opercule punches but no disc tags. The Peterson population estimate for returning hatchery adults to the lower ladder of Eagle Creek in 2007 and 2008 is 3,823 ± 696 95% CI and 2,566 ± 927 95% CI, respectively (Table 8). The total number of adult hatchery steelhead counted and recovered at the hatchery was 1,436 in 2007 and 970 in 2008.

An estimate of wild fish abundance was extrapolated from hatchery and wild fish trapping data in 2007. Using the ratio equation described in section 5.1.4, ((31 wild catch) x (3823 estimated hatchery abundance / 187 hatchery catch)); 634 ±116 95% CL wild fish were estimated at the lower ladder in 2007.

6.1.5 Length, Sex and Age Class Structure

Age at length data of wild and hatchery adult steelhead in Eagle Creek is presented in Table 9. Age 3 wild steelhead ranged between 60 cm and 69 cm while age 3 hatchery steelhead ranged between 59 cm and 65 cm. Age 4 wild steelhead ranged between 73 cm and 79 cm while age 4 hatchery steelhead ranged between 64 cm and 77 cm. In general, the majority of hatchery and wild steelhead returning to Eagle Creek were age 3 fish followed by age 4.

Scale analysis indicated that of the 92 wild fish sampled in 2005-2007, 53 were age 3 (22 males, 22 females and 9 unknown sex) and 35 were age 4 (9 males, 23 females, and 3 unknown sex). Seven samples were unreadable and not used in data analysis. Of the 1,240 hatchery steelhead adults that were sampled at the hatchery during 2005-2007, an average 54% were age 3 and 46% were age 4 (Table 9).

6.2 Smolt Migration Monitoring and Abundance

6.2.1 Radio Tagging and Tracking of Steelhead Smolts 2005-2008

Of the 195 hatchery steelhead smolts that were surgically implanted with coded radio- transmitters prior to volitional release from Eagle Creek NFH in 2005-2008 (Table 10), 18 fish shed their tags in the hatchery raceways and were excluded from all

analyses. Additionally, 16 smolts were not detected at fixed station receivers or through mobile tracking and were also excluded from data analyses.

Mean length, weight, and condition factor of radio-tagged hatchery smolts for each year is given in Table 10. The condition factor of smolts radio-tagged from the high density grouping in 2005 was significantly less than those of the low and medium density groups as determined by ANOVA ($F_{2, 61} = 32.3$, $p = 0.001$), and in 2006 mean length ($F_{2, 39} = 7.5$, $p = 0.001$) and weight ($F_{2, 39} = 6.02$, $p = 0.005$) of radio-tagged fish from the low density ponds was significantly greater than those from the medium and high density ponds. Due to equipment failure weights of individual fish were not recorded in 2007 therefore we were unable to calculate condition factors. At the 95% confidence level, no significant difference in fork length or condition factor of migrant and non-migrant steelhead smolts for any year was detected with ANOVA (Table 11). Linear regression analysis suggests there was no relationship between smolt size at time of release and migration time from the hatchery to the mouth of Eagle Creek for all study years (Figure 22).

2005

Of the 75 radio-tagged hatchery steelhead smolts, 8 were detected at the fixed telemetry station located near the mouth of Eagle Creek and determined to be migrant fish. Average migration time from the hatchery to the mouth was 223 ± 93 hours 95% CI or approximately 9 days. Fifty radio-tagged hatchery steelhead smolts were determined to be non-migrant fish. The last known locations of non-migrant smolts in Eagle Creek were: fourteen smolts upstream of the hatchery (rkm 21); twenty nine smolts at the fixed station receiver located at the hatchery (rkm 20.1); one downstream from the middle ladder (rkm 14.7); three smolts at Eagle Fern Camp (rkm 12.8); and three at the lower ladder (rkm 9.7). Twelve smolts shed their tags in the raceways prior to release and 5 were not detected at the fixed station receivers or through mobile tracking. The mean fork length and condition factor for migrant steelhead was 199 ± 10.4 mm 95% CI and 0.88 ± 0.16 95% CI and for non-migrant steelhead 196 ± 3.1mm 95% CI and 0.93 ± 0.05 95% CI (Table 11).

2006

Of the 45 radio-tagged hatchery steelhead smolts, 10 were detected at the fixed telemetry station located near the mouth of Eagle Creek and determined to be migrant fish. Average migration time from the hatchery to the mouth was 139 ± 68 hours 95% CI or approximately 6 days. Twenty eight radio-tagged smolts were determined to be non-migrant fish. The last known locations of non-migrant smolts in Eagle Creek were: 25 smolts at the fixed station receiver located at the hatchery (rkm 20.1); 2 smolts near the middle ladder (rkm 14.7); and one at Eagle Fern Park (rkm 11). Seven smolts were not detected at the fixed station receivers or through mobile tracking. The mean fork length and condition factor for migrant steelhead was 185 ± 9.4 mm 95% CI and 1.05 ± 0.04 95% CI and for non-migrant steelhead 181 ± 5.7 mm 95% CI and 1.05 ± 0.02 95% CI (Table 11).

2007

Of the 45 radio-tagged hatchery steelhead smolts, 27 were detected at the fixed telemetry station located near the mouth of Eagle Creek and determined to be migrant fish. Due to equipment malfunctions with the data-logging receiver at the hatchery, smolts that migrated past the hatchery receiver from April 20th-23rd were not recorded. We detected 12 smolts at the mouth receiver that were not recorded as having passed the hatchery receiver; therefore we assume these fish left the hatchery while the data-logger was not operational. These fish were included in the total count of smolts detected at the mouth receiver, but were excluded from migration timing analyses. Average migration time from the hatchery to the mouth for 15 of the radio-tagged smolts was 67 ± 24hours 95% CI or approximately 3 days. Nine radio-tagged smolts were determined to be non-migrant/residual fish. The last known locations of non-migrant smolts in Eagle Creek were: 5 smolts at the fixed station receiver located at the hatchery (rkm 20.1); 3 smolts in upper Eagle Creek above the lower ladder (rkm 10.2); and one in lower Eagle Creek approximately 4 rkm from the mouth. Six smolts shed their tags in the raceways prior to release, and 3 tags were not detected at the fixed station receivers or through mobile tracking. The mean fork length for migrant steelhead was 189 ± 4.1mm 95% CI and for non-migrant steelhead 187 ± 10.9 mm 95% CI (Table 11).

2008

In addition to the fixed telemetry stations located at the hatchery and mouth of Eagle Creek, we added a station at the middle ladder. This gave us more resolution to describe migrant behavior. Of the 30 radio-tagged hatchery steelhead smolts, 23 passed the hatchery receiver, 17 passed the middle ladder receiver, and 6 were detected at the mouth receiver. Four steelhead migrated past the middle ladder site without detection and subsequently were detected downstream of the ladder through mobile tracking. The hatchery receiver also failed to log one fish that was later detected downstream. Average migration time from the hatchery to the mouth for the 6 migrant smolts was 204 ±156 hours 95% CI or approximately 8.5 days. Twenty three radio-tagged smolts were determined to be non-migrant fish. The last known locations of non-migrant smolts in Eagle Creek were: 5 smolts between the hatchery (rkm 20.1) and middle ladder (rkm 14.7); 7 smolts at the middle ladder (rkm 14.7); and 11 between the middle ladder (rkm 14.7) and lower ladder (rkm 10.23). One smolt was not detected at the fixed station receivers or through mobile tracking. The mean fork length and condition factor for migrant steelhead was 191 ± 14.4 mm 95% CI and 1.1 ±0.07 95% CI and for non-migrant steelhead 178 ± 9.5 mm 95% CI and 1.25 ± 0.22 95% CI (Table 11).

6.2.2 Smolt Trap Operations and Abundance Estimates 2006 and 2008

Eagle Creek

In 2006, the trap was operated from March 26- May 4. During this time the hatchery volitionally released approximately 500,000 coho and 150,000-steelhead smolts. The hatchery release is approximately 10 rkm upstream from the smolt trap site. As a result, the smolt trap was often inundated with hatchery fish. Initially, only hatchery smolts were being captured, which allowed us to generate trap efficiency estimates in the early phase of trap operations. We discontinued marking the hatchery fish when wild fish began emigrating, however hatchery fish were still enumerated. We captured 3,052 hatchery steelhead and 6,143 hatchery coho in the trap, and marked 76 of the steelhead and 149 of the coho. Our trap efficiency for recapturing hatchery smolts ranged from 17-30%, and the estimated number of hatchery steelhead and coho smolts migrating to the smolt trap during our trapping period was 17,848 and 20,824, respectively. The majority of wild fish were age 0 *O. mykiss* (< 110mm) caught between April 24- 30th. We had limited success recapturing marked wild fish and were only able to generate a seasonal population estimate for naturally produced age 0 *O.mykiss* (Table 12). The estimated number of age 0 *O. mykiss* was 592 ± 357 95% CI. On May 4 the trap was removed following a vandalism incident and subsequent fish kill. Sixty-five wild fish including, coho, steelhead, lamprey, dace, and cutthroat trout, and 14,929 hatchery smolts were counted as mortalities.

Smolt trapping did not take place in 2007, but was resumed in 2008 with assistance from personnel with the US Forest Service. The hatchery underwent a forced release of coho and steelhead smolts during the first week in April. This allowed us to begin trapping operations in late April, thereby avoiding the capture of large numbers of hatchery fish. The trap was operated from April 22- June 6. Several factors, including smaller hatchery releases (300,000 coho and 100,000 winter steelhead), forced versus volitional release, and a delay in the start of trap operations, likely contributed to fewer hatchery fish being captured (1,612 coho and 174 steelhead). Population estimates generated from seasonal trap efficiencies for age 0 *O. mykiss* was 622 ± 478 95% CI, age 1 *O. mykiss* was 420 ± 175 95% CI, age 0 coho was 210± 126 95% CI, and age 1 coho was 742 ± 368 95% CI (Table 12). The majority of wild fish were age 1 *O. mykiss* caught between May 5th and 11th.

North Fork Eagle Creek

The estimated mean annual production of age 0 and 1 winter steelhead in North Fork Eagle Creek from 1998-2005 and 2008 was 4,875 and 2,118, respectively. The number of emigrating age 0 *O. mykiss* was lowest in 2003 (1,412) and 2008 (1,460) compared to estimates from 1999-2002 which ranged from 5,822-8,306 (Table 13). Smolt production (age 1) for all years was lower than juvenile fish (age 0) production.

Peak capture dates for juvenile steelhead were not available for all years, however the available data suggests that peak emigration occurred between mid-April and May.

6.3 Distribution, Summer Rearing Density, and Habitat Selection of Juvenile Fish

6.3.1 Habitat Surveys 2007

Water temperatures and mesohabitat composition of North Fork Eagle Creek and upper Eagle Creek are more closely related than those of lower Eagle Creek. Thermographs of lower Eagle Creek, upper Eagle Creek, and North Fork Eagle Creek are displayed in Figure 23. Temperatures in all reaches were significantly different as determined by ANOVA ($F_{2, 1047}$ = 184.8; P < 001) and Student-Newman-Keuls tests (P < 0.05). North Fork Eagle Creek and upper Eagle Creek experience cooler average water temperatures (15.3 ºC and 15.6 ºC, respectively) with lower Eagle Creek experiencing the highest water temperatures (17.4 ºC). Habitat data collected from the three stream reaches are summarized in terms of number of habitat units, length and surface area of the reach occupied by each habitat type (Table 14). Habitat unit composition of riffles, pools and glides is independent of stream reach (χ^2 = 7.85, df = 4, P = 0.097). On average, lower Eagle Creek is the widest reach (17.8 ± 0.69 m 95% CI) followed by upper Eagle Creek (14.6 ± 0.57 m 95% CI) and North Fork Eagle Creek (7.48 ± 0.25 m 95% CI).

6.3.2 Snorkel Surveys and Population Estimates 2007

Summer rearing population estimates of juvenile salmonids varied among species, reaches and habitat units (Table 15). Coho salmon and *O. mykiss* age 0 have the highest population estimates in upper Eagle Creek and North Fork Eagle Creek, whereas population estimates of O*. mykiss* age 1 were highest in lower Eagle Creek. The majority of residual hatchery winter steelhead were found in upper Eagle Creek and zero were observed in North Fork Eagle Creek. Estimates of residual hatchery winter steelhead abundance were 9.3 percent of the total estimated *O. mykiss* age 1 and 1.1 percent of the total estimated *O. mykiss* age 0 populations in mainstem Eagle Creek. Residual hatchery steelhead were 2.2 percent of the total population of juvenile coho in mainstem Eagle Creek.

6.3.3 Fish Distribution and Density 2007

In mainstem Eagle Creek the highest densities and abundances of *O. mykiss* age 0, coho, and residual hatchery winter steelhead were located in the upper reaches of Eagle Creek, upstream of the middle ladder. Fish densities and abundances in North Fork Eagle Creek were more evenly distributed than in Eagle Creek (Figures 24-26). Residual hatchery winter steelhead were first observed in lower Eagle Creek and

distributed above the hatchery to the upper falls. Residual hatchery winter steelhead were only observed in mainstem Eagle Creek and not in North Fork Eagle Creek (Figure 27). Densities for all species were unevenly distributed throughout the three reaches ($P < 0.001$) except *O. mykiss* age 1 which were evenly distributed throughout all reaches ($P = 0.40$; Figure 29). There was an increasing trend in *O. mykiss* age 1 densities as we traveled upstream in North Fork Eagle Creek. *O. mykiss* age 0 densities were highest in upper Eagle Creek and lowest in lower Eagle Creek (Figure 28). The highest densities of residual hatchery winter steelhead were found in upper Eagle Creek and the lowest in lower Eagle Creek, with none observed in North Fork Eagle Creek (Figure 30). Coho densities were highest in North Fork Eagle Creek and similar in the other two reaches (Figure 31). No juvenile hatchery coho were observed in Eagle Creek or North Fork Eagle Creek.

6.3.4 Wild Fish Displacement by Residual Hatchery Winter Steelhead

Residual hatchery winter steelhead were located in 15 of the 63 mesohabitat units that were sampled in Eagle Creek (Figure 32). The 15 habitat units were composed of two riffles in lower Eagle Creek and seven pools, three riffles, and three glides in upper Eagle Creek. The percentage of the estimated population of *O. mykiss* age 0, *O. mykiss* age 1, and coho salmon rearing in those same 15 units is 55%, 59%, and 55%, respectively. Due to the high percentage of wild fish rearing in the vicinity of residual hatchery winter steelhead we were unable to document any displacement of wild fish.

6.4 Disease Profile 2005-2007

In 2004, 32 cutthroat trout were collected in the Salmon - Huckleberry Wilderness about four miles upstream of Eagle Creek NFH. Necropsy examinations of the fish revealed all to be in good health. Normal ectoparasites were found on the skin (*Epistylis*) at low numbers. Thirty fish were tested for *R. salmoninarum*, using individual or pooled samples of kidney depending on size of fish. From this sampling, there were confirmed low positive detections of *R. salmoninarum* (four positive of seven individual samples, three positive of six pooled samples) in the fish (48-193 mm length) of this population. However, none of the fish had any clinical signs of bacterial kidney disease (BKD). There were no other viral, bacterial, or parasites detected.

From 2005-2007, one hundred twenty naturally produced fish (62 juvenile winter steelhead and 58 juvenile coho salmon) were sampled for fish pathogens (Appendix 2). Necropsy examinations of the fish revealed most to be in good health. There were no signs of disease in either the steelhead or coho salmon and IHNV, IPNV, VHSV, OMV, ISAV, *Aeromonas salmonicida*, *Yersinia ruckeri*, *Flavobacterium columnare*, *Flavobacterium psychrophilum* and *Myxobolus cerebralis* were undetected. The exception was a positive detection of *Renibacterium salmoninarum* in one of the 31 steelhead juveniles tested. This winter steelhead smolt, collected from upper Eagle

Creek in 2007, was confirmed as a low positive (as per the National Wild Fish Health Survey Manual). The fish, however, did not have any clinical signs of bacterial kidney disease (kidney lesions, distended abdomen, exophthalmia). Ectoparasites, common to Eagle Creek, were found on the skin (*Epistylis* and *Gyrodactylus*) and gills (*Nanophyetus* and *Loma*) in low numbers of fish and generally did not appear to be debilitating. In one case, hyperplasia of the kidney and spleen appeared in one steelhead juvenile afflicted with high numbers of *Nanophyetus* in the kidney.

The hatchery fish were sampled for the same pathogens listed above (Appendix 3). Very low levels of the *R. salmoninarum* antigen were detected by ELISA sampling in 37- 73% of the adults, resulting in a suspect finding of the bacterium. Confirmation by PCR (for bacterial DNA) was not done; however, testing of the ovarian fluid by the direct fluorescent antibody test (DFAT) did not detect bacteria in any of the fish. Unlike the ELISA and PCR methodologies which test for bacteria products, the DFAT allows specific microscopic visualization of the bacterium. This test was also used to monitor *R. salmoninarum* in the hatchery juveniles, as per the AFS Fish Health Blue Book inspection protocols, and the bacterium was not detected in the juveniles nor were there any signs of clinical BKD in the steelhead. Coldwater disease, caused by *F. psychrophilum*, was present in the hatchery steelhead juveniles in 2005 and 2006 and has an annual presence in the coho juveniles. *Ceratomyxa shasta* is typically detected in the adults (30% in 2006, 2007) although it is not present in the juvenile fish in the hatchery. *Myxobolus cerebralis* has never been detected in the hatchery adults or juveniles (either in the coho or steelhead). Light loads of *Gyrodactylis*, and *Epistylis*/*Scyphidia* were detected in the gills and on the skin during monthly sampling of the juveniles. Necropsy exams, administered to a sample of steelhead and coho smolts prior to release, revealed fish to be in good health and no pathogens were detected.

6.5 Genetic Analysis

6.5.1 Descriptive Statistics 2005-2007

We detected one *O. clarkii / O. mykiss* putative F1-hybrid individual, and one putative *O. clarkii* or backcrossed individual in the NFEC group. Two individuals exhibiting the cutthroat (homozygous) genotype were detected among the juveniles sampled from the hatchery raceways. These four hybrid and cutthroat samples were omitted from the dataset and from all analyses.

We observed a high level of variability among 16 loci evaluated in the Eagle Creek steelhead dataset (2005-2007). Numbers of alleles ranged from 5 at *μSsa289* in the lower Eagle Cr. (LEC) group, to 22 at *μOne13* in the upper Eagle Cr. (UEC) group (mean = 131 over loci and groups). Observed heterozygosity ranged from 0.522 at *μOts4* in the UEC group, to 0.951 at *μOmy1011* in the adult LEC group (mean = 0.764 over loci and groups). We observed five departures from expected genotypic proportions within groups among 80 total HWE tests; departures indicated excess

homozygous genotypes, which were not locus or group specific (Table 16). Among 2007 collections only, we observed one departure at *μOmy77 North Fork Eagle Cr. (*NFEC), and one departure at *μOne13* in natural origin recruits (NOR), both indicating homozygote excess. The number of private alleles among groups ranged from 1 in the hatchery (HAT) group to 12 in the NFEC group (Table 17). Allelic richness ranged from 11-13 across loci and collections, and the overall observed diversity was significantly greater in the NOR group compared to HAT ($P < 0.02$).

6.5.2 Population Genetic Structure Analysis

We observed significant genetic variation among the 5 sample groups (LEC, NFEC, UEC, NOR, HAT) of Eagle Creek steelhead evaluated from 2005-2007. The F_{st} values across loci ranged from -0.001 to 0.024 (Table 18) and the overall F_{ST} estimate of 0.010 across loci and groups was significant (95% CI 0.008-0.013). Pairwise F_{st} analyses resulted in large group-specific differences in variation, particularly between HAT and NOR collections. The greatest among-group variation overall was apparent between LEC and HAT, but significant year-to-year variation was most consistent between NFEC and HAT. Across collection years, the lowest among-group variation (or greatest genetic similarity) between HAT and NOR collections occurred in 2007; the exception was the "adult NOR" group, where variation was conversely largest in HAT/ NOR-2007 comparisons. For any given group, the largest random (temporal) variability among replicate collections occurred in UEC; in fact, pairwise F_{st} comparisons between UEC-2007 and the remaining two collections were significant, while UEC-2007 was not significantly different from the HAT-2005 and 2006 collections (Table 17, Figure 33). The least temporal variation among replicate collections was observed in NFEC.

Significant heterogeneity among HAT and NOR groups is corroborated by the relationship of pairwise genetic distances demonstrated in the topology of a neighbor-joining (NJ) phylogram (Figure 34). In regard to the replicate collections (2005-2007) included in this study, we observed nearly identical NJ topology utilizing either the full suite of 16 loci or a condensed suite of 10 standardized loci. Genetic distances gleaned from 2001 data (Maureen Waite, pers. comm.) including a Big Creek Hatchery sample and a sample of Clackamas River wild steelhead compliment the structure observed between 2005-2007 NOR and HAT collections (Figure 34). All Eagle Creek NFH groups cluster close together on one end of the topology with high bootstrap support (100%) suggesting significantly restricted geneflow with most NOR population components (groups). The collection from UEC-2007 is strikingly similar to the HAT collections in relation to the remaining NOR collections. The greatest similarity among NOR groups is seen on the branch shared by the UEC-2006, LEC-2006 and adult-NOR-2005 groups, and in a cluster containing the three NFEC replicate collections.

Among all the collections evaluated from 2005-2007 we observed proportional membership in K=3 inferred population clusters where Pr(K) = 1. Displayed graphically, the HAT collections are predominately identified by a single cluster, while NOR collections are represented by the remaining two population clusters (Figure 35). For Q

summed across clusters 1-2, the highest mean membership fidelity (89%) across NOR groups and collections was observed within the LEC 2005 collection. These results indicate high geneflow (random mating) or mixed membership among most NOR collections, and restricted geneflow between HAT and NOR. The most notable exception was observed among a proportion of individuals within the UEC 2007 collection. Interestingly, the majority of individuals in any given collection had a high membership coefficient; that is, they were distinctly (proportionally) either members of the HAT cluster or NOR clusters, with relatively few intermediate types. This suggests that the samples among NOR collections are comprised of either NOR fish or naturally produced HAT fish, but few introgressed individuals.

We observed a significant hatchery influence in genetic samples collected from naturally produced juveniles in upper Eagle Creek. The population membership plots likewise show that a large contingent of individuals in the upper Eagle Creek collections are represented by the hatchery population cluster (Figure 35), and that representation increased progressively from 2005 to 2007. The largest among-group genetic variation between the HAT and adult-NOR groups was observed in the 2007 NOR collection. These findings suggest a contingent of naturally produced hatchery-origin fish were found among the UEC-2007 progeny, and the 2005 - 2006 adult NOR samples, resulting in greater similarity to the HAT collections in statistical tests.

6.5.3 Baseline Assignment Tests

The overall assignment power is defined as the proportion of baseline individuals correctly assigned to group-of-origin in the jackknife re-sampling procedure. Individuals in the HAT group assigned to their group-of-origin (LOD > 0) with a mean accuracy of 94.5% across NOR comparisons (Table 18). Among the NOR groups, the highest mean proportion of correct assignments was observed in the NFEC group (81.5%). The lowest mean proportion of correct assignments occurred in the UEC group, which was influenced largely by the 2007 collection (41.5% accuracy). Across years, assignment accuracy was greatest among 2005 NOR collections; note that while UEC and LEC assignment accuracy fell from 2005 to 2007, accuracy increased in both the NFEC and adult NOR groups. These assignment results can be seen graphically as the distribution of relative assignment likelihood values (HAT/NOR) for all sample groups, using the method of Hendry et al. (2002; Figures 36-39). In this plot, individuals that fall on the line have an equal probability of HAT or NOR assignment (LOD score = 0).

7.0 Discussion

7.1 Adult Return Timing, Distribution, and Abundance

The V-trap fish weir was very effective for capturing fish that passed through the lower ladder; however, under certain flow conditions the lower falls on Eagle Creek are

likely passable to adult winter steelhead. Because of this, some fish may have avoided capture in the fish ladder by jumping the falls when flow conditions were favorable.

We observed an overlap in return timing of the wild and hatchery stocks, particularly in 2005, and spatial overlap in distribution of hatchery and wild winter steelhead in mainstem Eagle Creek occurred for all years. Although hatchery origin adult steelhead showed more temporal variability than wild steelhead in return timing, wild fish returns to Eagle Creek were earlier than what is typically reported for the Clackamas River Basin. The peak returns for wild fish at the lower ladder of Eagle Creek were mid-March (2005 and 2006) and mid-April (2007). Past reports of upstream migration for adult wild winter steelhead in the Clackamas River at the North Fork Dam show peak migration occurring in May; however, recent counts show more adults are being observed from November through April and fewer in June (Bartlett 2006).

We speculate from our observations of wild and hatchery fish movement in Eagle Creek that wild fish spent less time holding or wandering than hatchery fish. Generally, a wild fish moved into a spawning area, spawned (we presume) and then left the system. However, we did not record the distance travelled per day for individual fish and were unable to detect discrete differences in wild and hatchery fish movement. Likewise, we perceived wild male fish to exhibit more holding and wandering behavior than wild females; yet were unable to detect noticeable differences within wild fish movement and behavior. It should be noted that Shibahara and Lumianski (1995) found male and female wild steelhead in the Clackamas River basin showed marked differences in behavior. Females often held in a spot then moved rapidly downstream and out of tracking range within 24 hours after spawning. Males stayed in an area three times longer than females and in some cases would hold for several days, move and hold again. They speculate these male fish were experiencing competition for female mates and/or spawning multiple times with multiple females.

Not surprisingly, sections of the mainstem directly below the hatchery appear to be utilized by returning hatchery steelhead more readily than the remaining sections of the watershed, despite the opportunity for hatchery fish to use those other locations. Prolonged adult residence time in the upper Eagle Creek increases opportunity and likelihood that hatchery fish will eventually attempt to reproduce in the natural environment. In the past, and specifically during 2005, environmental conditions combined with hatchery practices may have been responsible for larger than normal numbers of hatchery steelhead spawning within the upper Eagle Creek region. In January and February 2005, extreme low water conditions resulting in low flows resulted in delayed migration of the hatchery steelhead return and a significant temporal overlap in their distribution with the natural winter steelhead population. Moreover, the early closure of the hatchery trap (in late February) likely left a surplus of hatchery fish on the spawning grounds in upper Eagle Creek.

The results from spawning ground surveys conducted in Eagle Creek provide additional evidence of hatchery fish spawning in the mainstem. Even though redd surveys were not conducted on the mainstem in 2005, hatchery fish were observed on

natural spawning grounds in 2006 and 2007. For example, in 2006, 29% of the winter steelhead observed on redds were positively identified as hatchery fish, compared with 5% that were identified as wild fish (Hutchinson et al. 2008). Likewise, three of four steelhead identified on redds in 2007 were hatchery fish. Additionally, the majority of redds were located in upper Eagle Creek, above the middle ladder, and in close proximity to where we observed the greatest distribution and movement of radio-tagged hatchery fish.

The proportion of hatchery fish spawning naturally (pHOS) are an important component to consider for impacts on wild populations. The Hatchery Science Review Group has recommended pHOS < 5% for segregated hatchery programs (HSRG 2009a). Based on the results of ODFW spawning ground and live fish surveys, the proportion of hatchery spawners in Eagle Creek may have exceeded 5% of the spawning population (pHOS > 5%) in 2006. Hutchinson et al. (2008) also noted a small spawning peak in February which is also the peak spawning period at the hatchery. The peak spawning period in Eagle Creek was in mid March to late April, therefore the spawning ground survey data also supports the hypothesis that hatchery fish most likely spawn with hatchery fish and wild fish with other wild fish (more on this issue in Section 7.5).

In the Eagle Creek watershed the North Fork Eagle Creek is of particular concern because it is believed to support a significant wild steelhead spawning population. Based on the telemetry data and information provided from our genetic study (discussed in Section 7.5), we believe there is a minimal risk of hatchery fish straying and spawning in the North Fork. We saw negligible movement of adult hatchery fish into North Fork Eagle Creek, (one female and two males). Also, no residual juvenile hatchery fish were detected in the North Fork. While we don't dismiss the possibility of hatchery fish spawning in North Fork Eagle Creek, we cannot assume it to be a high risk, and most likely pHOS <5%, from the evidence collected in 2005-2007.

We acknowledge that there were limitations with this study including the lack of visual confirmation of redd locations for radio-tagged fish. High flows and inaccessibility to many locations in the mainstem, were the main reasons visual confirmation was not attempted. Additionally, there are logistical problems associated with the capture, tagging and tracking of highly mobile fish. A core assumption in radio-tagging studies is that tagged fish are representative of the entire population and have the same behavior and response of untagged fish (White and Garrot 1990). However, tag insertion can disrupt normal upstream migration causing radio-tagged fish to remain at the tagging site or move downstream following release (Hooton and Lirette 1986; Burger et al. 1985). For the most part, hatchery and wild fish responded well to tagging procedures with most fish resuming upriver migration within 24 hours after being handled, although, 44% of wild fish tagged in 2007 did not resume upstream migration and were detected at the mouth receiver within 24 hours after release. The retreat of tagged fish has been observed in numerous studies (Milligan et al. 1984; Johnson et al. 1992 and 1993; Pahlke and Bernard 1996), and the impacts of tagging and handling fish are a concern for researchers particularly when the study requires handling of an ESA listed stock.

Despite these concerns, tagged steelhead in this study exhibited holding, traveling, and wandering behavior which is consistent with results from a tagging study conducted on wild Clackamas River steelhead in 1994 (Shibahara and Lumianski 1995).

Utilizing information from our study we were able to estimate wild fish abundance in Eagle Creek. Our estimates of wild fish at the lower ladder in 2007 were between 634 ±116 95% CL, based on the Peterson estimate and 139 (15-349 95% CI) based on CPUE. When you consider redd counts (375 redds were estimated for the Eagle Creek watershed upstream of the lower ladder) and knowing that hatchery fish were also observed producing redds, a realistic estimate of wild fish abundance in Eagle Creek is likely between these two means (139-634). Also note that Hutchinson et al. (2008) estimated 513 (264-683 95% CI) wild steelhead spawning abundance in the Lower Clackamas River based on redd counts in 2007. Based on these spawning ground surveys by ODFW, 62% of the total redds in the lower Clackamas River were observed in Eagle Creek or approximately 318 wild steelhead spawners in 2007 (164-423 95% CI). Regardless of the technique used to estimate abundance, it appears that the number of wild steelhead spawners in Eagle Creek has the potential to be greater than 250 fish (NOS > 250).

We were also able to determine abundance of hatchery steelhead in lower Eagle Creek. In 2007, we calculated 1,885 (64-502 95% CI) hatchery steelhead using the CPUE estimate and 3,823 (± 696 95%CI) using the Peterson estimate (and the hatchery rack return was 1,436). In 2008, we calculated 2,566 (± 927 95% CI) hatchery steelhead using the Peterson estimate and 970 returned to the hatchery. Based on past estimates of harvest for hatchery steelhead in Eagle Creek, as well as observations of hatchery steelhead spawning in upper Eagle Creek, the abundance estimates seem reasonable. However, there are a number of limitations with our data. We received occasional comments from anglers that the Floy disc tagged fish were easier to spot in Eagle Creek. This could lead to selective fishing on tagged fish and potentially over-inflate our Peterson mark-recapture estimate. Additionally, gear saturation, water quality, water depth, and fish behavior are all known to negatively affect catch rates and CPUE indices (Ney 1993). During the early part of the hatchery run (January and February) more fish were moving through the ladder and we were able to trap more fish using less effort. As our catch rates decreased in April and May, trapping effort at the ladder increased considerably; therefore our effort (hours fished) was not consistent over the course of the season. Also, since the trap was rarely fished overnight or during the weekends, we likely missed a considerable portion of both the hatchery and wild run. Finally, fish may have avoided capture in the trap by navigating/jumping the lower falls during high flow events or when stream conditions were favorable.

7.2 Smolt Migration Monitoring and Abundance

Based on radio telemetry data and visual observations of residual hatchery steelhead, we determined there is a high probability of occurrence for residual

steelhead from the hatchery population at Eagle Creek NFH. Although migrant steelhead left the system quickly, generally between 2-9 days; for most years, and in particular 2005, less than 50% of smolts were detected at the mouth of Eagle Creek two months after release. We recognize that "undetected" are most likely a combination of shed radio tags, non-detected radio tags, mortalities and/or residual fish. In reference to residual fish, while it is possible that smolts remaining in Eagle Creek longer than two months will eventually emigrate, it is highly unlikely based on widespread information regarding residual behavior in steelhead. For example, hatchery steelhead smolts released in Abernathy Creek, WA made quick downstream movements within the first two days following release; however, fish that remained in the creek longer than two months did not emigrate until the following fall or winter (Ostrand 2005). In addition, Viola and Schuck (1995) found that hatchery-reared juvenile steelhead failing to emigrate 30 days after volitional release would remain as residuals in the system. Given that hatchery steelhead smolts were visually observed in Eagle Creek three months after release, it is expected that smolts not emigrating within two months will remain as residual fish in Eagle Creek.

Generally, size at release, temperature and flow regimes, type of release strategy, and rearing environment are known to contribute to residual behavior in hatchery steelhead. Our assessment of steelhead smolts released from Eagle Creek NFH provides little quantitative evidence suggesting size at release affects migration timing of the fish and/or contributes to the number of residuals in the population. It has been documented that steelhead smolts emigrate faster and are less likely to remain as residuals when released at lengths between 190-199 mm and condition factors between 0.9-0.99 (Fessler and Wagner 1969; Folmar and Dickhoff 1981; and Tipping 1997). However, smolts migrating from Eagle Creek NFH had mean fork lengths between 176-203 mm and condition factors between 0.88 and 1.1 yet we found no correlation between size at release and migration time. Furthermore, we found no significant difference in condition factors of migrating and non-migrating smolts. This may be a function of our small sample size confounded with the combination of physical, biological and environmental variables that contribute to smolt migration.

Volitional releases are thought to reduce the number of residuals in a population by releasing smolts that are physiologically ready to migrate and removing non-migrating smolts from the raceways at the end of the release (Viola and Schuck 1995). Although steelhead smolts were volitionally released from Eagle Creek NFH through the 2007 release, the hatchery adapted a practice of forcibly releasing any non-migrating fish from the raceways into Eagle Creek at the end of the volitional release (pers. comm. with Doug Dysart). The practice of releasing non-migrating smolts may have contributed to the number of residuals in Eagle Creek during those release years. Hoping to reduce the number of residuals (as well as accommodate our smolt trapping in Eagle Creek), the hatchery switched to a forced release in 2008. In the future, it would be advantageous to evaluate forced vs. volitional release (with removal of non-migrating individuals) at Eagle Creek NFH to determine if release strategy contributes to residuals in Eagle Creek.

It is important to acknowledge some of the limitations in our evaluation of residual steelhead. First, we may have over-estimated the number of residual radio-tagged hatchery smolts in Eagle Creek. Some of the fish that we grouped as "non-migrants" may have been migratory fish that shed their tags, fish that were not detected by the datalogger at the mouth telemetry station, or mortalities. Second, we did not examine how physical factors (water flow and temperature), or physiological factors (smoltification), contribute to the number of residuals in the hatchery population. These parameters should be considered in future evaluations of migration and residual steelhead.

Smolt abundance

Estimating the number of naturally produced salmonid smolts in Eagle Creek was complicated by large numbers of hatchery released fish, few recaptures of marked fish, and vandalism of the smolt trap in 2006. The vandalism incident caused large mortalities of hatchery fish; thereby forcing us to remove the trap on May 4th, presumably during the peak of wild fish emigration. Additionally, Eagle Creek is a large stream and correct placement of the trap is essential for capturing sufficient numbers of fish for accurate population estimates. Despite these limitations, the production data we collected from smolt trap operations offers a glimpse of wild fish abundance above the North Fork confluence. We were more successful capturing juvenile fish (age 0) than smolts which is not uncommon. Thedinga et al. (1994) found that winter steelhead smolt yields were often underestimated because large migrants are able to avoid capture in the traps.

It is unlikely that our estimates are an accurate representation of the true abundance of wild fish in the Eagle Creek. Considering the majority of redds were located upstream of our trap site, we would expect to see greater numbers of wild fish in our trap. In addition, it's difficult to compare smolt and juvenile steelhead production estimates in Eagle Creek with those from other tributaries in the Clackamas River because Eagle Creek is larger than other tributaries, (Clear, Deep, Fish and North Fork Eagle Creek), in the basin; we have two non-consecutive years of data compared to multiple year data sets; and juvenile fish (age 0) are reported as *O. mykiss* by some agencies and winter steelhead by others. Regardless of the shortcomings in our data set, we have documented that Eagle Creek above the confluence with the North Fork Eagle Creek is a natural producer of coho salmon, steelhead and lamprey.

7.3 Distribution, Summer Rearing Density, and Mesohabitat Selection of Juvenile Fish

We have visually confirmed the presence of residual hatchery winter steelhead in Eagle Creek, and with a high degree of confidence, their absence in North Fork Eagle Creek. We were able to document residual hatchery winter steelhead rearing in the same habitat units as wild salmonids. However, with the highest numbers of both hatchery and wild fish distributed in upper Eagle Creek we were unable to document

any displacement caused by their presence. Though, there still exists a potential for competition knowing that the majority of observed wild *O. mykiss* and coho salmon rearing in Eagle Creek were observed in the same 15 habitat units as residual hatchery winter steelhead. Considering that upper Eagle Creek and North Fork Eagle Creek are similar reaches in terms of mesohabitat unit composition and stream temperature, other interesting findings with these data include the differences in rearing densities at the reach scale. The majority of juvenile coho salmon and *O. mykiss* age 0 are rearing in the North Fork Eagle Creek and upper Eagle Creek, respectively. Whereas, *O. mykiss* age 1 are more evenly distributed throughout Eagle Creek and to a point, North Fork Eagle Creek.

While a substantial proportion of natural production for wild winter steelhead is in North Fork Eagle Creek, natural production of wild steelhead was also found in the mainstem of Eagle Creek, particularly upstream of the middle ladder. Similar habitat and thermal regimes of North Fork Eagle Creek and upper Eagle Creek are likely factors influencing spawning and rearing in these reaches.

McMichael and Pearsons (2001) documented residual hatchery steelhead had migrated over 12 kilometers upstream from a release site on the Teanaway River, Wa. into areas containing ESA listed fish populations. Considering that North Fork Eagle Creek is thought to be the primary area of successful natural production of ESA listed salmonids in the Eagle Creek Basin, there was a concern that residual hatchery winter steelhead from Eagle Creek NFH would make a similar migration up the North Fork Eagle Creek. Our results suggest that residual hatchery winter steelhead did not migrate up North Fork Eagle Creek, however similar to McMichael and Pearsons (2001) we did document an upstream migration in Eagle Creek. Due to the impassible Upper Falls located above the hatchery, fish were only able to migrate upstream less than 0.5 kilometers, a fraction of what McMichael and Pearsons (2001) observed.

Given our study design, there are four scenarios that could explain our inability to document any displacement of wild salmonids from preferred mesohabitats by residual hatchery winter steelhead. First, studies suggest that hatchery fish pose the risk of negative hatchery-wild interactions by displacing wild fish (Vincent 1987; Hillman and Mullan 1989; McMichael et al. 1999); it is possible that this is not the case in Eagle Creek. Second, Jonasson et al. (1996) documented the highest densities of residual hatchery steelhead were located near the release site, similar to our study. Also consider, that Vincent (1987) concluded that releases of hatchery fish reduced populations of wild rearing fish in the vicinity of the release site. Therefore, any displacement of wild fish by residual hatchery winter steelhead in Eagle Creek likely would have been seen in the upper reaches near the hatchery. With the majority of both the wild salmonid population and the residual hatchery winter steelhead population located in upper Eagle Creek it is difficult to detect a displacement without pre-release data. Due to high spring flows and the associated turbidity we were unable to collect pre-release abundance data on wild fish rearing below the hatchery that would be required for a case-control comparison. Third, the studies that document a displacement of wild fish caused by hatchery fish are conducted shortly after (approx. 1

month or less) the release of the hatchery fish (Hillman and Mullan 1989; McMichael et al. 1999) when the abundance of hatchery fish is higher than that of the wild fish. It is possible that because we were evaluating a displacement caused by residual hatchery winter steelhead, which have resided in the stream for over two months, that any potential large scale displacement occurred closer to the time of release. Lastly, there may have been some scale issues. It is possible that the abundance of residual hatchery winter steelhead was not at a scale large enough to elicit a displacement response or that the elicited response is occurring at a smaller scale than the mesohabitat scale.

While the data contained in this section of the report is an important component to assessing ecological interactions in the Eagle Creek Basin, it is important to recognize that this is only one year of data and therefore may have limited applicability when making long term management decisions. In determining what type of impact the hatchery is having on juvenile fish abundance and density, a multiyear data set would be ideal and could help explain potential stochastic environmental factors occurring in the basin that can confound the results of a one year data set. The uncertainty with the calibration ratios calculated from the correlation between population estimates and diver counts, specifically in riffle habitat units, may have had an effect on the results in the this study. Also, population estimates of *O. mykiss* age 1 in lower Eagle Creek riffle habitats may be inflated due to a data outlier where in one riffle habitat unit 260 fish were estimated to be rearing directly below the North Fork Eagle Creek confluence. This one outlier inflates the lower Eagle Creek *O. mykiss* age 1 population estimate by more than 2,000 fish, or 40 percent. Regardless, juvenile hatchery residuals were not found in the North Fork Eagle Creek but were found in the mainstem of Eagle Creek. The highest density of residual hatchery juvenile steelhead was in upper Eagle Creek between the middle ladder and the upper falls near the hatchery.

A surplus of hatchery spawners in Eagle Creek that produce offspring which subsequently move throughout the watershed in search of foraging and protective habitat may have a measurable impact on the natural population regardless of reproductive (geneflow) restrictions between hatchery and wild fish. During a recent review of Eagle Creek NFH, a scientific panel expressed concern with the level of impact that a release of 150,000 hatchery steelhead may have on the ESA listed population (USFWS 2007). As a result of the review, the steelhead smolt release was reduced to 100,000 in spring of 2008. Also recommended in the review, is to determine if further reductions in steelhead hatchery production was warranted based on the conclusions of this study.

7.4 Disease Profile

The wild and hatchery steelhead of Eagle Creek appeared to be in good health with no signs of overt disease. In testing for the pathogens that cause bacterial kidney disease (BKD), coldwater disease (CWD), furunculosis, columnaris, enteric redmouth disease, whirling disease, infectious hematopoietic necrosis, and four other viral

diseases, only *R. salmoninarum*, causative agent of BKD, was detected. This was found in one of the 31 wild fish tested sampled at the Eagle Fern Camp, rkm 12.8, in 2007; the other findings of the bacterium were suspect positive in the wild steelhead (73-112 mm length) from NF Eagle Creek in 2005 as based on the detection of very low levels of the soluble protein by ELISA. None of the fish had signs of BKD. The soluble protein of *R. salmoninarum* was also detected at very low levels in 37-73% of the hatchery steelhead adults; however, there were no signs of disease nor was the actual bacterium detected by the direct fluorescent antibody test (DFAT) which allows for visualization of the bacteria when levels are elevated. While these tests indicate that the adult fish may carry low levels of the bacterium, it has not resulted in disease at the hatchery. The bacterium is vertically transmitted from the female to her eggs via the ovarian fluid with the result that progeny are potentially infected at hatch; however, tests for *R. salmoninarum* in the juvenile steelhead and coho salmon at Eagle Creek NFH were negative and these fish have never shown signs of BKD (with one exception). Detection of *R. salmoninarum* in a fish without disease signs is not unusual as the bacterium's mechanisms of pathogenicity generally dictates a long, latent period of infection that may or may not result in disease, depending on the species and the physiological and environmental conditions faced by the fish (Fryer and Sanders 1981, Evelyn 1993). *Renibacterium salmoninarum* may be more "detectable" than other fish pathogens because its mechanisms of pathogenicity allow it to survive for months or years within the immune system of the fish (Gutenberger et al. 1997), only causing disease when changes such as smoltification, spawning or elevated temperature adversely overwhelm the fish. As an obligate pathogen of fish, *R. salmoninarum* has limited capacity to survive outside of the salmonid host (Austin and Austin 1987) so its best option is maintain at low levels in the fish until conditions favor maximum reinfection. These factors may explain why *R. salmoninarum* is so frequently detected in fish sampled by the National Wild Fish Health Survey which has found positive and suspect positive findings in many salmonids (and some non-salmonids) throughout a wide range of watersheds in Oregon, Washington, and Idaho (National Wild Fish Health Survey database). This includes the wild cutthroat trout sampled in the Salmon-Huckleberry Wilderness in Eagle Creek about four miles above the hatchery. These fish, unaffected by hatchery and other human influences, had positive findings of *R. salmoninarum* in 2003, indicative of the ubiquitous nature of the bacterium in the Pacific Northwest.

Another endemic pathogen of concern to the salmonids of Eagle Creek is *Flavobacterium psychrophilum*, the bacterium causing CWD which seems to be most infectious during the colder months in late winter to spring. At the hatchery, the steelhead juveniles are far less susceptible to this bacterium than are the coho, possibly because the steelhead hatch in May, four months later than the coho. Culture conditions can aggravate infections of *F. psychrophilum*, and expression of acute disease at the Eagle Creek NFH seems to be induced by high early rearing densities or excess handling during the months of February to June when the water temperatures are less than 55^0F. Thus, the hatchery avoids these practices and in the years of 2005 to 2007, mortality from CWD disease in the coho was limited to low mortalities from the less transmissible form of chronic disease which results in deformities. The steelhead

experienced a highly unusual mortality from an acute infection of CWD in late June/July in the years 2006 and 2007. This resulted in two nursery tanks of steelhead, from waterborne bacteria infecting the head wounds obtained when the steelhead jumped at the newly plumbed water outlet. After the plumbing was corrected, the infections ceased. At time of release of the smolts from 2005-2007, both the coho and the steelhead were healthy. During these years, neither *F. psychrophilum* nor signs of CWD were detected in the wild steelhead and coho of Eagle Creek below the hatchery and the North Fork of Eagle Creek (2005). Given the limited sampling of the wild fish, it is quite possible that an acute or chronic infection of this, or indeed any pathogen other than *R. salmoninarum*, could have been missed because the debilitating effects of acute infections combined with the capability of native predators quickly remove sick fish from natural populations. However, CWD is more likely a problem for cultured fish than for the wild fish and with the good health of the hatchery fish at release; it is presumed that transmission of *F psychrophilum* to the wild fish is minimal.

The hatchery and wild steelhead populations generally had no to low levels of the parasites that are present in Eagle Creek. In the sampling of the wild fishes of Eagle Creek, the snapshot of the health profiles indicates that the parasites *Loma*, *Nanophyetus*, *Epistylis*, and *Gyrodactylis* are present. Significantly, the agent of whirling disease, found in 2001 in the nearby Clackamas River Basin (Clear Creek), was not found. All but *Epistylis* could cause debilitation and even an inability to survive should environmental conditions (such as warmer water temperatures) favor the parasite and overwhelm the physiological condition of the fish (Woo 2006). At least one wild steelhead, collected below the hatchery, was affected enough by its load of parasites to show pathological changes in the kidney and spleen. At the hatchery, however, there is no indication that the parasites found in Eagle Creek, generally limited to the ectocommensal sesslines (*Epistylis*/*Scyphidia*) and *Gyrodactylis* in the hatchery, present a health risk or a cause of mortality in the steelhead and coho. This is likely due to a steady supply of food and hence the limited possibility of transmission of parasite-infected fish flesh via the oral route.

Because of the good health of the steelhead and coho that rear at and return to Eagle Creek NFH, there appears to be little likelihood of disease transmission from the hatchery fish to the wild fish. Although the hatchery-reared fish are more susceptible to coldwater disease given the constraints of culture conditions, no pathogens other than *F. psychrophilum* were detected in the yearling steelhead and coho smolts from Eagle Creek.

In view of the fact that the Eagle Creek watershed is free from most viral and bacterial pathogens, the proposed off-station rearing of the Eagle Creek hatchery stock at Oak Springs SFH (ODFW facility) is of special concern. Even though the Oak Springs site is described as a pathogen free facility, all smolts reared in this facility need to be diligently tested before acclimation and release into Eagle Creek. The fish health profile on the direct stream release of hatchery spring Chinook juveniles into Eagle Creek by ODFW also needs to be monitored.

7.5 Genetic Analysis

In this evaluation we have addressed the principal, overarching question; are hatchery and natural-origin steelhead contributing to the production of wild steelhead trout in Eagle Creek, and if so, how is production spatially distributed within the watershed? Previous studies (USFWS unpublished) have confirmed that the Eagle Creek NFH brood stock has undergone significant genetic introgression with out-of-basin Big Creek hatchery steelhead, characterized by a relatively early return time. Native Clackamas River and Eagle Creek naturally produced stocks are typically considered late-run steelhead. Genetic differences or restricted gene flow between the two groups is largely the result of this temporal separation in run and spawn time. Hatchery brood stock is collected exclusively from early returning hatchery steelhead, a practice that helps maintain the discreteness of ESA-listed fish and the temporal separation of the stocks.

Our three year (2005-2007) genetic interactions evaluation of Eagle Creek steelhead provides evidence that natural productivity among hatchery steelhead and the wild Eagle Creek population component is distributed non-randomly on a spatial scale, and occurs at variable annual rates. Marked genetic differences were observed between the Eagle Creek NFH stock and naturally produced juveniles in lower Eagle Creek, North Fork Eagle Creek and adult wild collections. It is likely that naturally spawning steelhead utilizing the lower main stem and North Fork of Eagle Creek experience little competition or genetic interaction with early returning hatchery origin steelhead. Although significant genetic differentiation between the upper Eagle Creek natural production and hatchery collections was observed overall, upper Eagle Creek natural production was most similar to the hatchery group. This was particularly evident among fish (age 1) sampled from upper Eagle Creek in 2007. These findings suggest a contingent of naturally produced hatchery-origin fish were found among the upper EagleCreek-2007 progeny, and the 2005 - 2006 adult wild samples, resulting in greater similarity to the hatchery collections in statistical tests. In accordance with our results, and based on the temporal separation of the natural and hatchery origin population components, it is reasonable to infer that if hatchery steelhead in Eagle Creek do spawn naturally, they have a high likelihood of pairing with other hatchery steelhead. Furthermore, hatchery steelhead may experience poor natural reproductive success owing to the effects of domestication selection in the hatchery (Mclean et al. 2005). McLean et al. (2003) observed differential productivity among sympatric groups of hatchery and natural steelhead, in which smolt production by hatchery females was 4.4% to7.0% that of NOR females (see also Chilcote et al. 1986).

Leider et al. (1986) found that the spatial and temporal overlap among hatchery and wild steelhead on the Kalama River, WA was adequate to permit interbreeding. Undoubtedly, some degree of geneflow does occur between hatchery and wild winter steelhead in Eagle Creek. However, genetic introgression from hatchery fish into the natural population (resulting from hatchery x natural origin mate pairings) is not the unequivocal result of natural reproduction by hatchery steelhead. In addition to genetic introgression, other risk factors that threaten wild populations can occur in association

with hatchery operations and through competitive interactions between hatchery and wild fish within spawning and rearing habitats (Kostow et al. 2003). Although overlaps in return timing of hatchery and wild steelhead in Eagle Creek were only demonstrated in 2005, we recognize this as a moderate risk level to ESA listed fish in Eagle Creek. To reduce this risk, we recommend that hatchery management practices ensure collection of hatchery fish throughout the entire run to reduce the number of hatchery fish spawning naturally and utilize the earliest returning fish for hatchery brood stock to ensure temporal segregation. Other ways to reduce the risk of hatchery fish spawning naturally would be to reduce the number of hatchery adult returns by reducing smolt production and/or increased harvest exploitation of hatchery fish. As concluded by Kostow (2008), "even moderate decreases in the level of hatchery-wild fish interactions may be beneficial to wild populations".

8.0 Recommendations

When evaluating the program at Eagle Creek we tried to consider the full biological, social and economic value the steelhead fishery brings to the Clackamas River basin. The winter steelhead fishery provided by Eagle Creek National Fish Hatchery (NFH) offers a social and economic benefit to the Clackamas River watershed. The 5 year average harvest of steelhead in Eagle Creek was 1,023 for the period 1999-2003 (ODFW data as reported in USFWS 2007). Peak catches of hatchery fish occurred in December, January, February, and March prior to the bulk of the returning wild fish. Hatchery fish were also harvested in sport fisheries in the lower Clackamas and Willamette Rivers and may account for another 500 fish harvested annually (USFWS 2007). We need to balance the harvest benefits from hatchery production with the risks to ESA listed populations. In this study we evaluated adult distribution, natural production, impacts of residual steelhead, and disease and genetic information to assess the impact of hatchery released steelhead on wild steelhead in Eagle Creek and North Fork Eagle Creek. The native stock of winter steelhead returning to Eagle Creek are an important component in the recovery of listed and threatened fish species in the Clackamas River Basin. As cited in HSRG (2009b) the entire Clackamas winter steelhead (late) population is designated as a "Primary" population for recovery. We make the following recommendations, based on findings from our study, so that Eagle Creek steelhead provide an important contribution towards recovery.

We recommend that the North Fork Eagle Creek be identified as a wild fish reserve area with habitat protection and restoration measures along with continuing the restrictive harvest management practices currently in place.

We recommend the following hatchery practices at the 100,000 or lower smolt production level: spawn hatchery steelhead no later than March 15 and preferably all takes completed by the end of February in order to retain the early spawn and run timing of hatchery fish; keep the hatchery ladder open into April to remove hatchery fish from Eagle Creek; and evaluate the downstream migration rate of forced vs. volitionally

released juvenile fish from the hatchery (with removal of non-migrating individuals) to achieve quick outmigration of smolts released from Eagle Creek NFH. The downstream evaluation should include radio tagging, PIT (passive integrated transponder) tagging, physiological sampling, and snorkel/electrofishing to monitor over-summer residualism.

In this report we compared upper Eagle Creek to North Fork Eagle Creek and found upper Eagle Creek had a greater density of age 0 and larger abundance of age 1 winter steelhead. North Fork Eagle Creek is the primary area of natural spawning adult winter steelhead. The discrepancy between juvenile production and adult returns needs further evaluation. We recommend comparing productivity in North Fork Eagle Creek to that in upper Eagle Creek using spawner recruitment rates and smolt to adult recovery rates to assess the impact of naturally spawning hatchery fish on fitness and productivity of the natural population. This information will determine what life history stages, if any, are impacted by the winter steelhead hatchery program at Eagle Creek National Fish Hatchery.

There has been some discussion of ceasing propagation of winter steelhead at Eagle Creek NFH and rearing the stock at an off-station facility. The hatchery would continue to collect and spawn adult fish, but the eyed eggs would be transferred to ODFW's Oak Springs Fish Hatchery and reared through the smolt stage. These smolts would then be returned to Eagle Creek NFH for acclimation and release. Off station rearing would reduce costs associated with fish propagation such as staff and feed requirements. Oak Springs Fish Hatchery maintains a disease free status, so the risk of disease transmission would be minimal. If this proposal is implemented, monitoring for residual steelhead from these releases is recommended as well as monitoring adult returns at the hatchery and potential straying of Eagle Creek fish in tributaries of the lower Clackamas River Basin, in particular North Fork Eagle Creek. Straying behavior of male fish has been well documented, and the homing behavior of out of basin stocks is less precise than for locally adapted stocks (Barns 1976; Stabell 1981,1984).

As a result of fish health monitoring, the hatchery program at Eagle Creek NFH is at low risk of spreading disease to the wild steelhead and coho in Eagle Creek. We recommend continued periodic sampling to confirm this finding. We also recommend that all direct stream releases of hatchery fish into Eagle Creek be diligently monitored for health and disease (for example the spring Chinook releases into Eagle Creek by ODFW).

Other alternatives being considered for the winter steelhead production program at Eagle Creek NFH include: 1) replacing the current out of basin brood stock with native Clackamas River (or native Eagle Creek) winter steelhead; 2) replacing the current segregated program with an integrated "stepping stone" program where hatchery brood stock would be integrated with native Clackamas River hatchery brood stock propagated by ODFW, and 3) terminating the current steelhead program in favor of using the hatchery to support conservation, recovery, and reintroduction of native fish species in the lower Clackamas and Willamette Rivers. These alternatives, described in more detail in the USFWS (2007) Hatchery Review Team final report, would reduce or

eliminate the genetic and ecological risks associated with the current program. However, many challenges exist with switching to an integrated or all native brood stock program at Eagle Creek NFH. For example, a local brood stock program for steelhead would require the capability for two-years of on-station rearing to mimic the natural production cycle of the native Clackamas River population. Unfortunately, this is not possible at the present time due to reduced flows and increased water temperatures at the hatchery affecting fish rearing from June through August. If conservation alternatives are to be implemented at Eagle Creek NFH some of these alternatives may require a significant monetary investment, including considerable changes to the infrastructure of the current facility.

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9.0 Tables

Table 1: Number of hours per month the lower ladder was fished using V-trap fish weir in 2006 and 2007.

Year	Jan.	Feb.	March	April	May	June	Total hours fished
2006	54.5	67.5	99	94	120	0	*435*
2007	77	61	79	184	338.5	95	*834*

Table 2: Sample frame of available habitat units, number of habitat units selected for first phase snorkel surveys and number of habitat units selected for second phase calibration estimates, summer 2007.

Stream	Riffles[a]	Pools[b]	Glides[c]	Total
Lower Eagle Creek				
Total Number of Units	81	31	53	165
Phase 1 Snorkel Survey	8	8	11	27
Phase 2 Calibration Estimates	1	0	0	1
Upper Eagle Creek				
Total Number of Units	106	64	49	219
Phase 1 Snorkel Survey	10	16	10	36
Phase 2 Calibration Estimates	1	2[d]	2	5
North Fork Eagle Creek				
Total Number of Units	212	121	118	451
Phase 1 Snorkel Survey	21	30	24	75
Phase 2 Calibration Estimates	2	3	2	7

[a] Riffles were sampled at a rate of 1/10
[b] Pools were sampled at a rate of 1/4
[c] Glides were sampled at a rate of 1/5
[d] Phase 2 calibration estimates were conducted using multiple pass electrofishing with the exception of one pool on Eagle Creek in which a mark-recapture was conducted.

Table 3: Total numbers of genetic samples collected for each reach and sample year in Eagle and North Fork Eagle Creek.

Genetic Group	Year	Juvenile (n)	Smolts (n)	Total (n)
1.) Hatchery (HAT)	2005	0	56	56
	2006	0	48	48*
	2007	0	51	60
2.) Lower Eagle Creek	2005	0	50	50
(LEC)	2006	26	3	29*
	2007	15	9	24*
3.) N. Fork Eagle Creek	2005	46	5	51
(NFEC)	2006	27	42	69
	2007	0	47	47*
4.) Upper Eagle Creek	2005	0	50	50
(UEC)	2006	69	25	94
	2007	17	48	65
5.) Adult (NOR)	2005	----	----	42*
Natural Origin Recruits	2006	----	----	29*
	2007	----	----	29*
	Totals	200	434	743

Target sample size n=50 smolts and/or juveniles per year. (*) did not meet target.

Table 4: The estimated monthly catch of adult winter steelhead at the lower ladder on Eagle Creek in 2006.

2006	Catch	Effort	CPUE	Total hours in Month	Estimated Catch and (95% CI)
Wild					
January	0	54.5	0	744	0
February	4	67.5	0.059	672	40 (2-78)
March	12	99	0.121	744	90 (47-133)
April	11	94	0.117	720	84 (18-151)
May	3	120	0.025	744	19 (14-24)
June	0	0	0	720	0
Totals	***30***	***435***		***4344***	***233 (80-386)***
Hatchery					
January	28	54.5	0.513	744	382(0-1243)
February	67	67.5	0.992	672	667(214-1120)
March	41	99	0.414	744	308(12-604)
April	7	94	0.074	720	54 (6-101)
May	0	120	0	744	0
June	0	0	0	720	0
Totals	***143***	***435***		***4344***	***1411 (232-3069)***

Table 5: The estimated monthly catch of adult winter steelhead at the lower ladder on Eagle Creek in 2007.

2007	Catch	Effort	CPUE	Total hours in Month	Estimated Catch and (95% CI)
Wild					
January	2	77	0.026	744	19 (0-98)
February	2	61	0.032	672	22 (0-69)
March	3	79	0.038	744	28 (0-67)
April	7	184	0.038	720	27 (5-47)
May	16	338	0.047	744	35(3-61)
June	1	95	0.01	720	8[a]
Totals	***31***	***834***		***4344***	***139 (15-349)***
Hatchery					
January	67	77	0.87	744	647 (0-2565)
February	93	61	1.525	672	1025 (0-2104)
March	20	79	0.253	744	188 (56-290)
April	6	184	0.032	720	23 (8-36)
May	1	338	0.003	744	2 (0-7)
June	0	95	0.00	720	0
Totals	***187***	***834***		***4344***	***1885 (64-5002)***

[a] The ladder was fished only once in June 2007; therefore the upper and lower confidence intervals are the same as the mean estimate

Table 6: Last known locations of radio-tagged adult steelhead in Eagle Creek, 2005-2007.

Year	Origin	Sex	No. Tagged	Last Detection Hatchery receiver [a]	Last Detection North Fork receiver	Last Detection Mouth receiver	Last Detection Lower Ladder	Last Detection above Lower Ladder [b]	Last Detection below Lower Ladder
2005	Hatchery	Male	14	6	0	5	1	1	1
		Female	13	4	0	5	2	2	0
		Unknown	24	11 [c]	0	3	8	2	0
	Natural	Male	7	2	1	0	2	2	0
		Female	12	1	3	6	2	0	0
		Unknown	5	3	0	1	1	0	0
2006	Hatchery	Male	22	11	0	5	4	2	0
		Female	13	2	1	7	1	1	1
	Natural	Male	11	3	0	5	2	1	0
		Female	9	1	0	4	2	1	1
2007	Hatchery	Male	3	0	1	0	1	1	0
		Female	16	4	0	7 [d]	2	2	1
	Natural	Male	9	0	1	7	1	0	0
		Female	7	0	0	6	0	0	1

[a] Hatchery receiver is located approximately 100' downstream of hatchery fish ladder
[b] Mobile tracking events that occurred between lower ladder and middle ladder of Eagle Creek
[c] Detections include tags that were recovered during biosampling and by anglers near the hatchery.
[d] Four of these fish were also detected in the Clackamas River through mobile tracking.

Table 7: Summary of winter steelhead spawning ground surveys conducted in Eagle Creek and North Fork Eagle Creek by Oregon Department of Fish and Wildlife and Bureau of Land Management. For a detailed description of survey reaches in Eagle Creek please see Hutchinson et al. 2008. Downstream and upstream reach boundaries for North Fork Eagle Creek are identified by river kilometer.

	Downstream Reach Boundary	Upstream Reach Boundary	Times Surveyed	Survey Length (miles)	Total # of adult fish counted on redds	Marked	Unmarked	Unknown	Redd/km
Eagle Creek									
2006									
	rkm 1.06	rkm 2.5	9	1.3	13	0	3	10	8.1
	rkm 10.6	rkm 14.5	9	0.7	15	1	2	12	18
	rkm 14.5	rkm 25	9	0.7	35	10	2	23	52.5
2007									
	rkm 10.6	rkm 14.5	5	0.7	5	0	2	3	12.4
	rkm 14.5	rkm 25	5	1.1	2	2	0	0	13
	rkm 14.5	rkm 25	5	0.8	2	1	0	1	22.5
North Fork Eagle Creek									
2005									
	rkm 0.4	rkm 2.0	2	1	3	n/a	n/a	n/a	12
	rkm 2.0	rkm 3.6	1	1	0	n/a	n/a	n/a	2
	rkm 3.6	rkm 4.4	1	0.5	0	n/a	n/a	n/a	9.3
	rkm 4.4	rkm 7.2	1	1.75	0	n/a	n/a	n/a	18
2006									
	rkm 0.4	rkm 2.0	2	1	0	n/a	n/a	n/a	12
	rkm 2.0	rkm 3.6	2	1	0	n/a	n/a	n/a	5.5
	rkm 3.6	rkm 4.4	2	0.5	0	n/a	n/a	n/a	5
	rkm 4.4	rkm 7.2	1	1.75	0	n/a	n/a	n/a	18
2007									
	rkm 0.4	rkm 2.0	7	0.5	0	0	0	0	13
	rkm 2.0	rkm 3.6	6	0.8	0	0	0	0	1.5

Table 8: Population estimate for adult steelhead at the lower ladder on Eagle Creek. Confidence Intervals (95%) are reported in parentheses.

Year	Adult winter steelhead marked at Lower Ladder	Returning adults to hatchery	Marked winter steelhead recaptured at hatchery	Population Estimate
2007	180	1,436	67	3,823 (± 696)
2008	44	970	16	2,566 (± 927)

Reported angler catch of tagged fish was 18 in 2007 and 2 in 2008.

Table 9: Age class structure and mean length of wild steelhead trapped at the lower ladder and hatchery fish sampled at Eagle Creek NFH.

Year	Origin	Sex	Age 3 returns	Mean Length (cm)	Std. Dev.	Age 4 returns	Mean Length (cm)	Std. dev
2005	Wild	Male[a]	10	68	2.22	2	73	4.94
		Female	4	69	2.87	11	79	4.66
		Unknown	9	66	5.55	3	77	5.50
2006		Male	3	60	5.13	5	76	5.31
		Female	6	63	3.18	7	75	5.50
2007		Male	9	65	6.97	2	79	11.3
		Female	12	65	3.2	5	75	2.96
2005	Hatchery	Male	73	62	3.62	64	64	4.94
		Female	14	64	3.95	171	75	3.37
		Unknown	8	59	2.38	1	72	
2006		Male	309	64	3.37	43	72	4.96
		Female	131	64	3.50	64	73	3.51
2007		Male	551	65	3.61	191	77	4.82
		Female	226	64	3.13	468	75	3.69

[a] Age was not determined for three wild male steelhead

Table 10: Mean length, weight, and condition factor of radio-tagged hatchery smolts from low, medium, and high-density groups. Steelhead smolts that shed their tags in the hatchery raceways were excluded from analysis.

Year	Density Group	*n*	Mean Length (mm)	Mean Weight (g)	Condition Factor (95% CI)
2005	Low	20	196	79.9	1.05 *(±0.04)*
	Medium	21	199	78.6	0.99 *(±0.03)*
	High	22	192	53.5	0.75 *(±0.08)* [a]
2006	Low	15	192 [b]	74 [c]	1.04 *(±0.02)*
	Medium	15	175	57	1.04 *(±0.04)*
	High	15	177	61	1.07*(±0.02)*
2007	Low	15	194	n/a	n/a
	Medium	11	187	n/a	n/a
	High	13	184	n/a	n/a
2008	n/a	30	181	69	1.16 *(±0.04)*

[a] Condition factor of smolts from the high density group was significantly less than smolts from the low and medium density groups (ANOVA; F=32.3; df 2, 61; p=0.001).
[b] Mean length of radio-tagged fish from the low density group was significantly greater than those from the medium and high density groups (ANOVA; F=7.5;df 2, 39; p=0.001).
[c] Mean weight of radio-tagged fish from the low density group was significantly greater than those from the medium and high density groups (ANOVA; F=6.02;df 2, 39; p=0.005).

Table 11: Mean migration time, fork length and condition factor (with 95% confidence intervals in italics) for smolts released from Eagle Creek NFH and detected at the telemetry station at the mouth of Eagle Creek in 2003-2008. The number of fish that shed their tags in the raceways prior to release is in (italics), and these fish were excluded from all analyses. No siginficant difference in fork length or condition factor of migrant and mon-migrant steelhead smolts was detected with ANOVA.

Year	Number of smolts	Mean migration time (hrs)	Mean fork length (mm)	Mean Condition Factor (95% CI)
2003				
Migrant	16	40	176	1.00 *(±0.04)*
Non-Migrant	16	n/a	175	1.00 *(±0.03)*
2004				
Migrant	14	117	191	1.13 *(±0.04)*
Non-Migrant	8	n/a	200	1.11 *(±0.06)*
2005 *(12)*				
Migrant	8	223 *(±93)*	199 *(±10.4)*	0.88 *(±0.16)*
Non-Migrant	50	n/a	196 *(±3.1)*	0.93 *(±0.05)*
2006 *(0)*				
Migrant	10	139 *(±98)*	185 *(±9.4)*	1.05 *(±0.04)*
Non-Migrant	28	n/a	181 *(±5.7)*	1.05 *(±0.02)*
2007 *(6)*				
Migrant	27	67[a] *(±24)*	189 *(±4.1)*	n/a
Non-Migrant	9	n/a	187 *(±10.9)*	n/a
2008 *(0)*				
Migrant	6	204 *(±156)*	191 *(±14.4)*	1.1 *(±0.07)*
Non-Migrant	24	n/a	178 *(±9.5)*	1.25 *(±0.22)*

[a] Migration time for 15 of 27 smolts detected at the mouth

Table 12: Population estimates for smolts trapped in the rotary screw trap on Eagle Creek, 2006 and 2008.

Year	Species	Caught	Marked	Recaptured	Trap Efficiency	Estimated Migrants and 95%CI	Morts
2006	Hatchery Steelhead	3052	76	13	17%	17,848 (±7,702)	1095
	Hatchery Coho	6143	149	44	30%	20,824 (±4,969)	13,834
	Age 0 O.mykiss (<110 mm)	80	37	5	14%	592 (±357)	48
	Age 1 O.mykiss (>110 mm)	12	4	0			3
	Age 0 Wild coho(< 100 mm)	23	1	0			7
	Age 1 Wild coho (>100 mm)	13	8	0			1
	Wild Steelhead (<150 mm)	1	0	0			0
	Wild Steelhead(>150 mm)	8	5	0			3
	Cutthroat trout	2	1	0			1
	Longnose Dace	4	0	0			2
	Lamprey	5	0	0			0
2008	Hatchery Steelhead	174	0	0			0
	Hatchery Coho	1612	0	0			0
	Age 0 Wild coho (<100 mm)	15	14	1	7%	210 (±126)	0
	Age 1 Wild coho (>100 mm)	96	85	11	13%	742 (±368)	0
	Age 0 O.mykiss (<110 mm)	61	51	5	10%	622 (±478)	0
	Age 1 O.mykiss (>110 mm)	21	20	1	5%	420 (±175)	0
	Wild Steelhead(>150 mm)	34	34	0			0
	Cutthroat trout	2	0	0			0
	Lamprey	8	0	0			0

Table 13: Estimated number of juvenile steelhead migrating from North Fork Eagle Creek, 1998-2008. A 95% confidence interval is expressed as a percentage of the population estimate. Population estimates for 2006-2007, and confidence intervals for the 2008 estimates were not available at the time of this report. Peak capture dates were only available for some years.

Year	Age 0 winter steelhead	*95% CI*	Peak capture date	Age 1 winter steelhead	*95% CI*	Peak capture date
1998	2511	16	April 24th	1496	145	April 24th
1999	8162	22	May 31st	3750	10	May 24th
2000	5822	35	n/a	2248	40	n/a
2001	8306	14	n/a	1507	11	n/a
2002	5861	23	n/a	2319	25	n/a
2003	1412	18	n/a	1119	23	n/a
2004	6131	8	n/a	1141	20	April 18th
2005	4210	12	n/a	3737	27	April 23rd
2006	n/a	n/a	n/a	n/a	n/a	n/a
2007	n/a	n/a	n/a	n/a	n/a	n/a
2008	1460	n/a	n/a	1741	n/a	n/a

Data obtained from Fisheries Partnerships in Action Accomplishments reports for Clackamas River working group 1999-2006 and personnel communication with Tom Horning USFS.

Table 14: Summary of habitat unit characteristics of lower Eagle Creek, upper Eagle Creek, and North Fork Eagle Creek, summer 2007.

Stream Reach	Riffles	Pools	Glides	Total
Lower Eagle Creek				
Number of Habitat Units	81	31	53	165
% of Total Habitat	49	19	32	100
Length of Habitat Units (m)	5,630	1,380	3,407	10,417
% of Total Stream Length	54	13	33	100
Area of Habitat Units (m^2)	106,997	25,122	60,318	192,437
% of Total Area	56	13	31	100
Upper Eagle Creek				
Number of Habitat Units	106	64	49	219
% of Total Habitat	48	29	23	100
Length of Habitat Units (m)	6,584	2,659	2,214	11,457
% of Total Stream Length	58	23	19	100
Area of Habitat Units (m^2)	107,869	37,417	33,016	178,302
% of Total Area	60	21	19	100
North Fork Eagle Creek				
Number of Habitat Units	212	121	118	451
% of Total Habitat	47	27	26	100
Length of Habitat Units (m)	9,839	2,535	2,474	14,848
% of Total Stream Length	66	17	17	100
Area of Habitat Units (m^2)	77,125	20,399	18,140	115,664
% of Total Area	67	17	16	100

Table 15: Population estimates of juvenile fish in Lower Eagle Creek (LEC), Upper Eagle Creek (UEC), and North Fork Eagle Creek (NFEC) calculated from two phase snorkel surveys conducted during the summer of 2007. Confidence intervals (95%) are reported in parentheses.

Species	*O. mykiss* age 0			*O. mykiss* age 1			Residual Hatchery Winter Steelhead			Coho Salmon		
Habitat Type	LEC	UEC	NFEC	LEC	UEC	NFEC	LEC	UEC	NFEC	LEC	UEC	NFEC
Glides	2,949 (± 2,160)	10,708 (± 2,124)	3,162 (± 3,157)	712 (± 263)	454 (± 250)	677 (± 167)	0	282 (± 250)	0	958 (± 908)	1,975 (± 887)	2,460 (± 1,429)
Pools	948 (± 2,957)	18,421 (± 4,046)	2,581 (± 5,664)	112 (± 49)	637 (± 85)	247 (± 150)	0	215 (± 85)	0	255 (± 762)	6,283 (± 1,079)	5,397 (± 1,582)
Riffles	9,255 (± 885)	30,015 (± 1,318)	10,870 (± 3,080)	4,491 (± 283)	2,030 (± 500)	1,501 (± 1,315)	102 (± 283)	187 (± 500)	0	15,626 (± 784)	11,090 (± 1,215)	14,471 (± 2,940)
Totals	13,152 (± 3,342)	59,143 (± 4,459)	16,613 (± 6,954)	5,315 (± 348)	3,121 (± 508)	2,425 (± 1,254)	102 (± 348)	685 (± 508)	0	16,839 (± 1,267)	19,348 (± 1,697)	22,328 (± 3,486)

Table 16: Descriptive statistics among the Eagle Creek Steelhead genetic collections for combined years 2005-2007. Column headings are defined as follows: n is the number of individuals, A is the number of alleles, AR is the allelic richness, 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} in the index of inbreeding, and θ is the unbiased estimate of Wright's F_{ST} (Weir and Cockerham 1984). Values with the symbol (*) indicate statistical significance (Rice 1989; α = 0.05 adjusted byα/k).

	HAT (2005-2007)							LEC (2005-2007)						
Locus	N	A	AR	AP	H_E	H_O	F_{is}	n	A	AR	AP	H_E	H_O	F_{is}
µOcl1	161	14	14	--	0.897	0.814	0.093*	101	15	15	--	0.816	0.812	0.004
µOgo3	164	8	7	--	0.627	0.561	0.105*	103	8	8	--	0.554	0.573	-0.034
µOgo4	164	9	9	--	0.797	0.768	0.037	103	10	10	--	0.781	0.777	0.005
µOki23	164	13	13	--	0.893	0.890	0.003	103	14	14	--	0.858	0.854	0.004
µOmy1011	164	16	15	--	0.874	0.854	0.024	103	17	17	--	0.887	0.951	-0.073
µOmy77	163	12	12	--	0.855	0.871	-0.019	103	14	14	--	0.871	0.874	-0.004
µOmy7i	163	10	10	--	0.734	0.804	-0.096	101	14	14	1	0.764	0.762	0.002
µOne13	164	16	15	--	0.830	0.793	0.045	103	19	19	--	0.858	0.845	0.016
µOne14	164	9	9	--	0.785	0.823	-0.049	102	10	10	--	0.800	0.735	0.081
µOts1	164	11	11	1	0.723	0.695	0.038	103	14	14	--	0.713	0.583	0.183*
µOts100	159	12	12	--	0.901	0.925	-0.026	100	14	14	1	0.883	0.860	0.027
µOts3	164	7	7	--	0.605	0.591	0.022	102	9	9	--	0.682	0.667	0.023
µOts4	164	7	7	--	0.579	0.555	0.042	103	8	8	--	0.618	0.602	0.026
µSsa289	163	6	6	--	0.634	0.687	-0.085	103	5	5	--	0.579	0.534	0.078
µSsa407	164	15	14	--	0.878	0.884	-0.007	103	18	18	--	0.812	0.883	-0.089
µSsa408	163	16	15	--	0.871	0.840	0.035	103	18	18	--	0.922	0.913	0.010
Over All	163	11	11	1	0.780	0.772	0.010	102	13	13	2	0.775	0.764	0.014

Table 16 conti.

	NFEC (2005-2007)							UEC (2005-2007)						
Locus	N	A	AR	AP	H_E	H_O	F_{is}	n	A	AR	AP	H_E	H_O	F_{is}
µOcl1	168	17	16	1	0.868	0.851	0.019	209	15	14	--	0.865	0.809	0.066
µOgo3	167	7	7	--	0.584	0.557	0.047	208	8	7	1	0.586	0.587	-0.001
µOgo4	168	10	10	--	0.837	0.845	-0.009	209	8	8	--	0.801	0.780	0.026
µOki23	168	14	13	1	0.834	0.839	-0.007	209	14	13	--	0.867	0.813	0.062
µOmy1011	168	18	17	1	0.882	0.893	-0.013	209	18	17	--	0.875	0.871	0.005
µOmy77	168	17	16	1	0.863	0.798	0.076	209	15	14	--	0.869	0.842	0.031
µOmy7i	167	14	14	--	0.767	0.731	0.048	209	14	13	--	0.810	0.861	-0.064
µOne13	167	19	17	--	0.864	0.868	-0.005	208	22	19	1	0.841	0.865	-0.029
µOne14	167	12	10	3	0.746	0.707	0.052	209	11	11	--	0.793	0.794	-0.002
µOts1	168	13	12	--	0.718	0.643	0.105*	209	14	12	--	0.686	0.660	0.038
µOts100	167	17	15	1	0.893	0.886	0.008	206	16	14	--	0.875	0.845	0.035
µOts3	168	10	9	2	0.629	0.595	0.054	209	9	8	--	0.671	0.627	0.065
µOts4	167	9	9	--	0.646	0.635	0.017*	209	7	7	--	0.517	0.522	-0.008
µSsa289	167	6	6	--	0.616	0.635	-0.031	209	6	5	--	0.592	0.565	0.047
µSsa407	168	19	17	2	0.855	0.869	-0.016	209	18	16	--	0.851	0.813	0.045
µSsa408	167	19	19	--	0.924	0.904	0.021	207	18	17	--	0.911	0.928	-0.018
Over All	168	14	13	12	0.783	0.766	0.022	209	13	12	2	0.776	0.761	0.019

Table 16 conti.

	NOR(2005-2007)							Overall (2005-2007)							
Locus	N	A	AR	AP	H_E	H_O	F_{is}	n	A	AR	AP	H_E	H_O	F_{is}	F_{st} (θ)
μOcl1	96	14	14	--	0.848	0.760	0.103	147	15	15	1	0.859	0.809	0.058	0.024
μOgo3	100	7	7	--	0.542	0.550	-0.015	148	8	7	1	0.578	0.565	0.023	-0.001
μOgo4	100	9	9	--	0.790	0.780	0.013	149	9	9	--	0.801	0.790	0.014	0.010
μOki23	100	16	16	1	0.852	0.860	-0.009	149	14	14	2	0.861	0.851	0.011	0.013
μOmy1011	100	15	15	--	0.878	0.830	0.055	149	17	17	1	0.879	0.880	0.000	0.007
μOmy77	99	15	15	--	0.872	0.848	0.027	148	15	14	1	0.866	0.847	0.022	0.009
μOmy7i	99	13	13	1	0.771	0.737	0.043	148	13	13	2	0.769	0.779	-0.013	0.008
μOne13	100	17	17	--	0.836	0.790	0.055	148	19	18	1	0.846	0.832	0.016	0.013
μOne14	100	10	10	--	0.760	0.730	0.039	148	10	10	3	0.777	0.758	0.024	0.008
μOts1	100	15	15	--	0.751	0.720	0.041	149	13	13	1	0.718	0.660	0.081	0.006
μOts100	100	18	18	2	0.867	0.870	-0.004	146	15	16	4	0.884	0.877	0.008	0.008
μOts3	100	8	8	--	0.674	0.710	-0.053	149	9	9	2	0.652	0.638	0.022	0.003
μOts4	99	7	7	--	0.587	0.596	-0.016	148	8	8	--	0.589	0.582	0.013	0.007
μSsa289	99	7	7	1	0.620	0.596	0.039	148	6	6	1	0.608	0.603	0.008	0.006
μSsa407	100	15	15	--	0.814	0.810	0.005	149	17	17	2	0.842	0.852	-0.012	0.013
μSsa408	99	18	18	--	0.926	0.929	-0.004	148	18	18	--	0.911	0.903	0.009	0.014
Over All	99	13	13	5	0.774	0.757	0.022	148	13	13	22	0.778	0.764	0.017	**0.010**
L95% CI	--	--	--	--	--	--	--	--	--	--	--		--	--	***0.008***
U95% CI	--	--	--	--	--	--	--	--	--	--	--		--	--	***0.013***

Table 17: The top matrix provides the pairwise Fst values for all comparisons across temporal replicate collections. Significant among-group variation is indicated in the bottom matrix, and is based on 20,000 permutations and an adjusted P-value (*0.0005) multiple tests.

	HAT 06	HAT 07	LEC 05	LEC 06	LEC 07	NFEC 05	NFEC 06	NFEC 07	NOR 05	NOR 06	NOR 07	UEC 05	UEC 06	UEC 07
HAT05	0.0099	0.0076	0.0287	0.0270	0.0181	0.0167	0.0251	0.0195	0.0173	0.0215	0.0370	0.0277	0.0225	0.0103
HAT06		-0.0003	0.0275	0.0247	0.0151	0.0179	0.0181	0.0145	0.0155	0.0164	0.0301	0.0228	0.0186	0.0075
HAT07			0.0243	0.0214	0.0112	0.0159	0.0154	0.0098	0.0123	0.0149	0.0260	0.0191	0.0162	0.0039
LEC05				0.0000	0.0040	0.0075	0.0071	0.0070	0.0039	0.0003	-0.0004	-0.0001	0.0022	0.0153
LEC06					0.0037	0.0065	0.0029	0.0060	0.0022	0.0007	-0.0012	0.0014	0.0019	0.0106
LEC07						0.0061	0.0074	0.0040	0.0022	-0.0005	0.0099	0.0030	0.0016	0.0027
NFEC05							0.0038	0.0019	0.0035	0.0007	0.0110	0.0092	0.0086	0.0116
NFEC06								0.0007	0.0051	0.0030	0.0057	0.0077	0.0055	0.0125
NFEC07									0.0029	0.0003	0.0088	0.0079	0.0054	0.0075
NOR05										0.0015	0.0088	0.0040	0.0016	0.0078
NOR06											0.0025	0.0026	0.0015	0.0105
NOR07												0.0015	0.0051	0.0183
UEC05													0.0018	0.0114
UEC06														0.0075

	HAT06	HAT 07	LEC 05	LEC 06	LEC 07	NFEC 05	NFEC 06	NFEC 07	NOR 05	NOR 06	NOR 07	UEC 05	UEC 06	UEC 07
HAT05	*	NS	*	*	*	*	*	*	*	*	*	*	*	*
HAT06		NS	*	*	*	*	*	*	*	*	*	*	*	NS
HAT07			*	*	NS	*	*	*	*	*	*	*	*	NS
LEC05				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
LEC06					NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LEC07						NS	NS	NS	NS	NS	NS	NS	NS	NS
NFEC05							NS	NS	NS	NS	NS	*	*	*
NFEC06								NS	NS	NS	NS	*	NS	*
NFEC07									NS	NS	NS	NS	NS	NS
NOR05										NS	NS	NS	NS	NS
NOR06											NS	NS	NS	NS
NOR07												NS	NS	*
UEC05													NS	*
UEC06														*

Table 18: Proportion of correct assignments by year and group using the jackknife re-sampling procedure. A positive LOD score for HAT (ECNFH) individuals from the designated "critical" population indicated a correct assignment, while a negative LOD represented correct assignments among NOR individuals within independent comparisons with HAT. The HAT samples were pooled across years. (2005-2007).

Group	2005	2006	2007	mean	HAT
Upper Eagle Creek	84.0	75.5	41.5	67.0	93.3
Lower Eagle Creek	90.0	72.4	70.8	77.7	96.3
North Fork Eagle Creek	76.5	82.9	85.1	81.5	95.1
Adult Wild Fish	69.0	75.9	82.8	75.9	93.3
mean	79.9	76.7	70.1		94.5

10.0 Figures

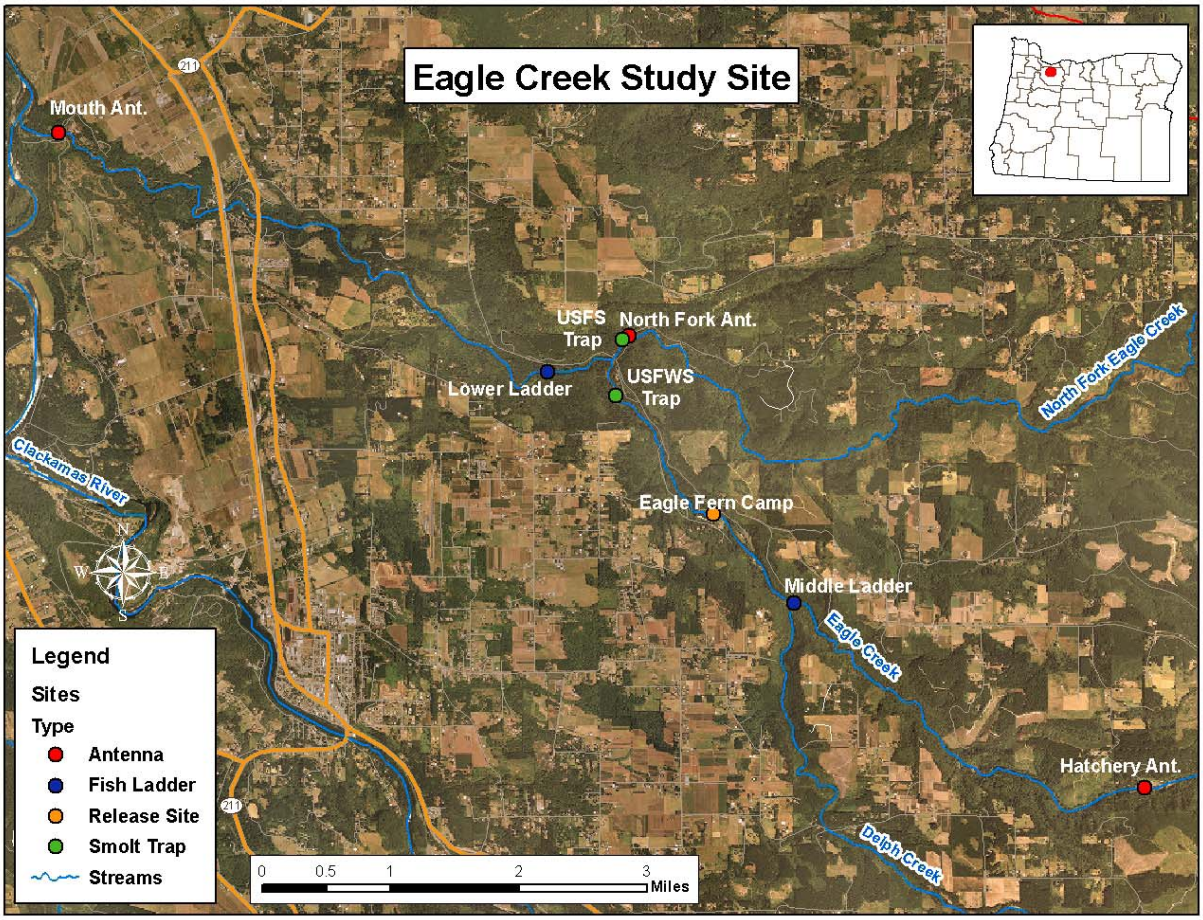


Figure 1: Map of Eagle Creek and North Fork Eagle Creek study area. Stationary telemetry receivers were located at the mouth, on the North Fork, and at the hatchery. Smolt traps were operated in Eagle Creek (above the confluence with North Fork Eagle Creek) and in North Fork Eagle Creek. Adult winter steelhead were radio and Floy tagged at the lower ladder.

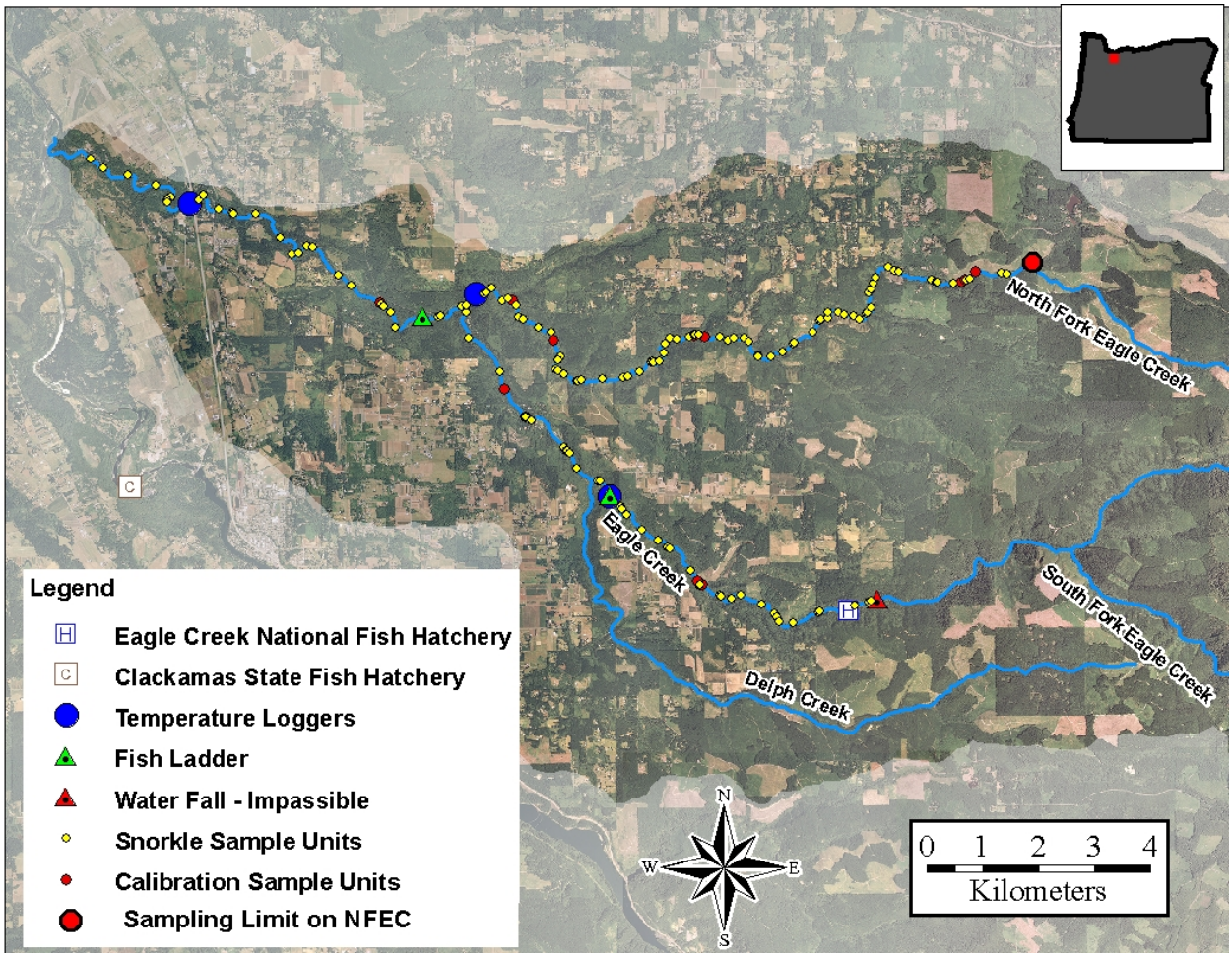
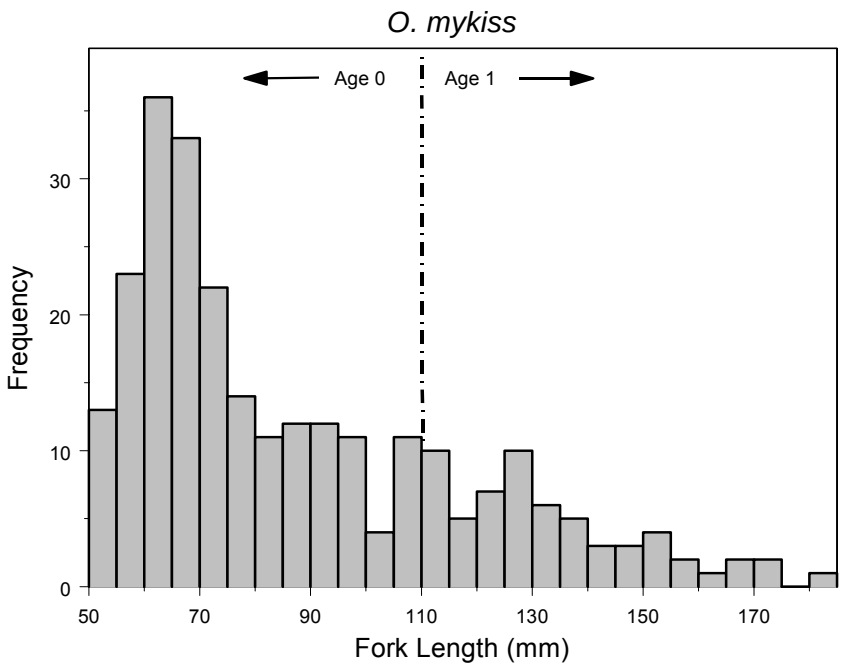


Figure 2: Map showing the spatial distribution of first phase snorkel units and second phase diver calibration units in Eagle Creek and North Fork Eagle Creek.



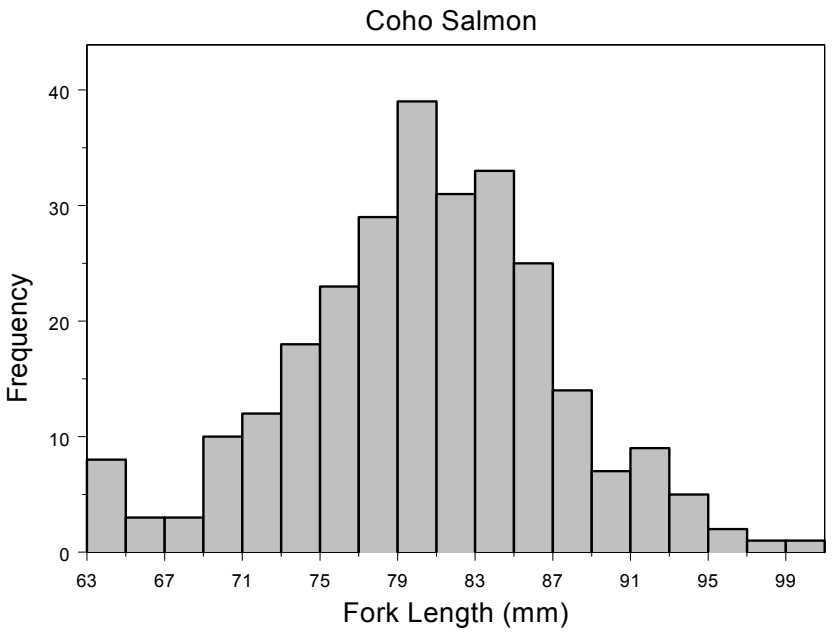


Figure 3: Length-frequency distribution of O. mykiss and coho salmon captured from Eagle Creek and North Fork Eagle Creek while electrofishing, summer 2007. O. mykiss age 0 and O. mykiss age 1 fish are separated by a fork length of 110mm which was verified by scale analysis.

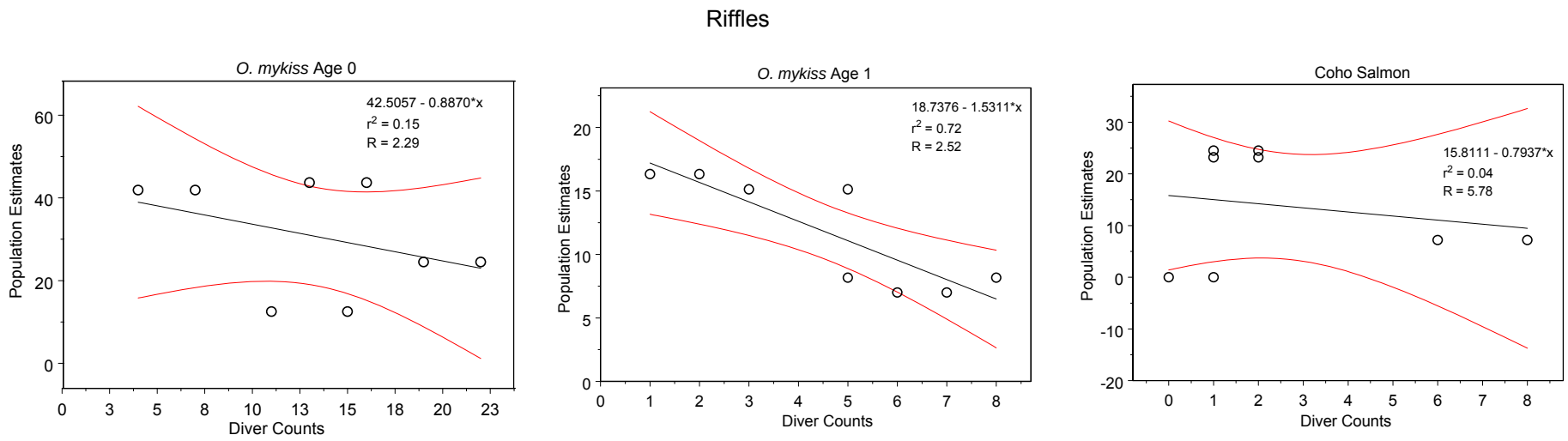


Figure 4: Results from second phase snorkeler calibrations in riffle habitat for O. mykiss and coho salmon, summer 2007. The black line is the estimated regression of the "true" fish population explained by single pass diver counts. The red lines represent the 95% confidence bounds for the estimated mean. The equation for the regression line, correlation coefficient (r^2), and calibration ratio (R) are given for each species.

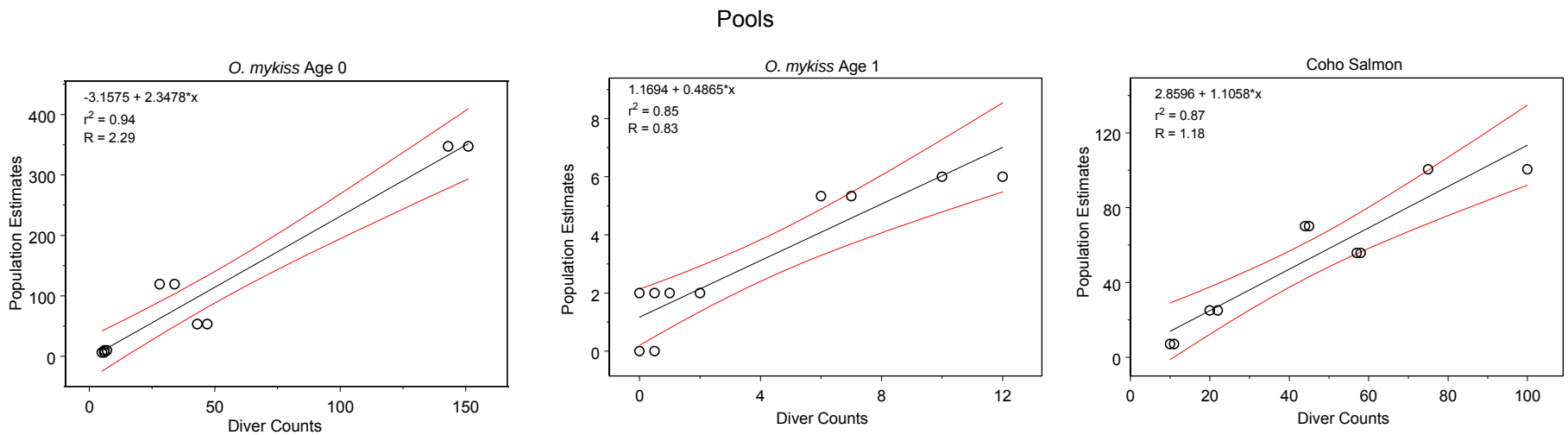


Figure 5: Results from second phase snorkeler calibrations in pool habitat for O. mykiss and coho salmon, summer 2007. The black line is the estimated regression of the "true" fish population explained by single pass diver counts. The red lines represent the 95% confidence bounds for the estimated mean. The equation for the regression line, correlation coefficient (r^2), and calibration ratio (R) are given for each species. To allow for visualization of all data some points are jittered along the x-axis.

Glides

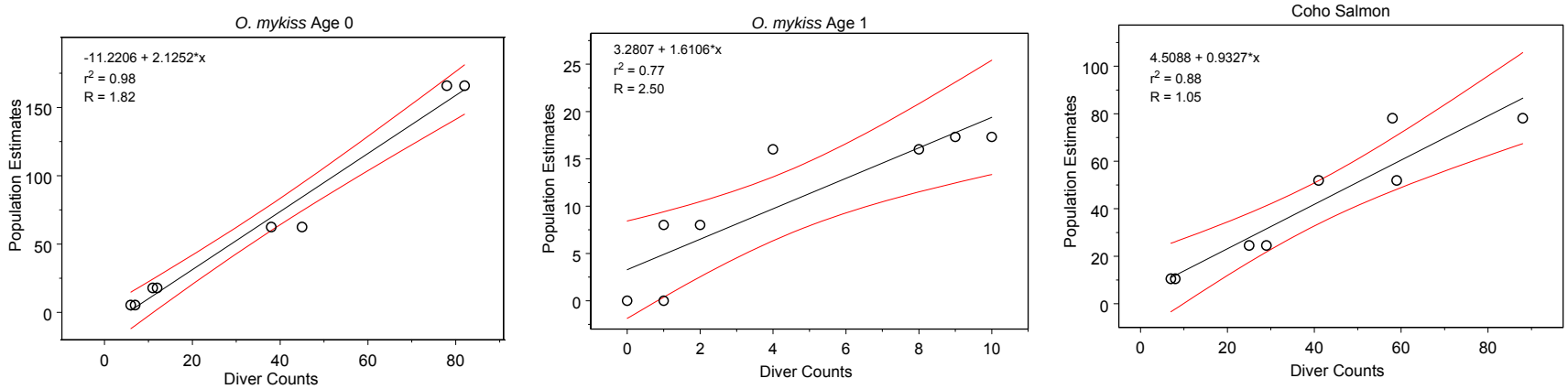


Figure 6: Results from second phase snorkeler calibrations in glide habitat for O. mykiss and coho salmon, summer 2007. The black line is the estimated regression of the "true" fish population explained by single pass diver counts. The red lines represent the 95% bounds for the estimated mean. The equation for the regression line, correlation coefficient (r^2), and calibration ratio (R) are given for each species. To allow for visualization of all data some points are jittered along the x-axis.

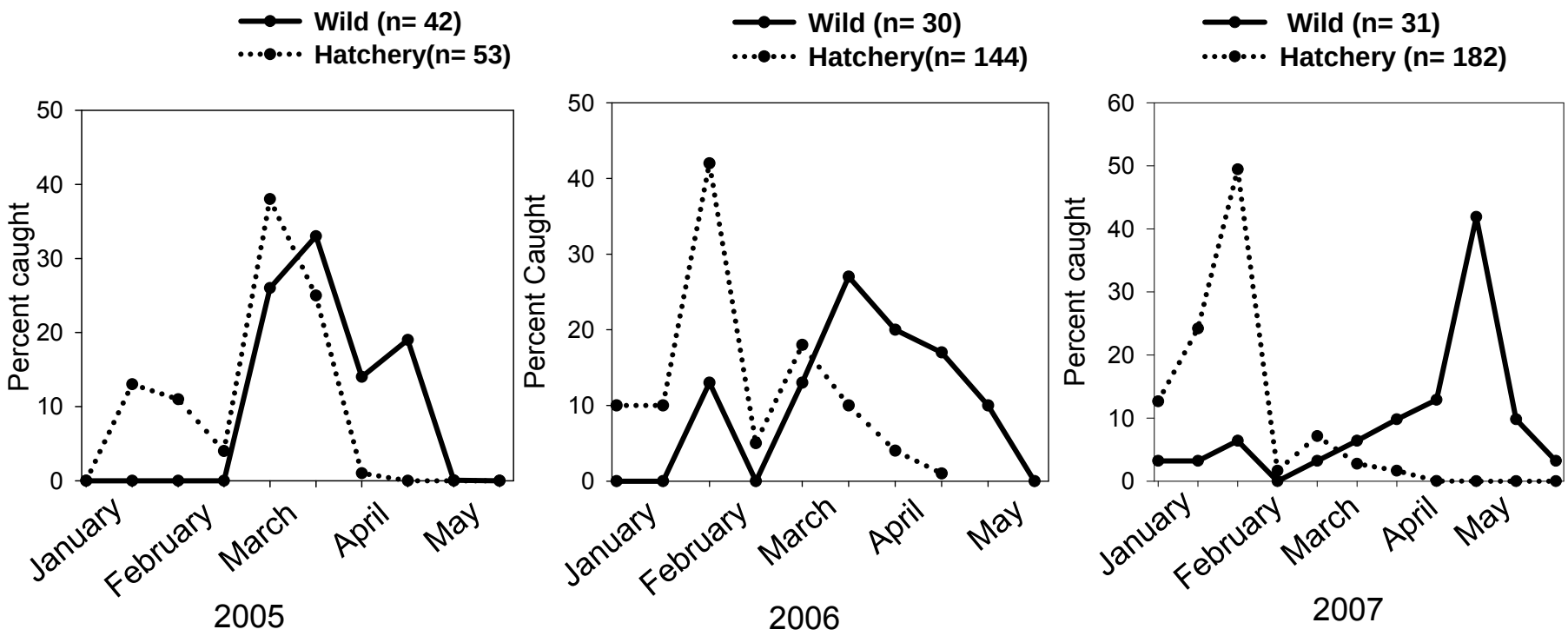


Figure 7: Monthly catch of adult winter steelhead at the lower ladder on Eagle Creek 2005-2007.

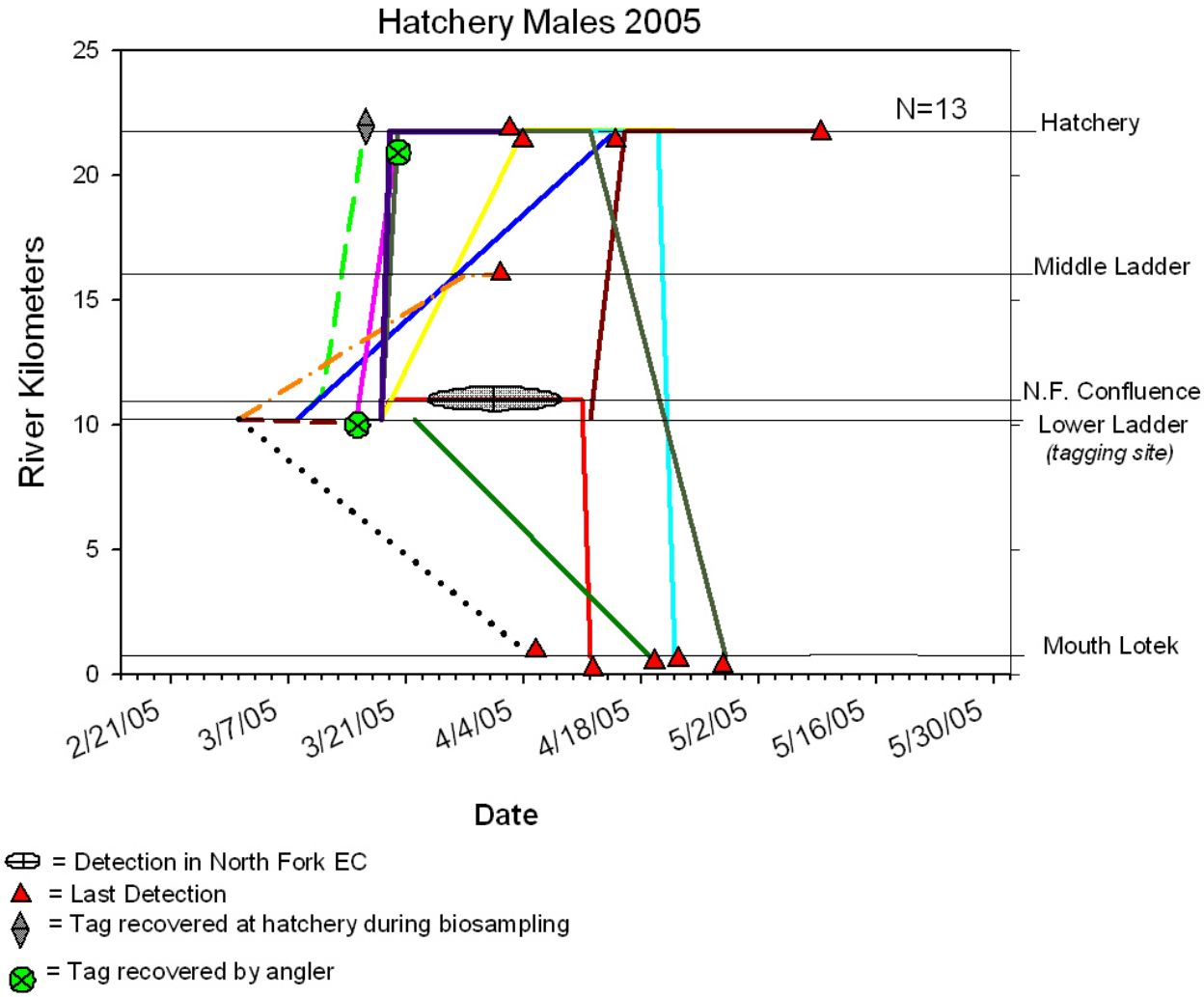


Figure 8: Distribution and movement of radio-tagged hatchery males in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.

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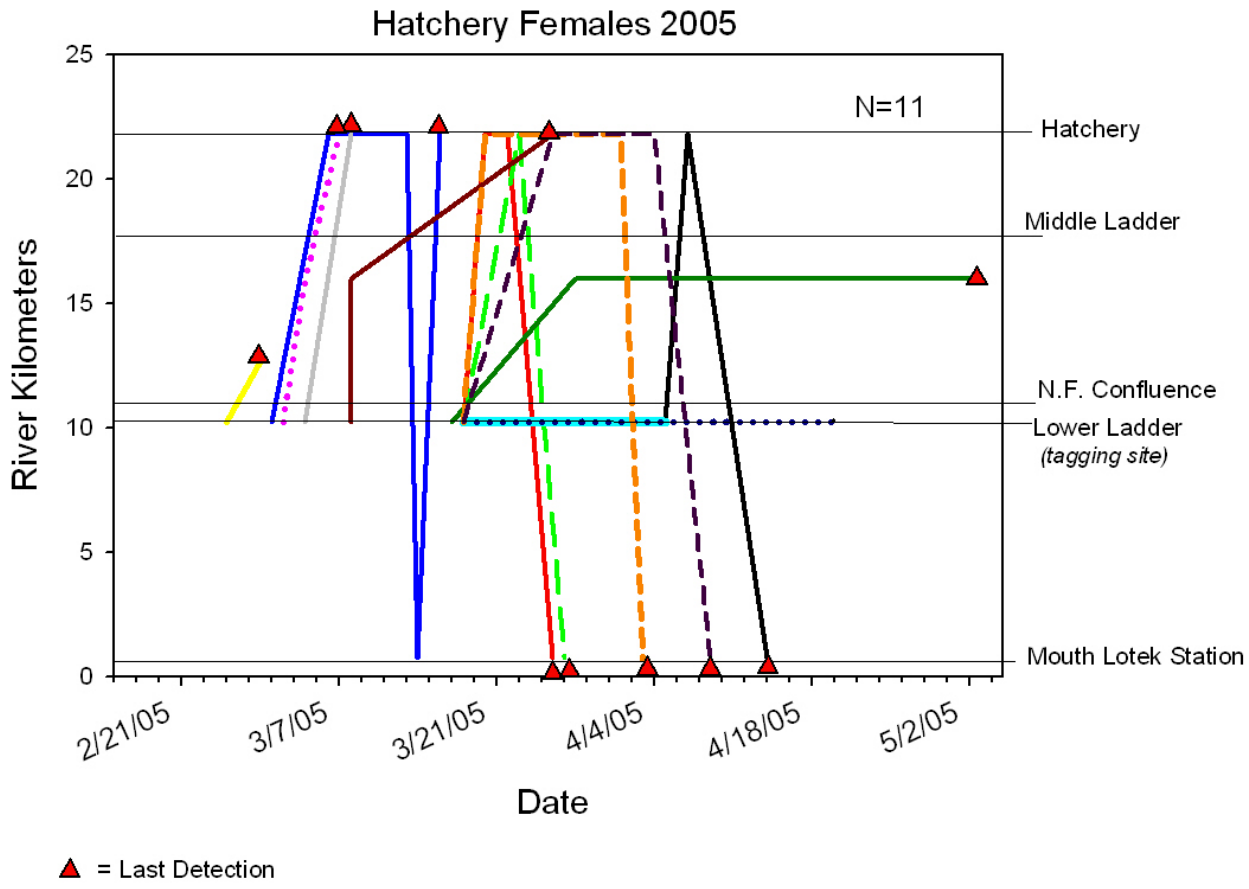


Figure 9: Distribution and movement of radio-tagged hatchery females in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.

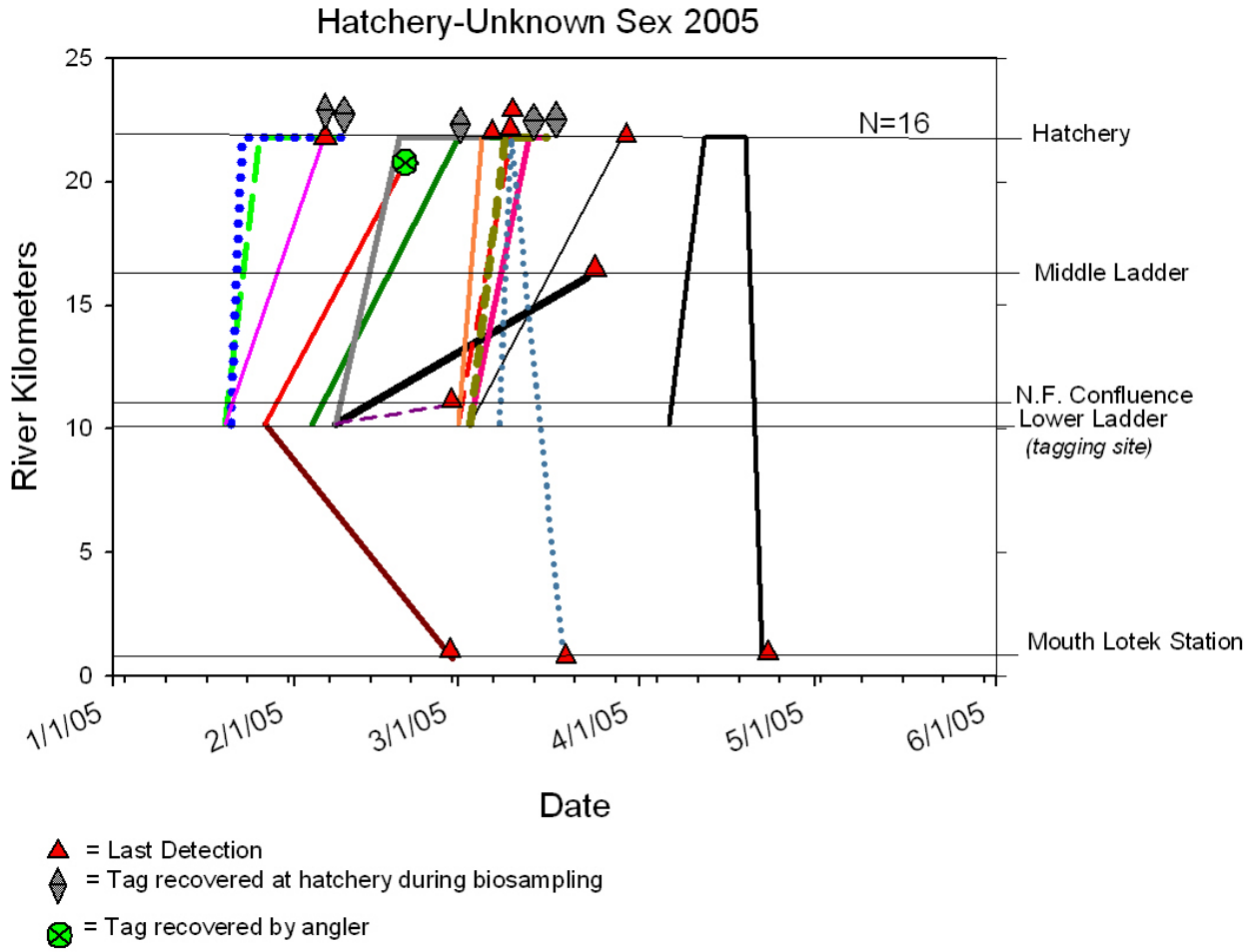


Figure 10: Distribution and movement of radio-tagged hatchery steelhead of unknown sex in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.

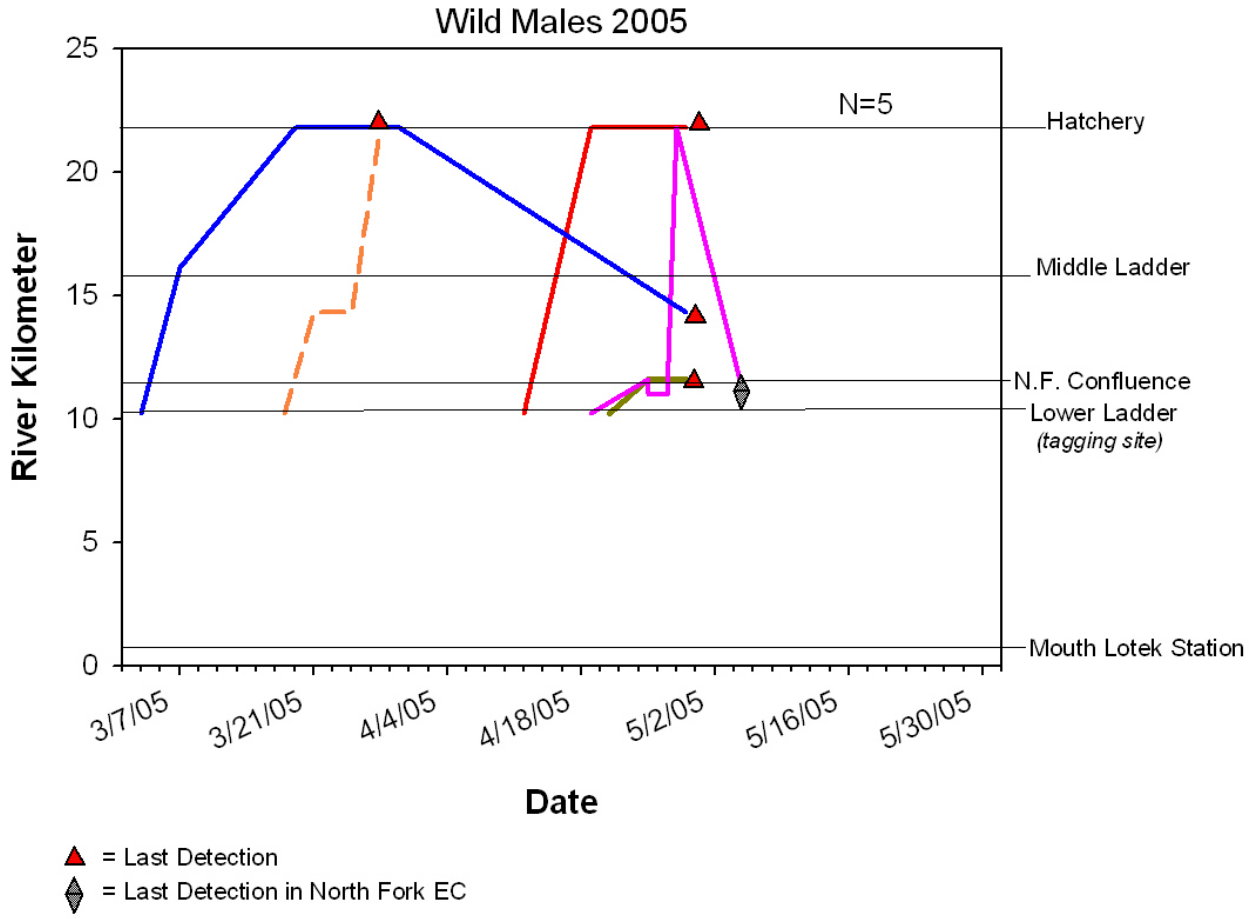


Figure 11: Distribution and movement of radio-tagged wild male steelhead in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.

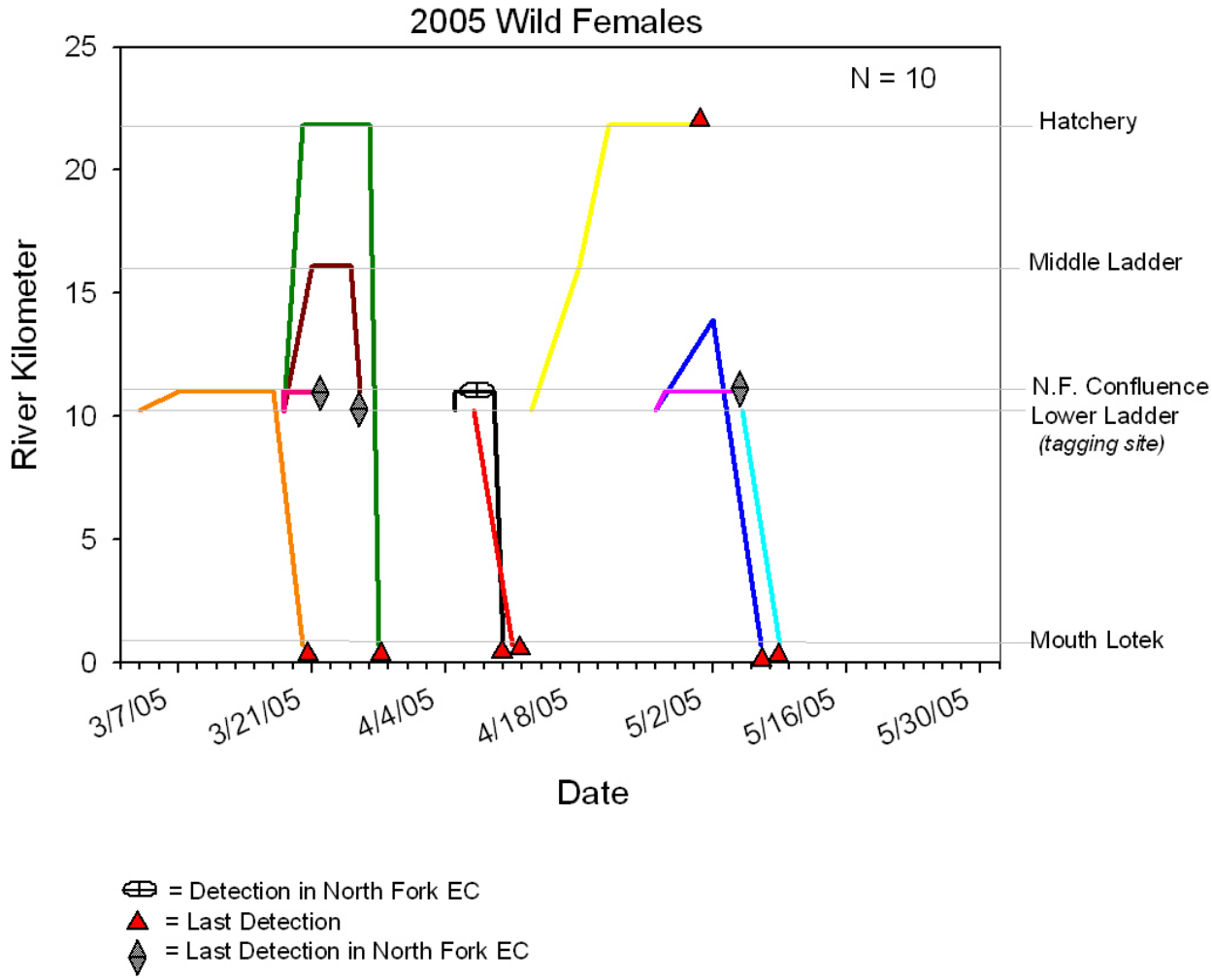


Figure 12: Distribution and movement of radio-tagged wild female steelhead in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.

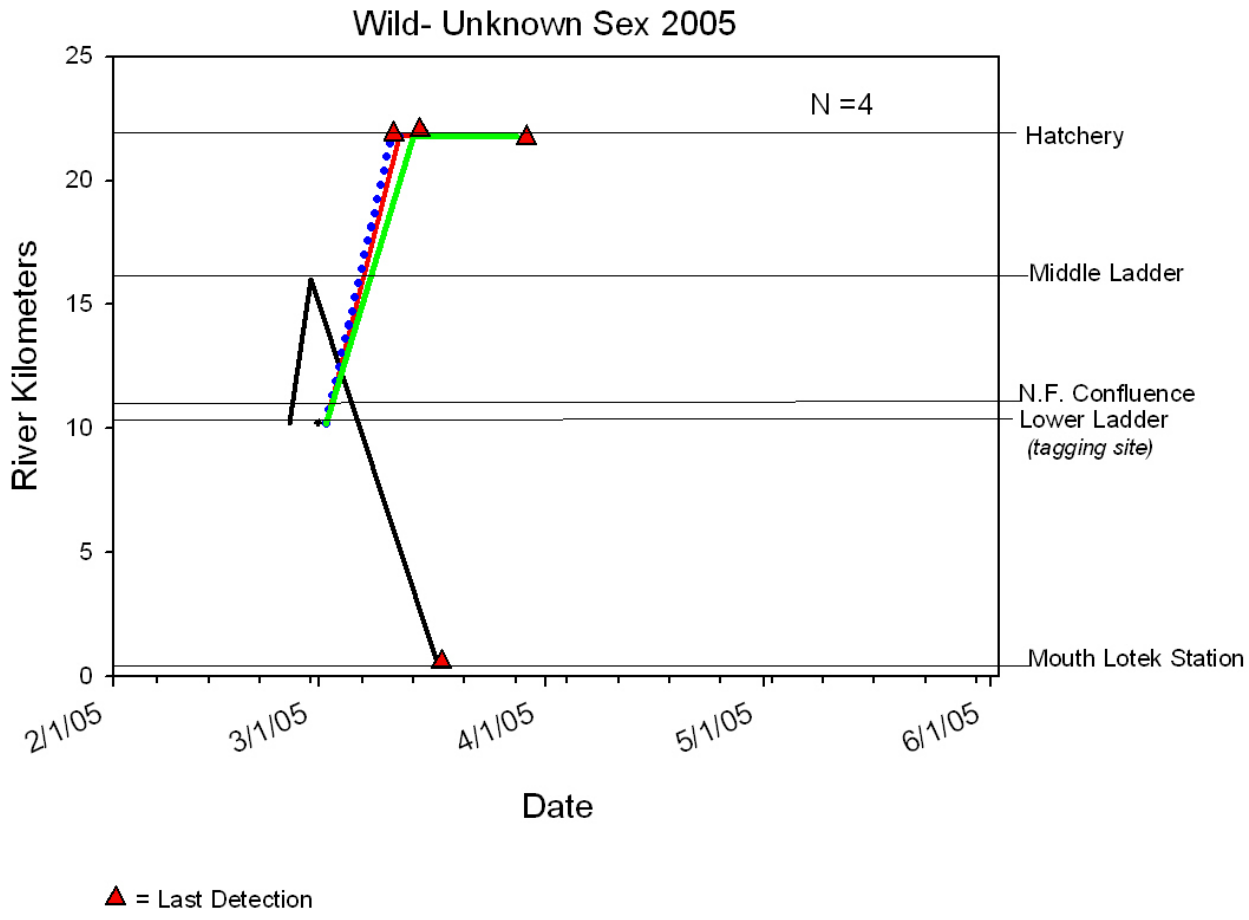


Figure 13: Distribution and movement of radio-tagged wild steelhead of unknown sex in 2005. Steelhead that shed tags at the lower ladder are not included on this graph.

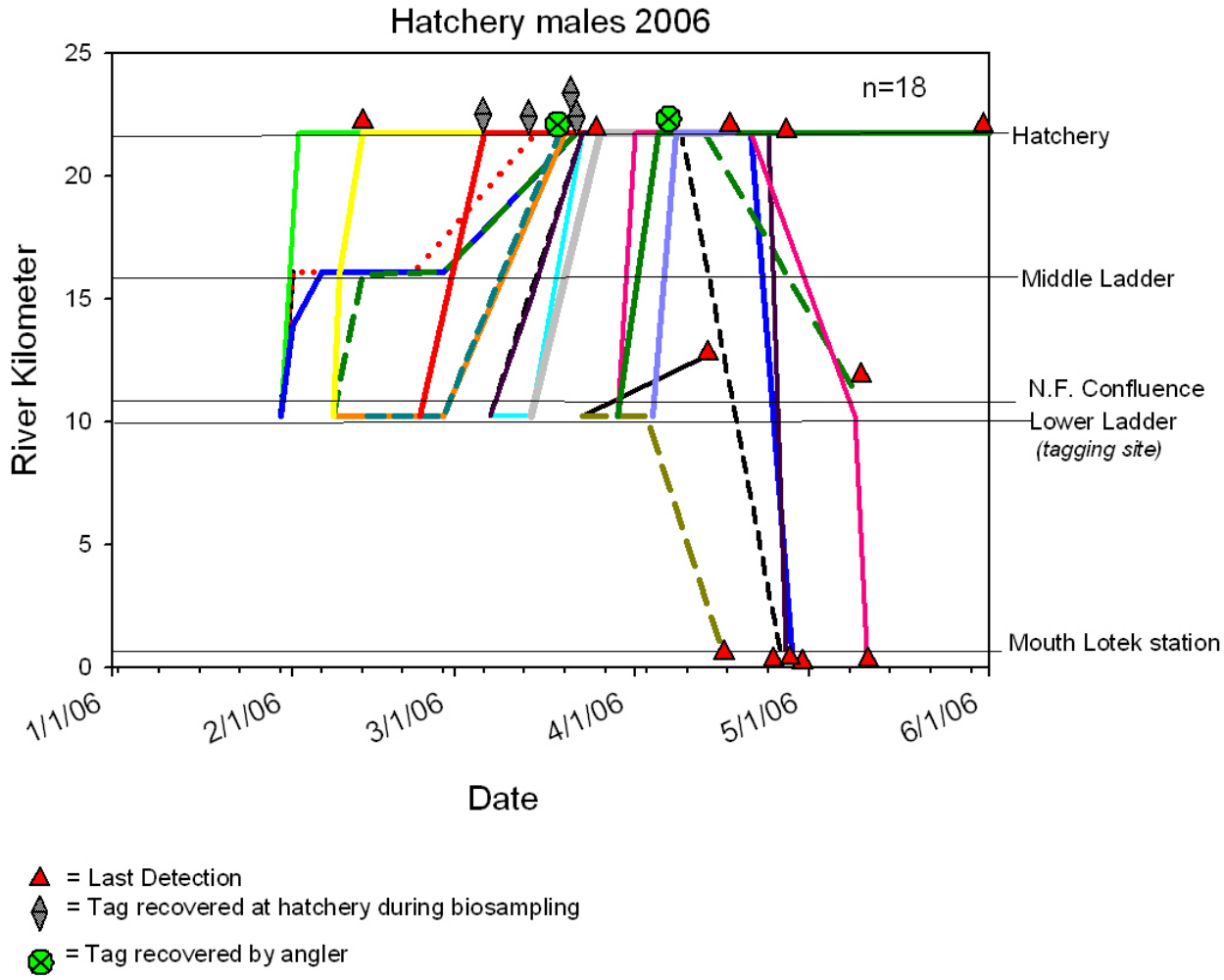


Figure 14: Distribution and movement of radio-tagged hatchery males in 2006. Steelhead that shed tags at the lower ladder are not included on this graph.

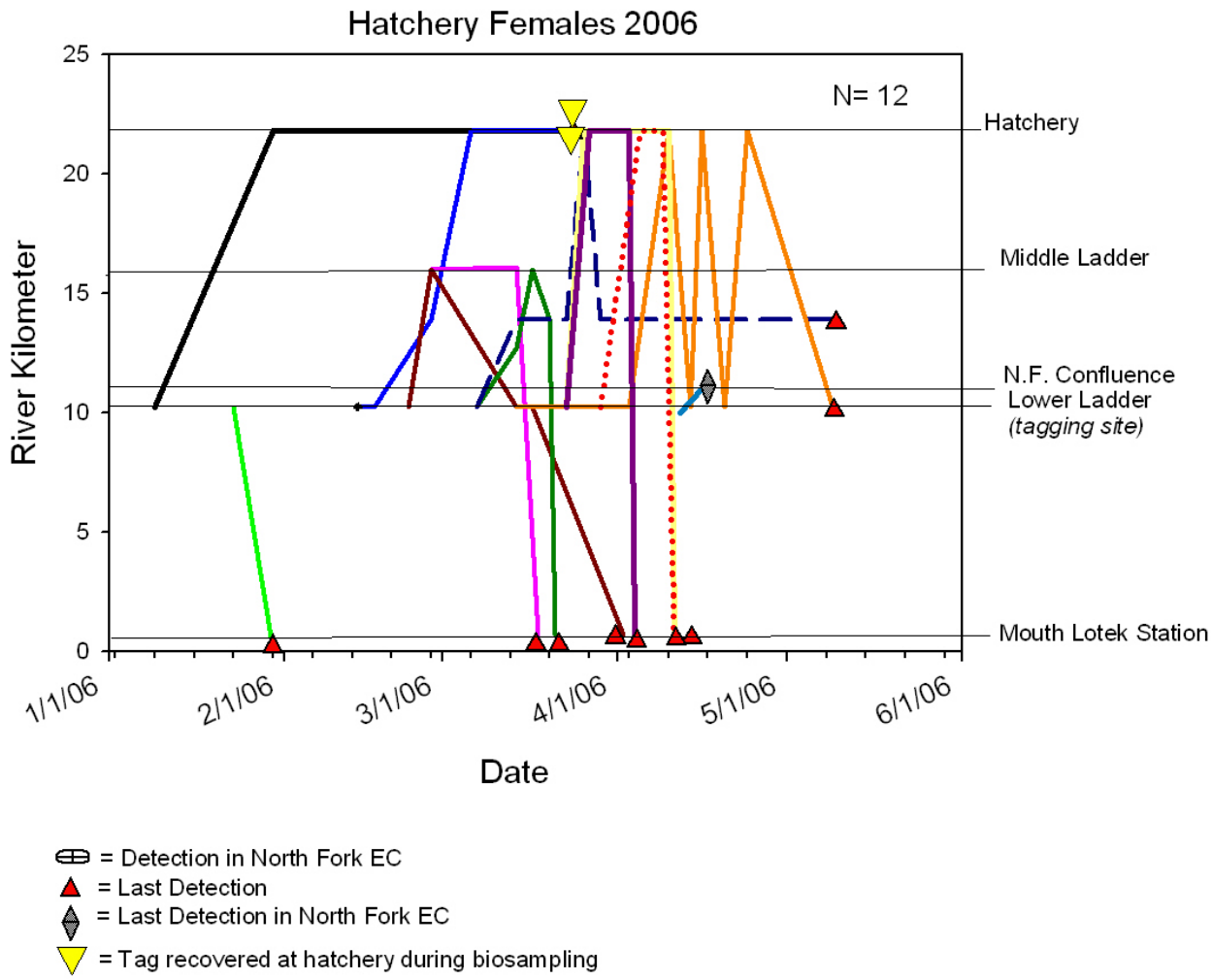


Figure 15: Distribution and movement of radio-tagged hatchery females in 2006. Steelhead that shed tags at the lower ladder are not included on this graph.

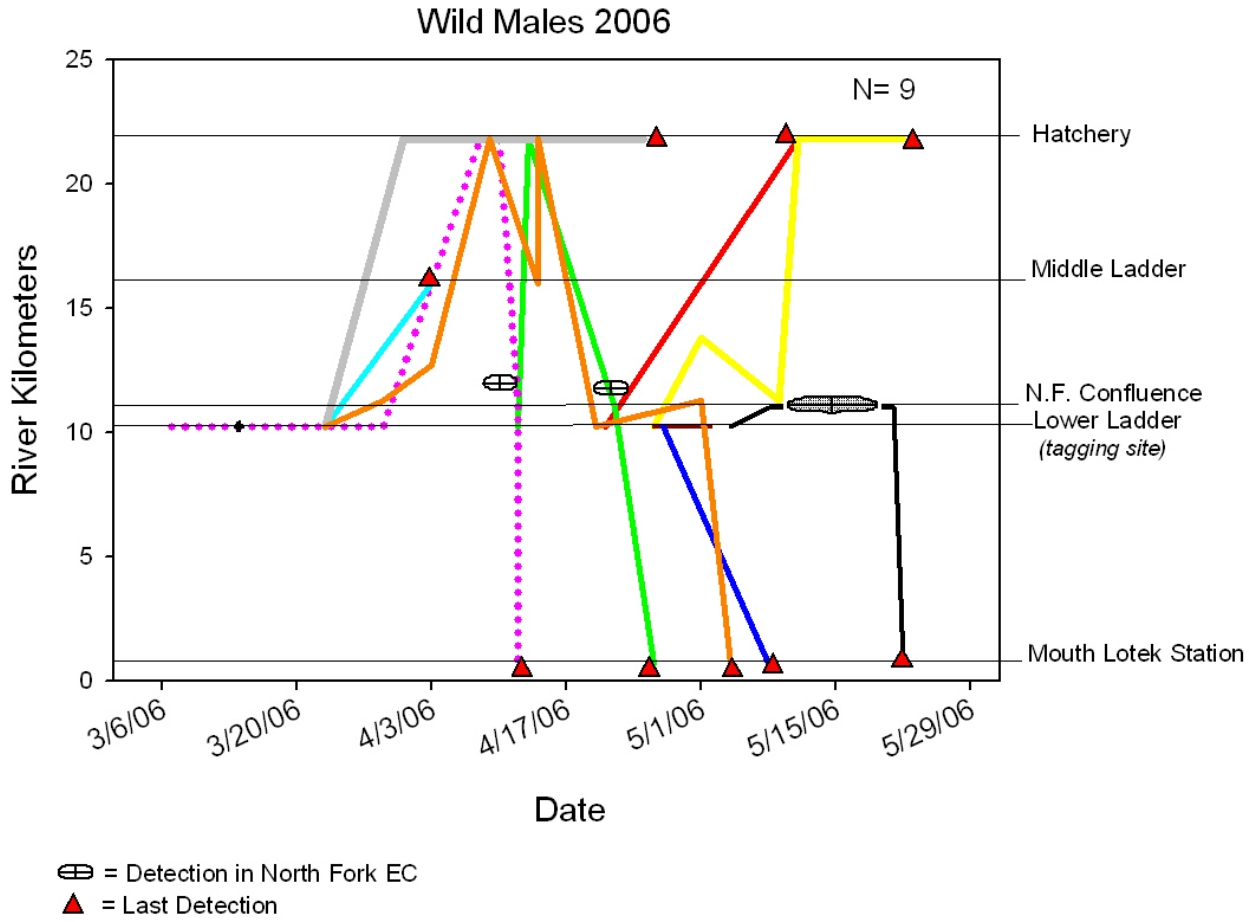


Figure 16: Distribution and movement of radio-tagged wild males in 2006. Steelhead that shed tags at the lower ladder are not included on this graph.

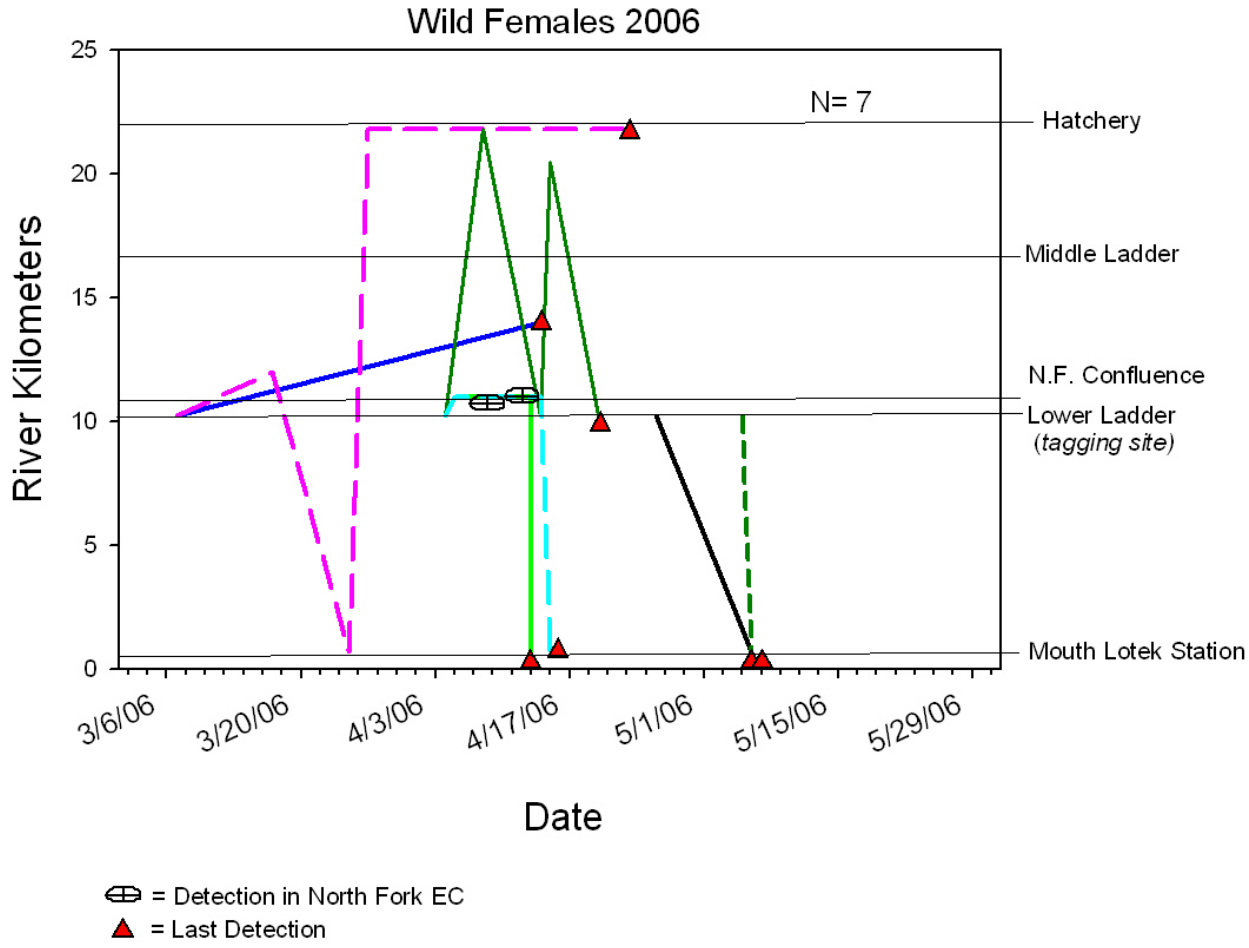


Figure 17: Distribution and movement of radio-tagged wild females in 2006. Steelhead that shed tags at the lower ladder are not included on this graph.

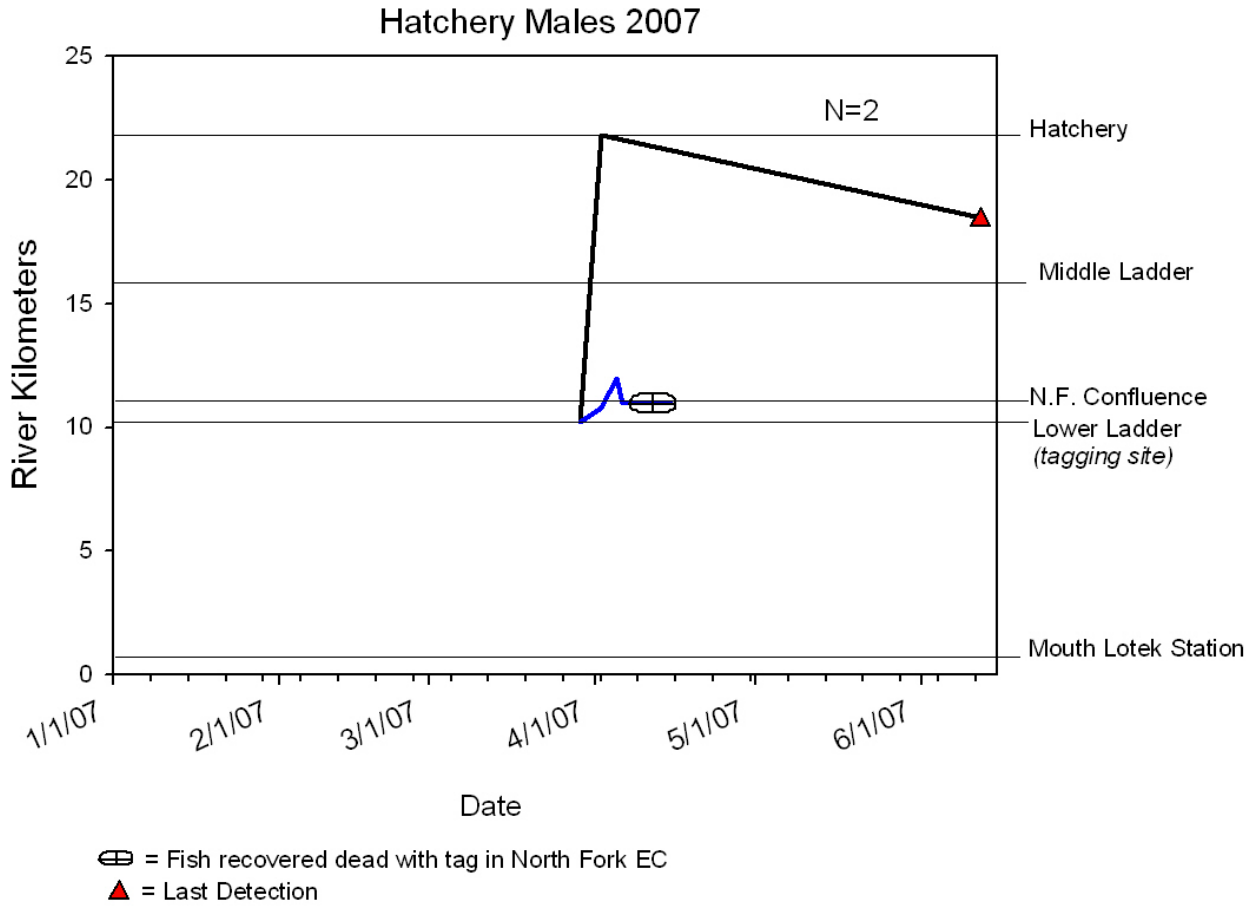


Figure 18: Distribution and movement of radio-tagged hatchery males in 2007. Steelhead that shed tags at the lower ladder are not included on this graph.

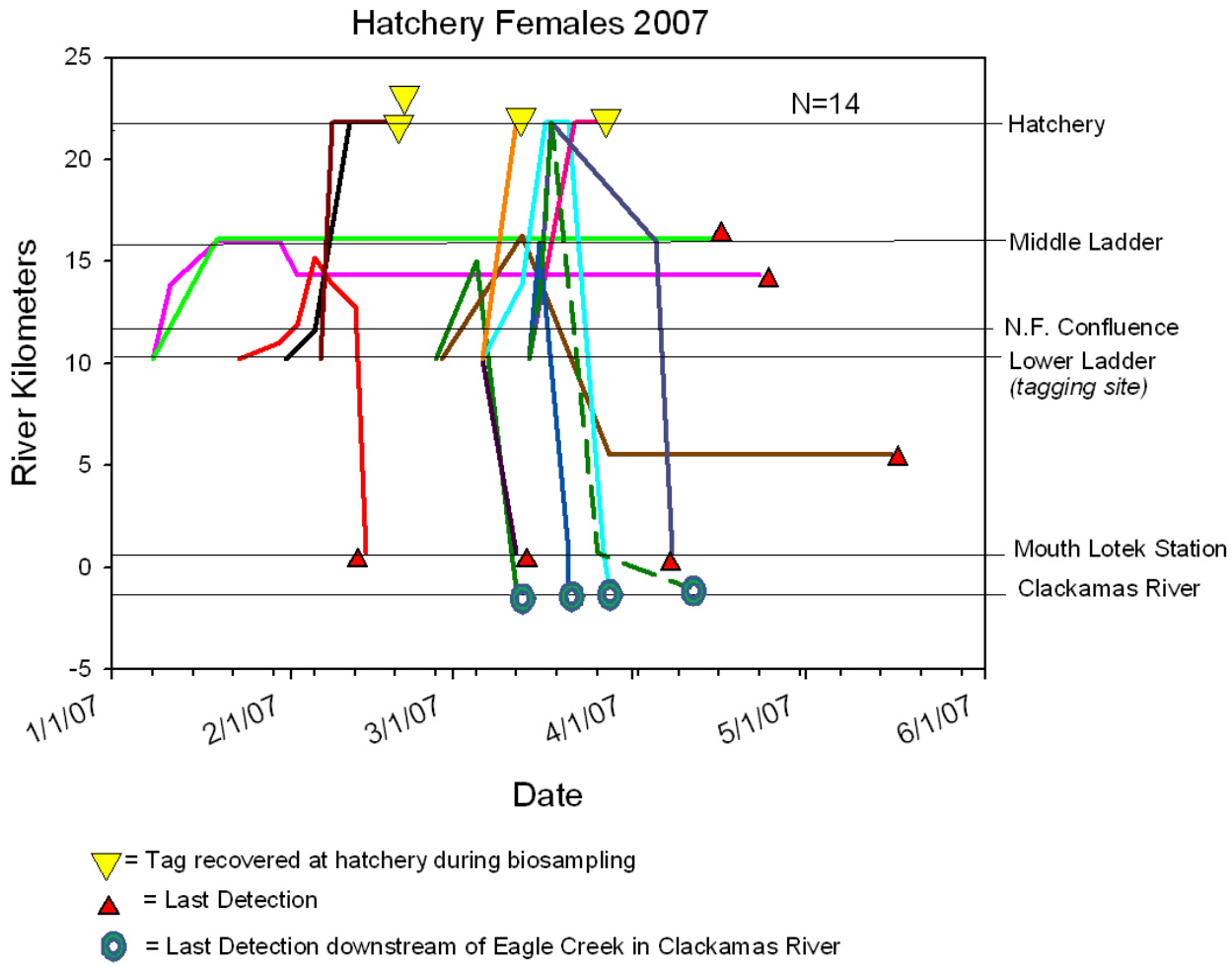


Figure 19: Distribution and movement of radio-tagged hatchery females in 2007. Steelhead that shed tags at the lower ladder are not included on this graph.

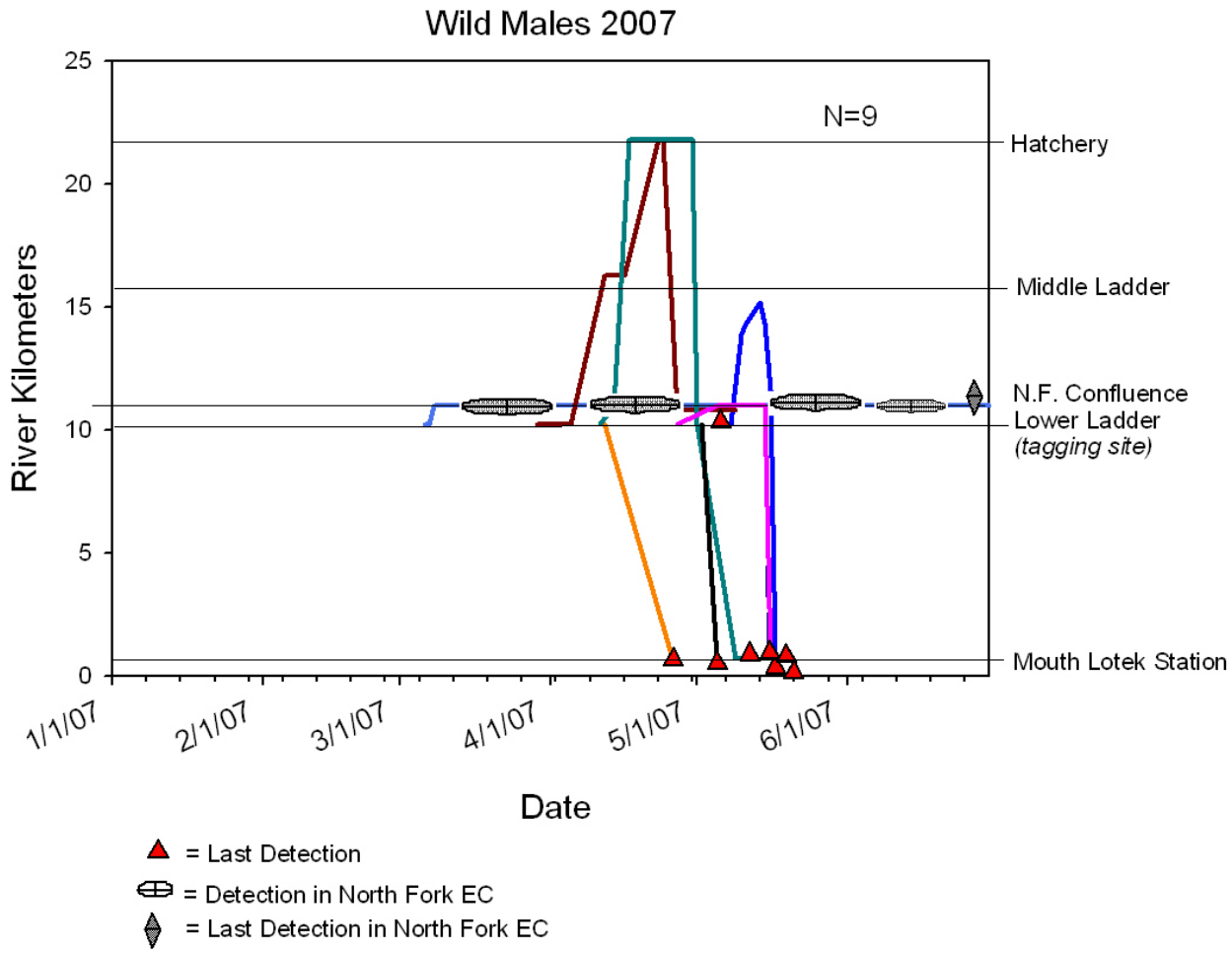


Figure 20: Distribution and movement of radio-tagged wild males in 2007. Steelhead that shed tags at the lower ladder are not included on this graph.

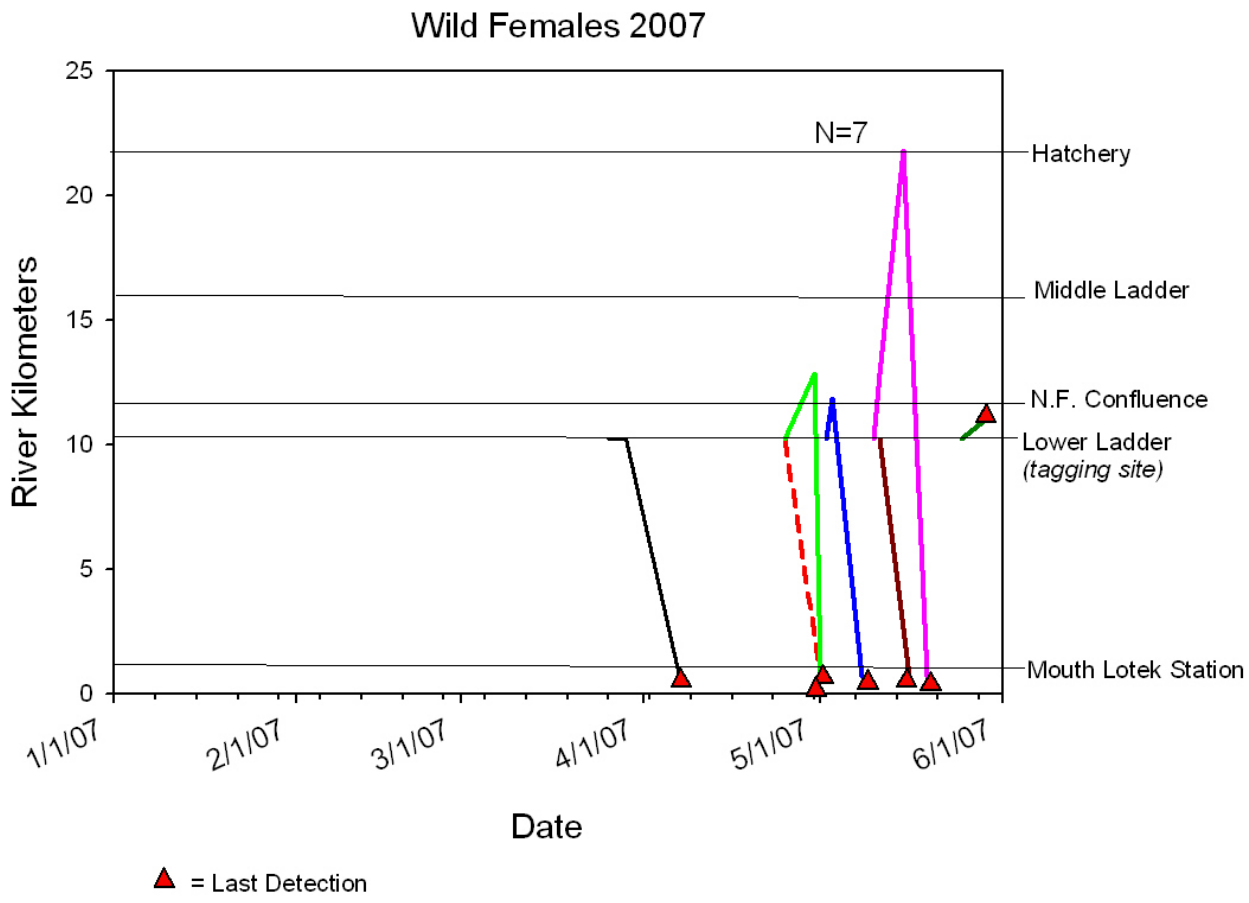


Figure 21: Distribution and movement of radio-tagged wild females in 2007. Steelhead that shed tags at the lower ladder are not included on this graph.

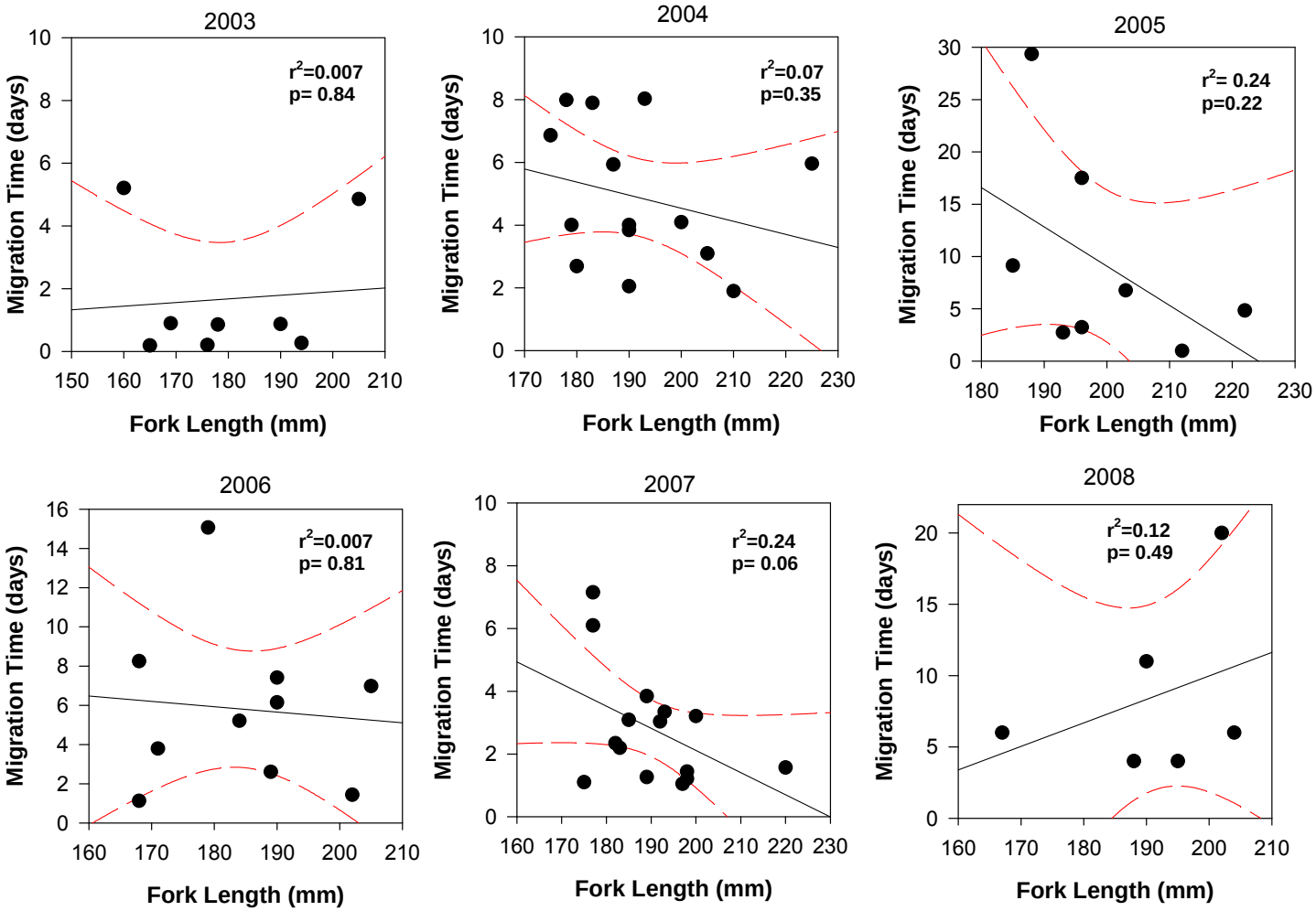


Figure 22: Migration time (in days) from Eagle Creek NFH to the mouth of Eagle Creek for winter steelhead smolts released in 2003-2008. A linear regression showed no relationship between fork length and migration time for any of the years evaluated. The black lines represent the estimated regression of smolt migration for a given fork length, and the red lines represent the 95% confidence limits. The correlation coefficient (r^2) and p-value are given for each year.

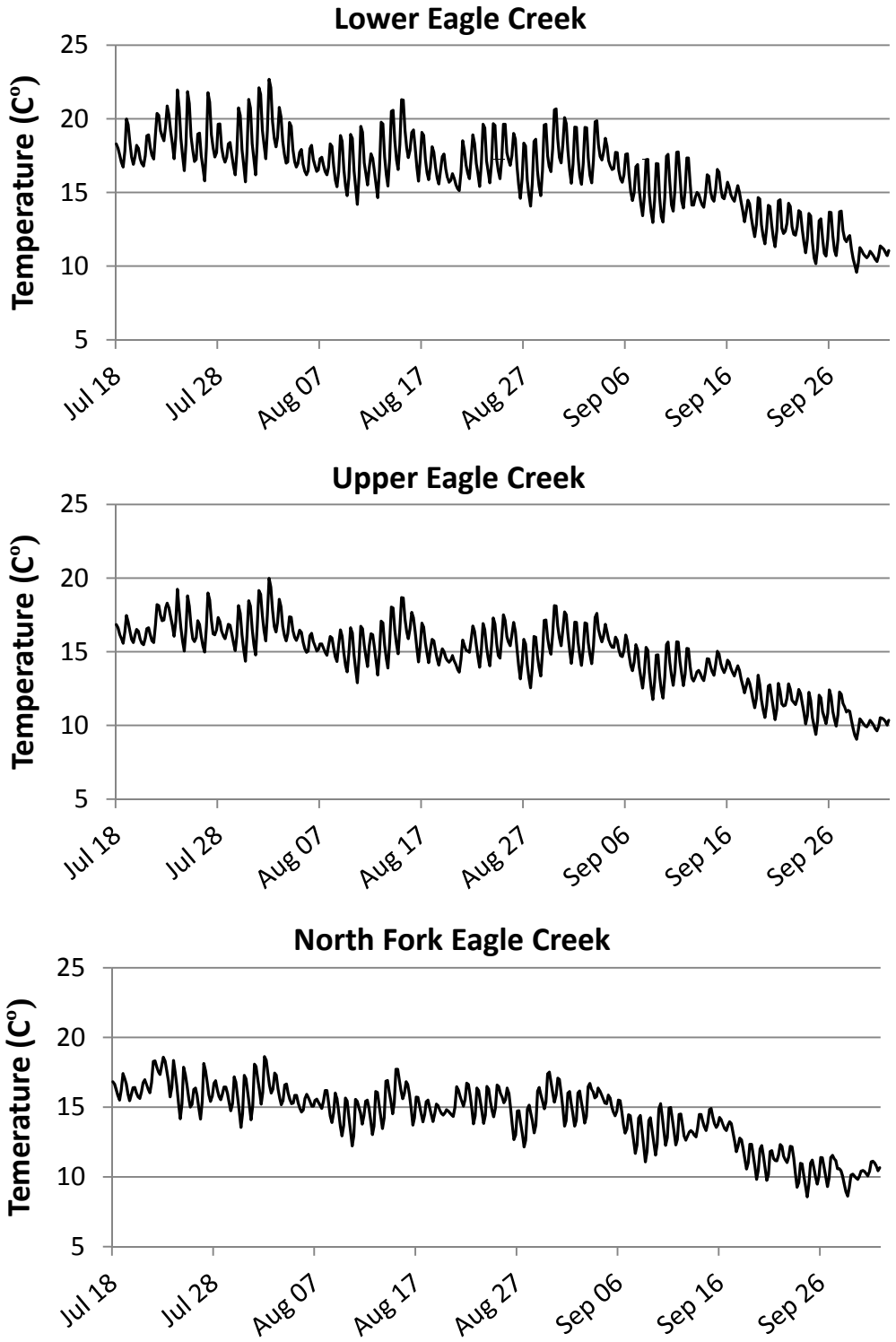


Figure 23: Thermographs of the three study reaches from July 18th to October 2nd 2007. Snorkel surveys were conducted between July 10th and September 14th 2007.

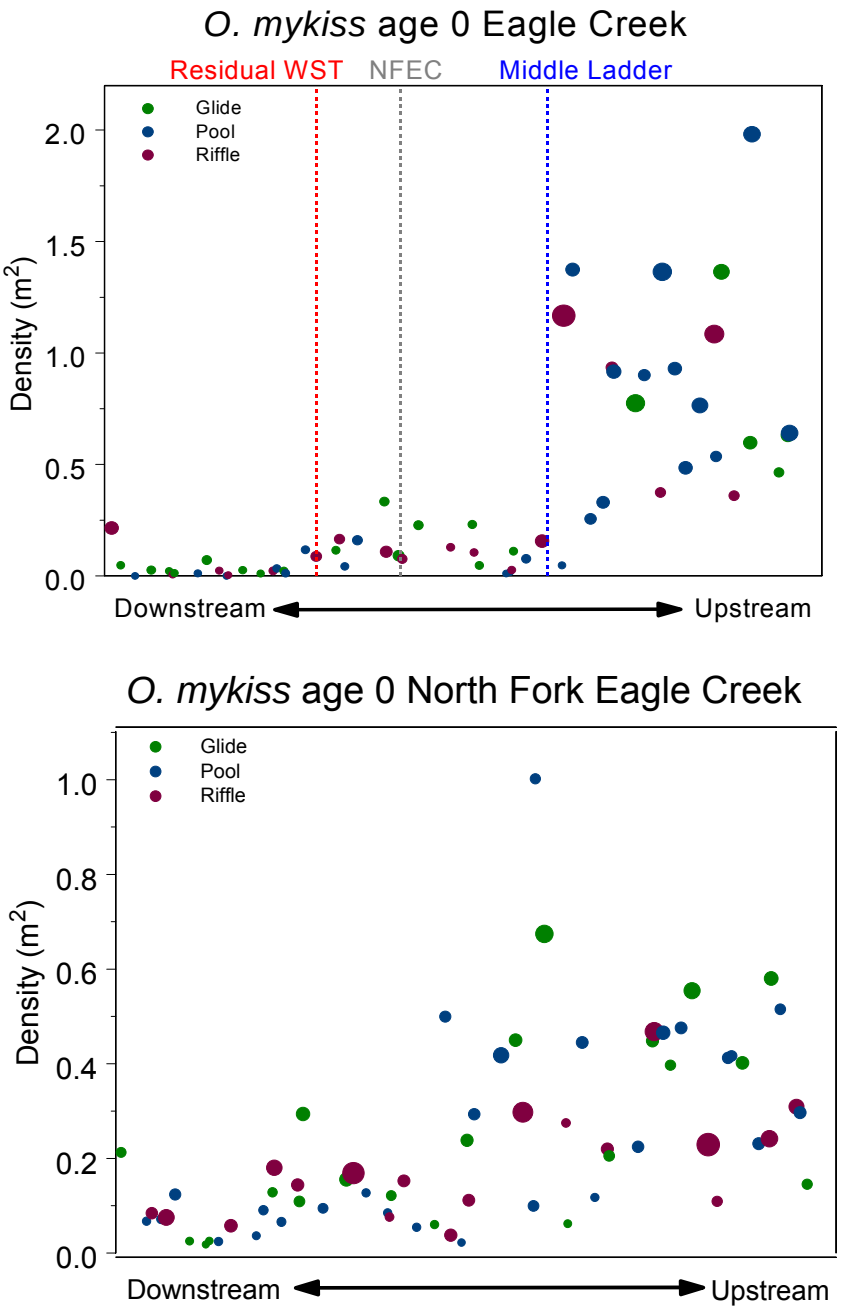


Figure 24: Bubble scatter plots of O. mykiss age 0 densities from sampled habitat units of Eagle Creek and North Fork Eagle Creek, summer 2007. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 1,085 fish in the Eagle Creek plot and 135 fish in the North Fork Eagle Creek plot. Three points of reference are labeled in the Eagle Creek plot: first detection of residual hatchery winter steelhead (red line), the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line).

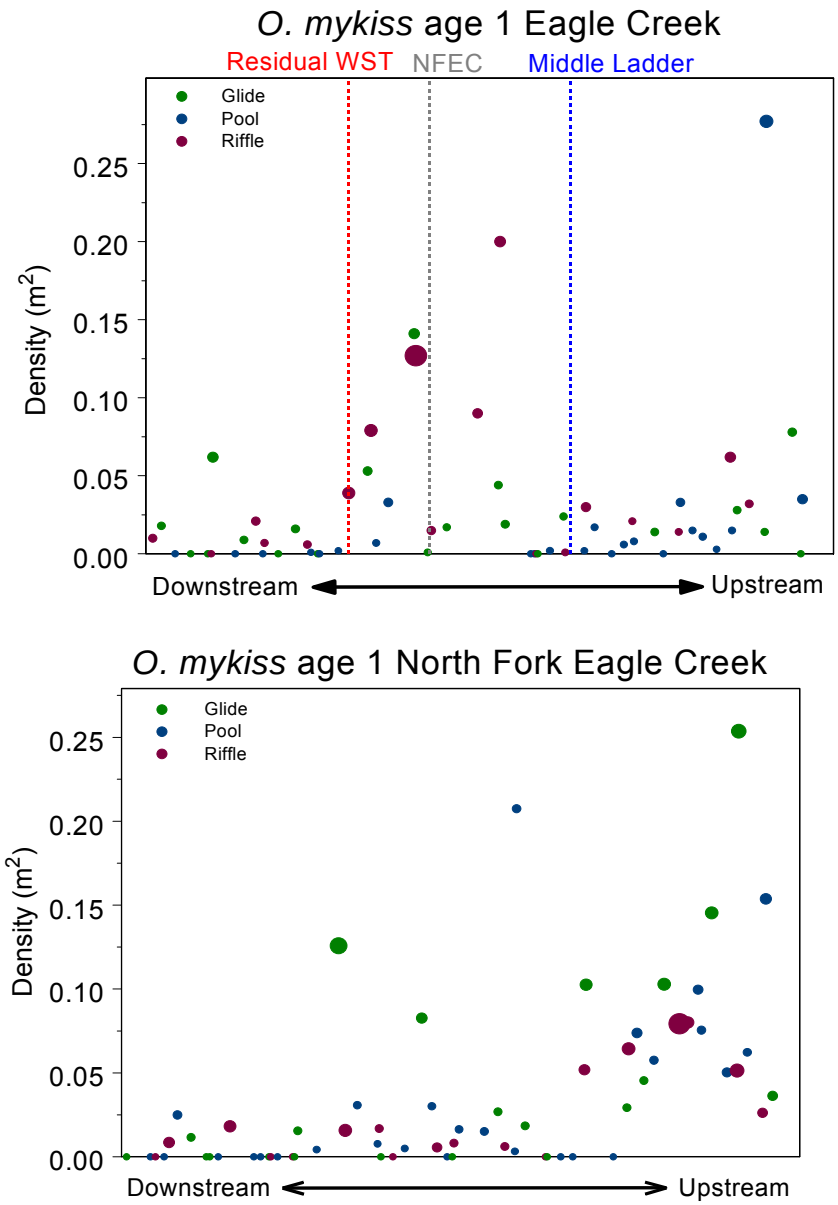
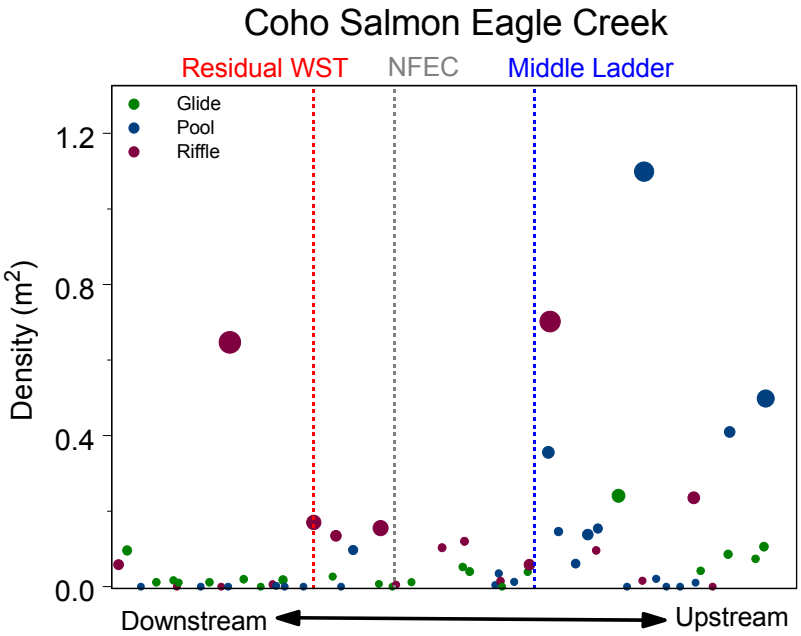


Figure 25: Bubble scatter plots of O. mykiss age 1 densities from sampled habitat units of Eagle Creek and North Fork Eagle Creek, summer 2007. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 260 fish in the Eagle Creek plot and 43 fish in the North Fork Eagle Creek plot. Three points of reference are labeled in the Eagle Creek plot: first detection of residual hatchery winter steelhead (red line), the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line).



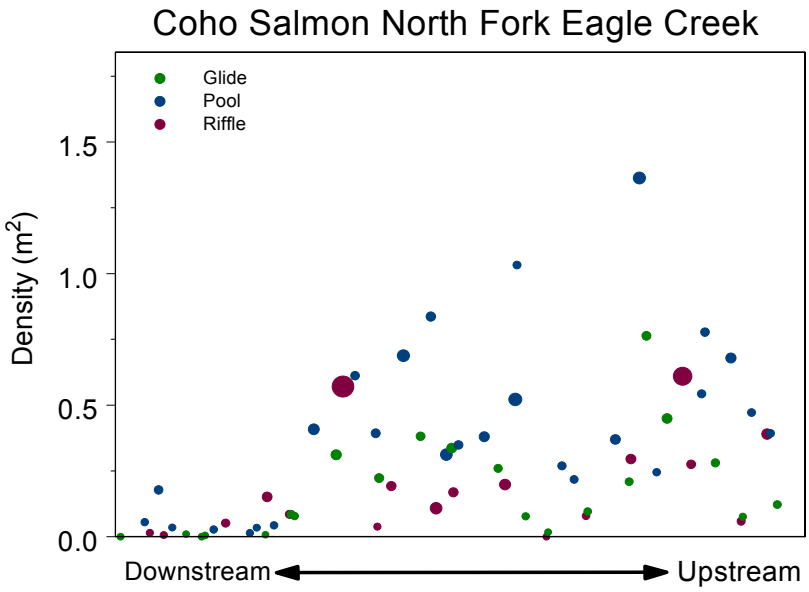


Figure 26: Bubble scatter plots of coho salmon densities from sampled habitat units of Eagle Creek and North Fork Eagle Creek, summer 2007. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 457 fish in the Eagle Creek plot and 722 fish in the North Fork Eagle Creek plot. Three points of reference are labeled in the Eagle Creek plot: first detection of residual hatchery winter steelhead (red line), the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line).

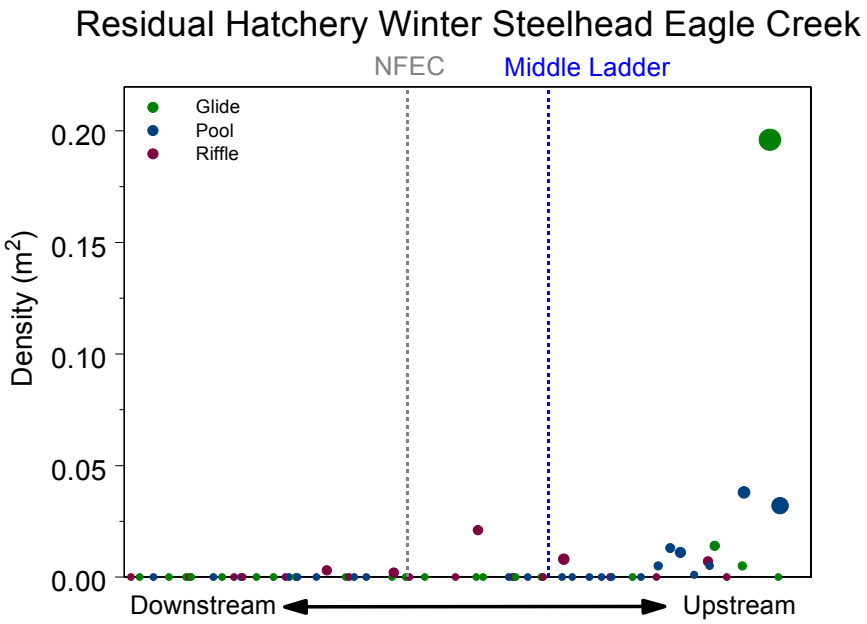


Figure 27: Bubble scatter plots of residual hatchery winter steelhead densities from sampled habitat units of Eagle Creek, summer 2007. Residual hatchery winter steelhead were not observed in North Fork Eagle Creek. The size of the bubble represents the abundance of fish in the habitat unit relative to other points on the plot. The largest circle represents 50 fish. The two points of reference labeled in this plot are the confluence with North Fork Eagle Creek (grey line), and the middle ladder (blue line).

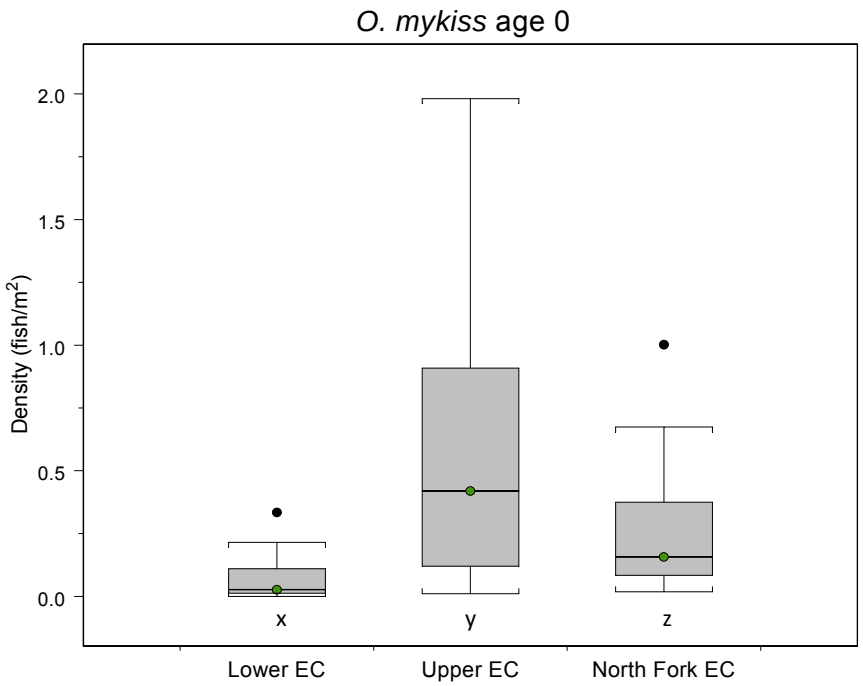


Figure 28: Box-and-whisker plots of O. Mykiss age 0 densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA detected density differences between stream reaches (χ^2 = 37.1, $P < 0.001$) and different letters above the x-axis represent significant differences ($P < 0.05$) in pairwise comparisons.

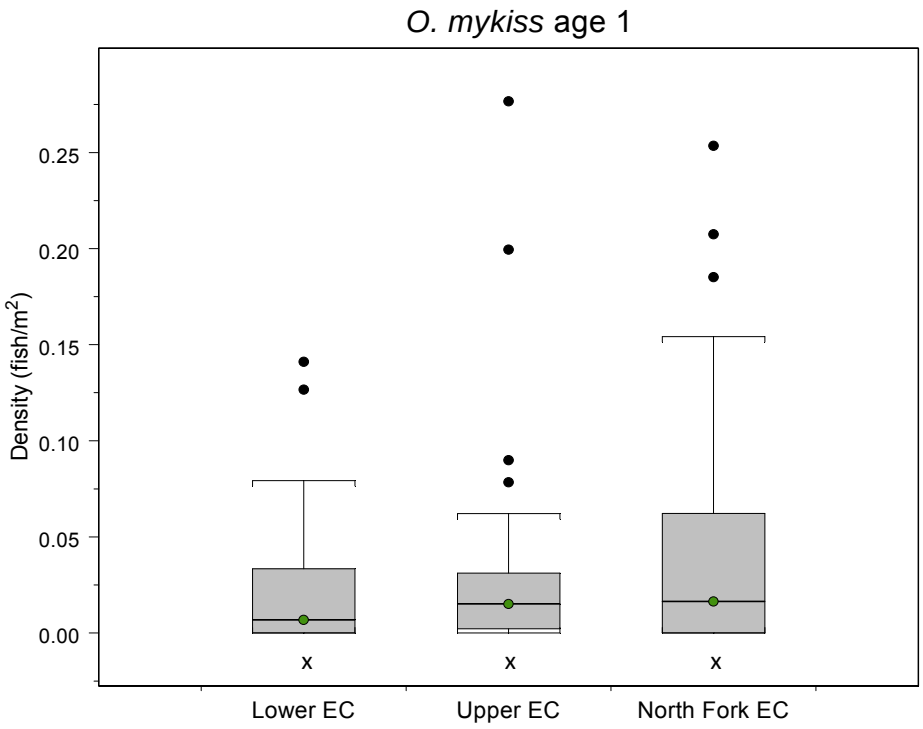


Figure 29: Box-and-whisker plots of O. Mykiss age 1 densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA did not detected density differences between stream reaches (χ^2 =1.82, P = 0.40) therefore the same letter is used above the x-axis to represent no significant differences (P < 0.05) in pairwise comparisons.

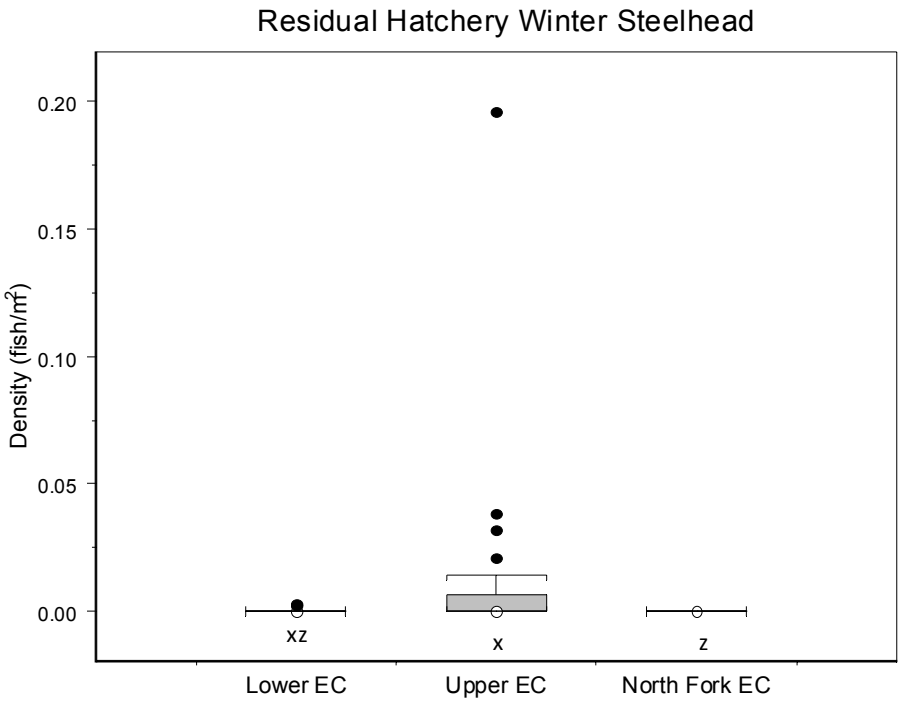


Figure 30: Box-and-whisker plots of residual hatchery winter steelhead densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA detected density differences between stream reaches ($\chi^2 = 33.7$, $P < 0.001$) and different letters above the x-axis represent significant differences ($P < 0.05$) in pairwise comparisons.

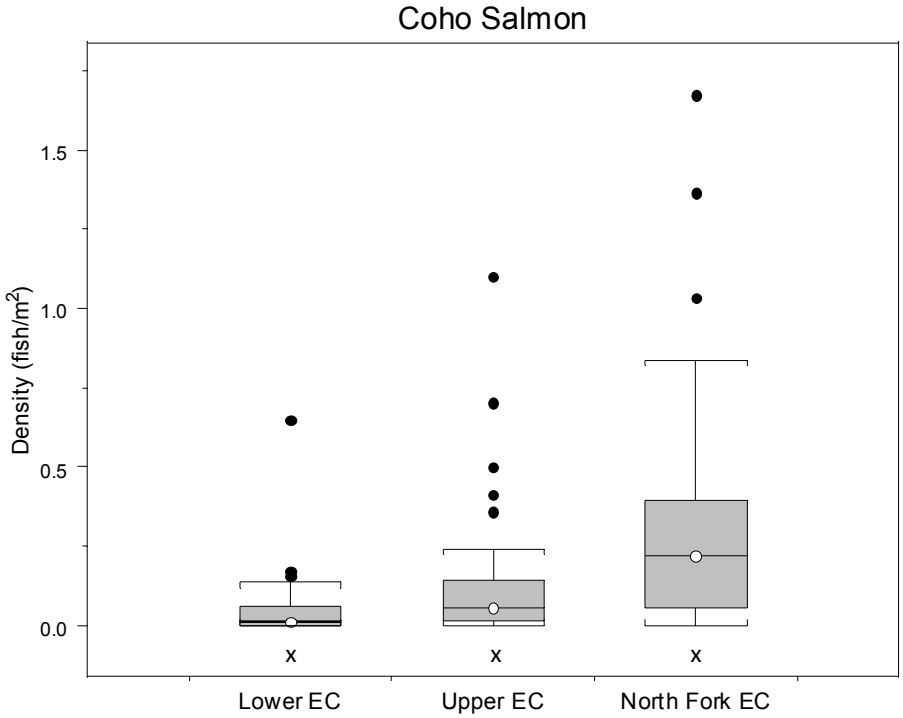


Figure 31: Box-and-whisker plots of coho salmon densities in Lower Eagle Creek (EC), Upper EC, and North Fork EC, summer 2007. The ends of each box are the 25th and 75th quartile range and the horizontal line with the open circle is the median. The whisker ends are all data points that fall within the distance calculated as 1.5 times the interquartile range. A closed circle that lies beyond the whiskers represents an outlier. Non-parametric ANOVA detected density differences between stream reaches ($\chi^2 = 30.8$, $P < 0.001$) and different letters above the x-axis represent significant differences ($P < 0.05$) in pairwise comparisons

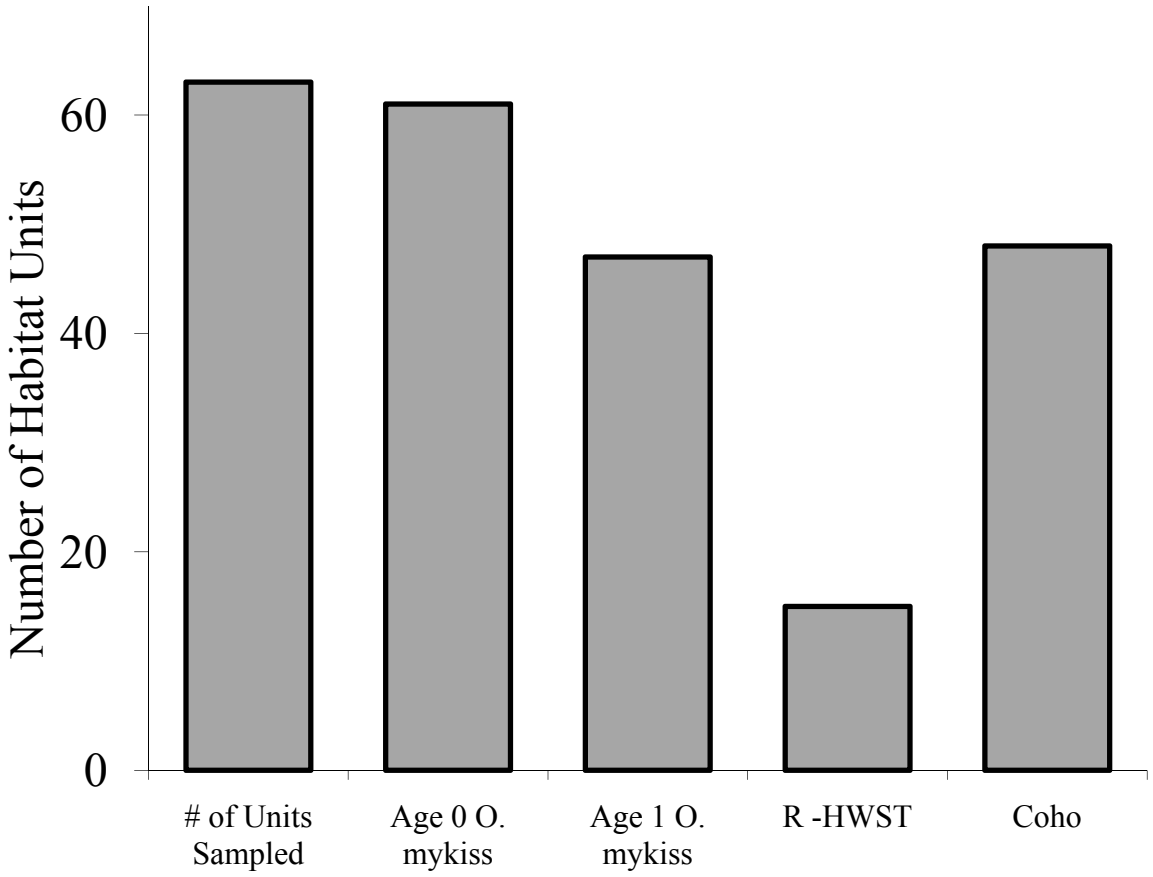


Figure 32: Number of sampled habitat units in Eagle Creek where fish species were observed, summer 2007. Residual hatchery winter steelhead is abbreviated R-HWST.

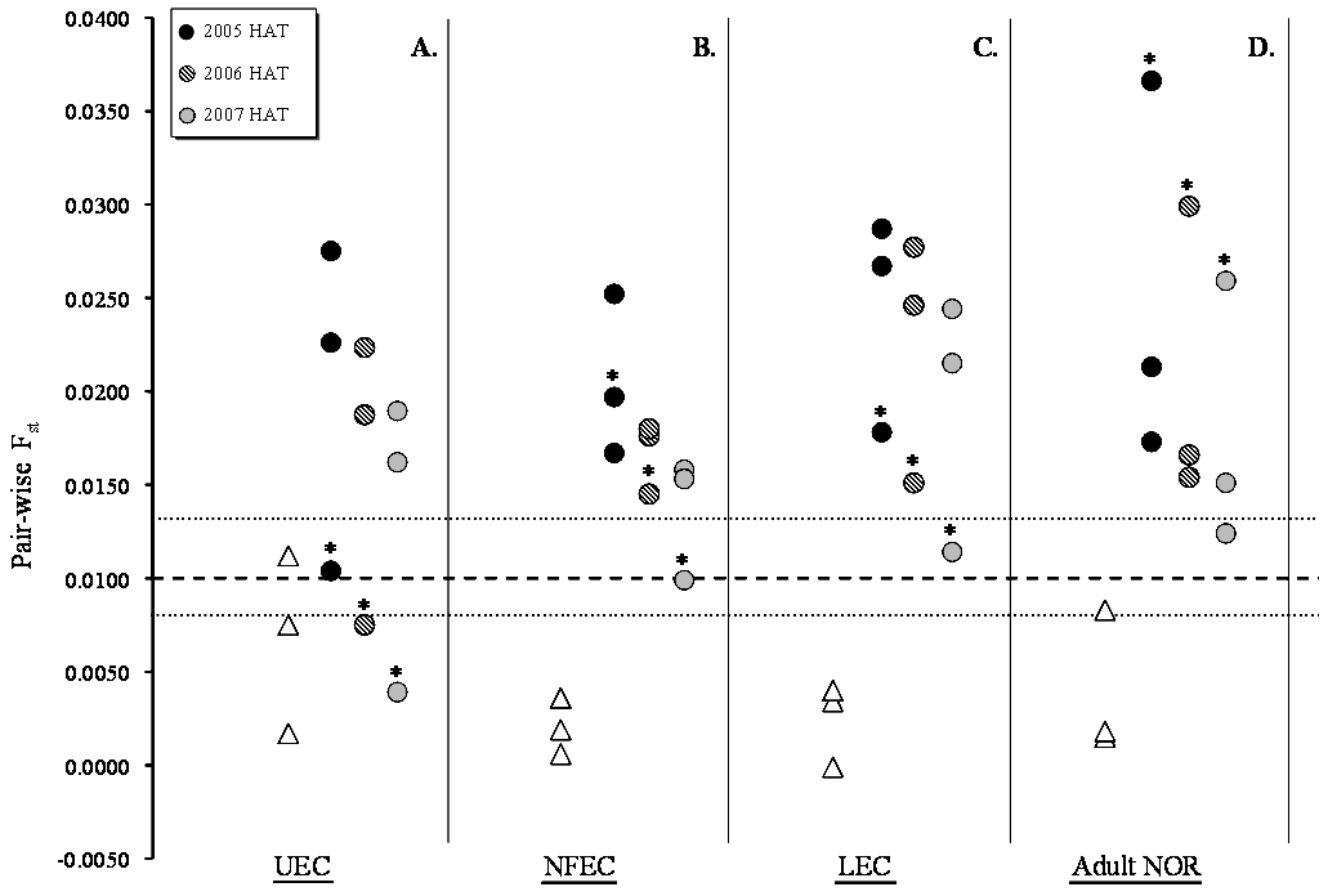


Figure 33: Distribution of pairwise among-group variation (Fst) displayed graphically for each pair of collections across years (2005-2007). Comparisons are arranged by NOR group. The symbol Δ indicates pairwise Fst comparisons between temporal replicate collections in four NOR groups (A-D), and (*) indicates all HAT pairwise comparisons with NOR-2007 collections. The dashed line marks the global F_{ST} across all collections, and the dotted lines indicate the 95% confidence interval for significance.

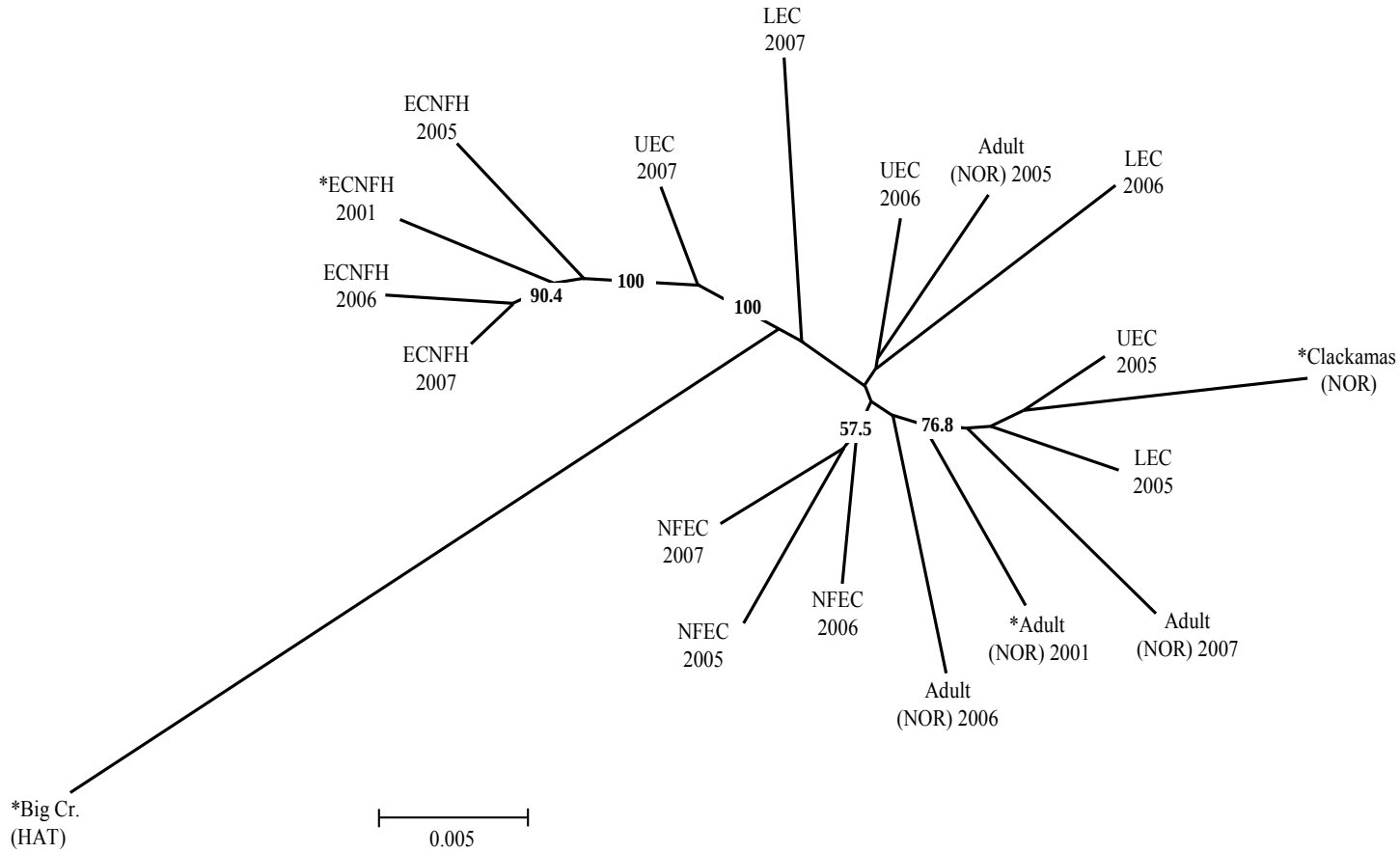


Figure 34: Un-rooted neighbor-joining tree: The phylogram topology was constructed from pairwise genetic (chord) distances (Cavalli-Sforza & Edwards 1967), and includes the 2005-2007 Eagle Creek steelhead collections evaluated in this study, and 2001 SPAN standardized data (*) contributed by Maureen Waite and Paul Moran from NOAA fisheries. Bootstrap support among 1000 replicate data sets is shown between branch nodes, indicating concordance level among loci for each branch in the topology.

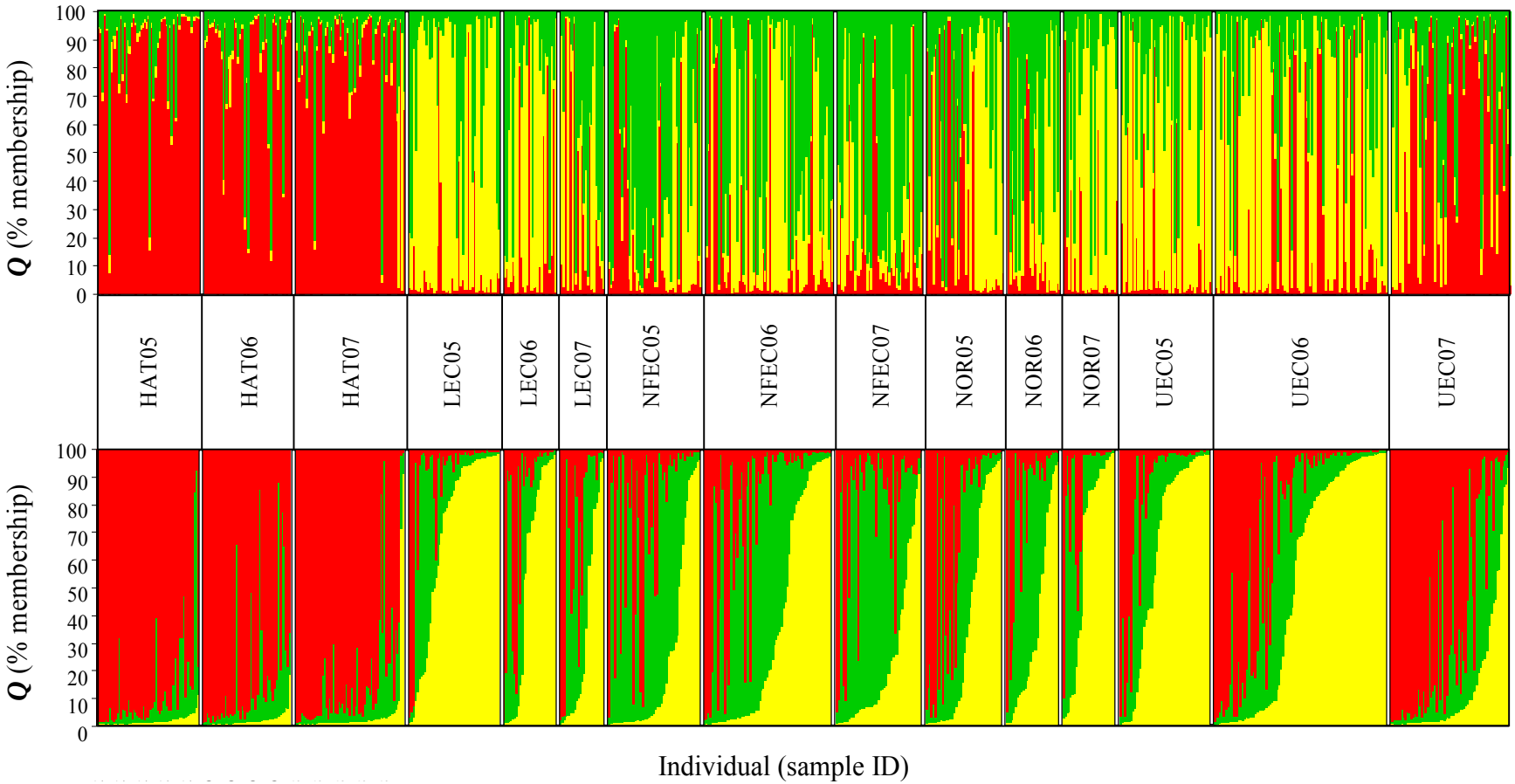
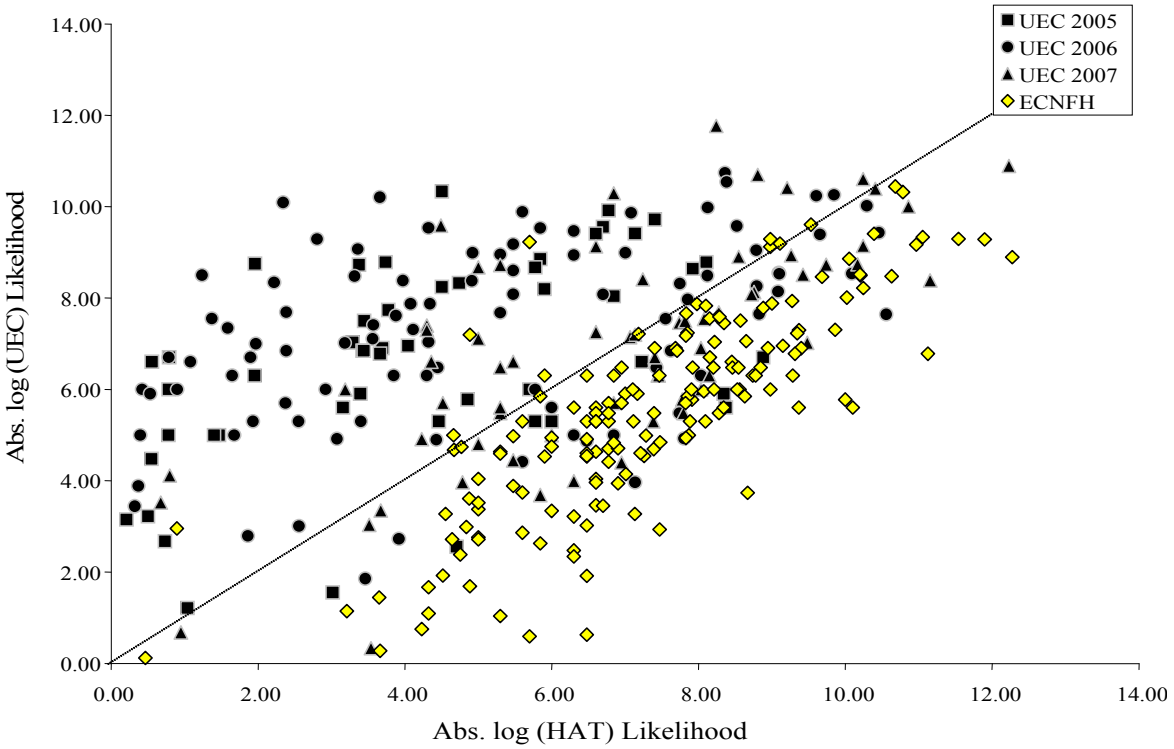


Figure 35: Bar plot of proportional population membership from the program STRUCTURE version 2.2. The K=3 inferred population clusters are each represented by a different color (e.g. the red cluster found largely among HAT samples). Proportional membership is displayed within each of 15 collections evaluated, where the x-axis is individual fish. The upper plot displays samples (individual fish) within collections arranged by time of return/capture. The bottom plot is arranged by collection and *Q* membership among clusters.



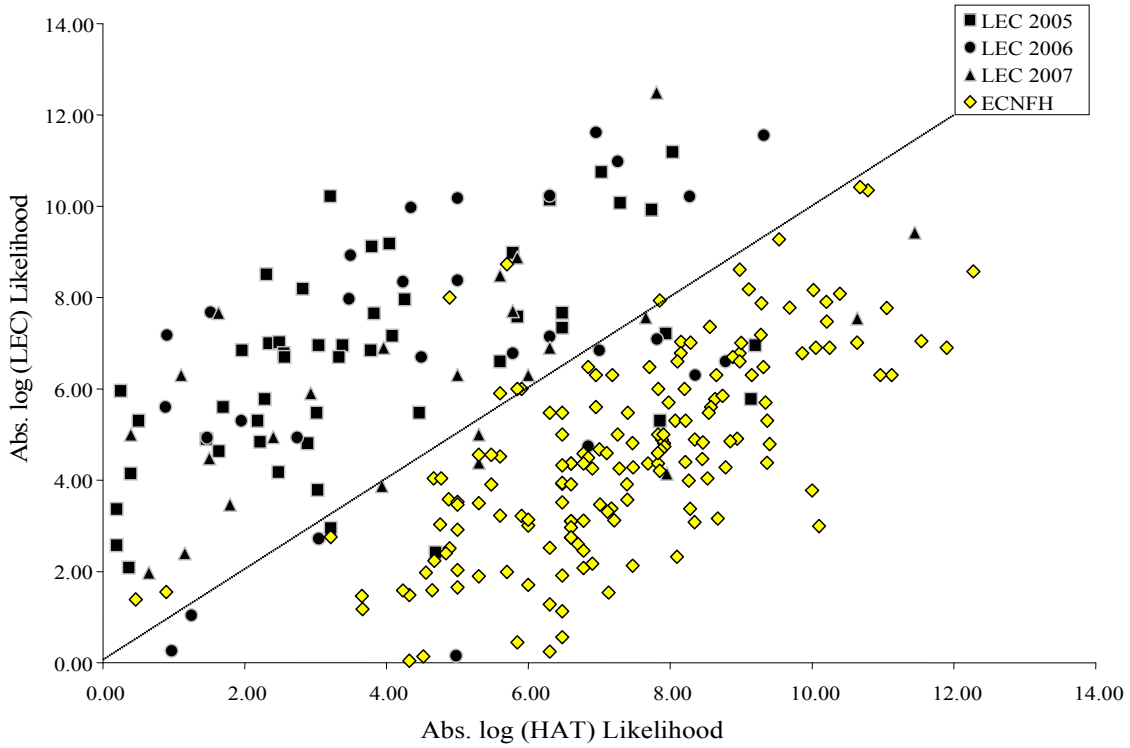
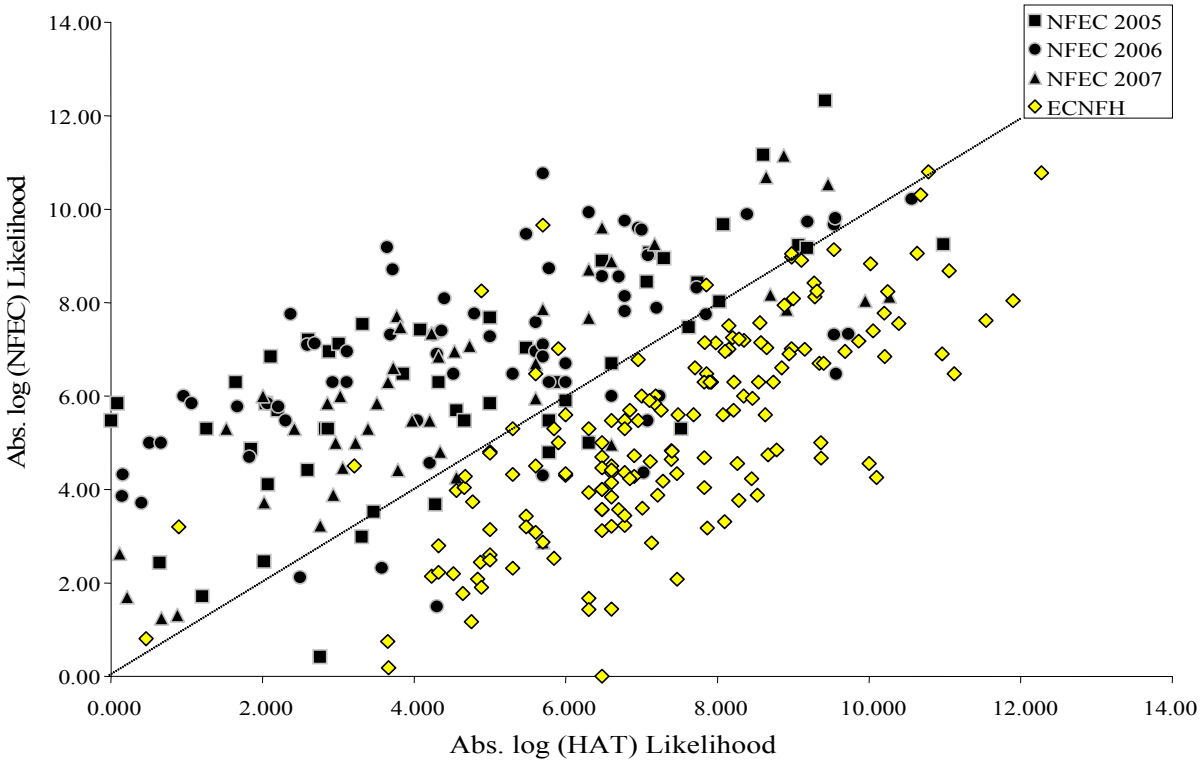
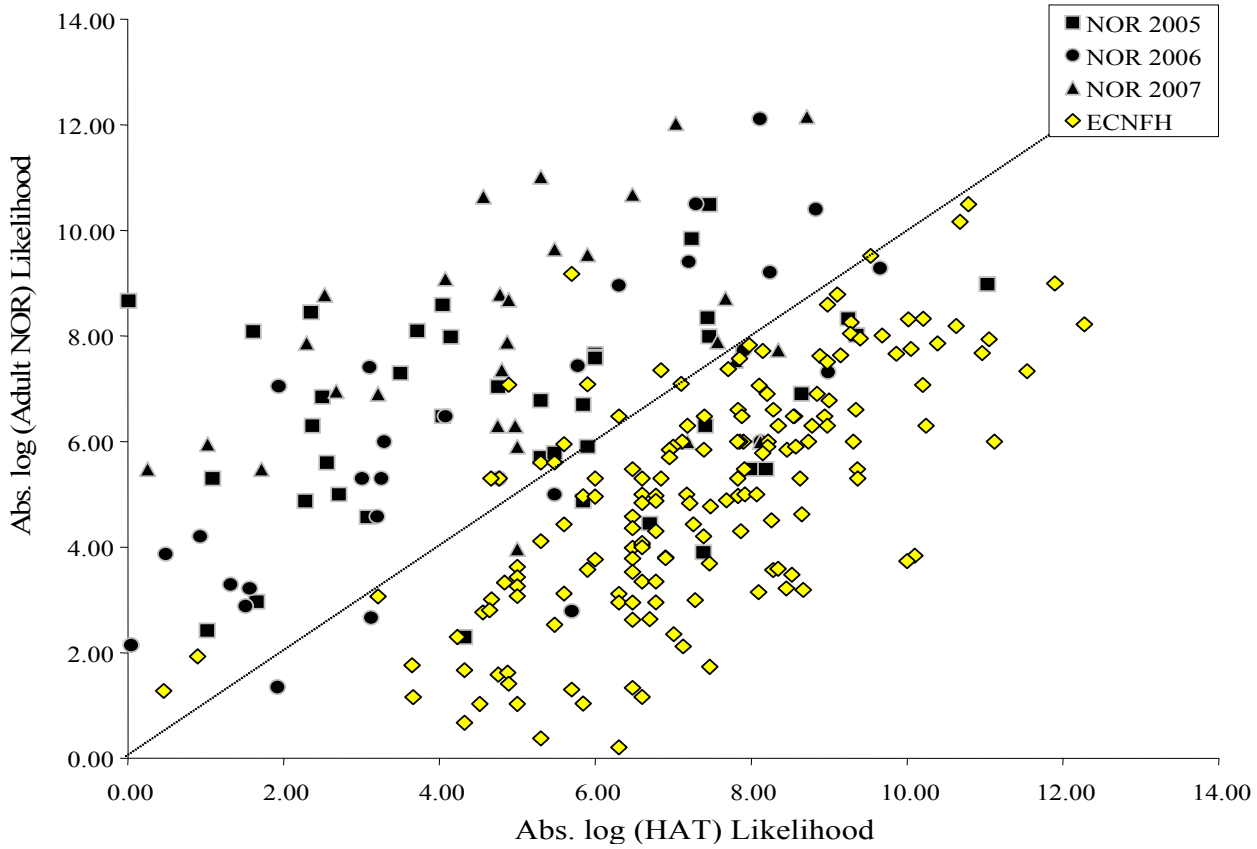


Figure 36-39: Population assignment likelihood plots for all groups and replicate collections. Likelihood values are derived from the jackknife re-sampling method. Data is presented as the absolute value of the log likelihood for assignment to HAT and a given NOR group (XY coordinates); the log likelihood ratio is the LOD score (Table 18). The diagonal line delineates where an individual is equally likely to be of NOR origin as HAT origin. Comparisons are: a) UEC & HAT, b) LEC & HAT, c) NFEC & HAT, and d) adult NOR & HAT.


NFEC 2005
NFEC 2006
NFEC 2007
ECNFH
Abs. log (NFEC) Likelihood
Abs. log (HAT) Likelihood
0.000
2.000
4.000
6.000
8.000
10.000
12.000
14.00
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00


NOR 2005
NOR 2006
NOR 2007
ECNFH
Abs. log (Adult NOR) Likelihood
Abs. log (HAT) Likelihood
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00

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Appendix 1: List of loci amplified for the Eagle Creek analysis (2005-2007). The symbol (*) designates a locus that is standardized in the Stephen Phelps Allele Nomenclature (SPAN) standardization effort. The annealing temperatures and MgCl2 concentrations are optimized PCR conditions for the conservation genetics program at Abernathy Fish Technology Center.

Locus	Cite/Source	Annealing ^{o}C	$[MgCl_2]$ mM
Ocl1	Condrey & Bentzen 1998	59	1.5
Ots1	Banks *et al.* 1999	59	2
Ots3	Banks *et al.* 1999	48	2
*Ots4**	Banks *et al.* 1999	53	2
*Ots100**	Banks *et al.* 1999	57	2
*Omy7iNRA**	Nichols *et al.* 2003	59	2
Ogo3	Olsen *et al.* 1998	59	2
*Ogo4**	Olsen *et al.* 1998	59	1.5
*Oki23**	Smith *et al.* 1998	59	2
*Ssa289**	McConnell *et al.*1995	50	2
*Ssa407**	Cairney *et al.* 2000	60	2
*Ssa408**	Cairney *et al.* 2000	59	2
*Omy1011UW**	Spies *et al.* 2005	59	2
Omy77	Morris *et al.* 1996	59	2
One13	Scribner *et al.* 1996	58	2
*One14**	Scribner *et al.* 1996	60	2

Appendix 2: Results from wild fish health survey conducted on juvenile salmon and steelhead in Eagle Creek from 2004-2007. Unless otherwise noted, all fish were tested for the following fish pathogens: infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), infectious salmon anemia virus (ISAV), *Aeromonas salmonicida* (AS, furunculosis), *Yersinia ruckeri* (YR, enteric redmouth disease), *Flavobacterium columnare* (CD, columnaris), *Flavobacterium psychrophilum* (CWD, coldwater disease), *Renibacterium salmoninarum*, (RS, bacterial kidney disease), and *Myxobolus cerebralis* (MC. whirling disease). Larger fish were screened for RS using the enzyme-linked immunosorbent assay (ELISA); a positive finding of RS denotes that ELISA results were confirmed by a second test, the polymerase chain reaction assay (PCR). All methods for detecting and identifying fish pathogens were based on standardized procedures from the AFS Fish Health Blue Book (USFWS and AFS-FHS 2007) and the National Wild Fish Health Survey – Laboratory Procedures Manual (Puzach 2006).

2004-2007 National Wild Fish Health Survey Summary (Eagle Creek)

Case Number	Date	Species	Location	# of fish sampled	Length (mm)	Weight (g)	Findings
2004 W04-022	11/13/03	Cutthroat trout	Salmon-Huckleberry Wilderness, Eagle Creek, 3-4 miles above NFH	32	NA	NA	The fish had *Epistylis* on the skin (low). Seven out of 13 samples (individual and pooled samples) were positive for RS by ELISA and confirmed by PCR. Five fish tested, negative for *Ceratomyxa shasta*
2005 W05-017	11/10/04	Steelhead	Eagle Fern Park	5	NA	NA	The fish had *Epistylis* and *Gyrodactylus* on the skin (high). *Nanophyetus* and *Loma* in the gills (low). Not tested: *RS*.
W05-087	6/07/05	Steelhead	NF Eagle Creek, Forest Service Trap	9			*Gyrodactylus* on skin (low). Suspect positive for RS (3+/3 pooled samples, not confirmed by PCR).
W05-089	6/07/05	Steelhead	NF Eagle Creek, Forest Service Trap	3			The fish appeared to be in good health, and no pathogens were detected. One fish tested, negative for RS.
W05-090	6/07/05	Coho salmon	NF Eagle Creek, Forest Service Trap	1			The fish appeared to be in good health, and no pathogens were detected.
W05-125	8/02/05	Steelhead	NF Eagle Creek	8			The fish appeared to be in good health and no pathogens were detected (3 fish tested, negative for RS).

W05-126	8/02/05	Coho salmon	NF Eagle Creek	5			Heavy loads of *Nanophyetus* (gills and skin) and *Epistylis* (skin). Two of five fish suspect for RS by ELISA.
W05-129	8/02/05	Steelhead	Eagle Fern Park	2	41 36	0.91 0.54	The fish appeared to be in good health, and no pathogens were detected. Not tested: RS, WD

2006 W06-134	6/27/06	Coho salmon	Mouth of Eagle Creek	24	NA	NA	Fish appeared to be in good health, and no pathogens were detected. Three fish tested, negative for RS.
W06-135	6/27/06	Steelhead	Eagle Fern Park	3	NA	NA	Fish appeared to be in good health, and no pathogens were detected. One fish tested, negative for RS.
W06-143	7/25/06	Steelhead	Lower Ladder	10	NA	NA	Fish appeared to be in good health, and no pathogens were detected. *Pseudomonas maltophilia* detected in one fish. Not tested: RS, CS
W06-148	8/03/06	Coho salmon	Mouth of Eagle Creek	6	69 70 85 87 80 79	5.35 5.41 8.61 9.64 6.30 6.83	Fish appeared to be in good health, and no pathogens were detected. Not tested: RS
W06-152	8/03/06	Steelhead	Lower Ladder	2	99 44	12.21 1.14	Fish appeared to be in good health, and no pathogens were detected. Heavy loads of *Loma* (gill) and *Nanophyetus* (kidney) in one fish. One fish tested, negative for RS.
W06-153	8/03/06	Fall Chinook salmon	Mouth of Eagle Creek	1	68	3.68	Fish appeared to be in good health, and no pathogens were detected. *Cystidicola* (low) found in swim bladder. Not tested: RS

2007 W07-043	5/02/07	Steelhead	Middle Ladder	3	73 82 92	5.12 6.72 9.27	Fish appeared to be in good health, and no pathogens were detected.
W07-044	5/02/07	Coho salmon	Middle Ladder	10	48 46 46 44 40 39 35 47 40 42	1.75 1.31 1.21 1.02 0.94 0.69 0.47 1.24 0.76 1.01	Fish appeared to be in good health, and no pathogens were detected. Low loads of *Loma* and *Nanophyetus* (gills); I and unknown myxobolid spore (head) found in fish. Not tested: RS.
W07-048	5/10/07	Coho salmon	Eagle Fern Park	10	58 53 50 58 51 51 56 52 58 42	3.07 1.94 2.00 3.01 2.06 3.01 2.94 2.10 2.78 1.03	Fish appeared to be in good health, and no pathogens were detected. Caudal fin eroded in one fish. Not tested: RS
W07-049	5/10/07	Steelhead	Eagle Camp	2	116 92	20.67 10.52	One fish tested, positive for RS by ELISA and PCR.
W07-050	5/17/07	Steelhead	Above Middle ladder	11	101 86 87 81 84 90 94 98	11.21 8.88 8.42 7.02 7.7 9.7 10.9 11.7	Fish appeared to be in good health, and no pathogens were detected. Nine fish tested, negative for RS.

					93 77 94	11.1 6.5 11.2	
W07-053	5/31/07	Steelhead	Below hatchery	4	126 105 135 101	20.2 13.8 39.9 15.0	Fish appeared to be in good health, and no pathogens were detected. One fish had hemorrhaged caudal peduncle, swollen kidney, enlarged spleen and *Nanophyetus* in kidney Low levels of *Nanophyetus* and *Loma* in gills of three fish. Three fish tested, negative for RS, CS. Four fish tested, negative for viruses and MC;
W07-054	5/31/07	Coho salmon	Below hatchery	1	75	5.7	Fish appeared to be in good health, and no pathogens were detected. Not tested: RS.

Data collected and analyzed by USFWS Lower Columbia River Fish Health Center, 201 Oklahoma Rd. Willard, WA 98605.

Appendix 3: Results from fish health survey conducted on hatchery adults returning to Eagle Creek NFH and hatchery smolts prior to release from the hatchery in 2005-2007. Fish were tested for the following fish pathogens: infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV), *Oncorhynchus masou* virus (OMV), infectious salmon anemia virus (ISAV), *Aeromonas salmonicida* (AS, furunculosis), *Yersinia ruckeri* (YR, enteric redmouth disease), *Flavobacterium columnare* (CD, columnaris), *Flavobacterium psychrophilum* (CWD, coldwater disease), *Renibacterium salmoninarum*, (RS, bacterial kidney disease), and *Myxobolus cerebralis* (MC. whirling disease).All methods for detecting and identifying fish pathogens were based on standardized procedures from the AFS Fish Health Blue Book (USFWS and AFS-FHS 2007). For detection of RS in the hatchery fish, both the enzyme-linked immunosorbent assay (ELISA) and direct fluorescent antibody test (DFAT) is used for the adults and the DFAT alone is used for the juvenile fish.

2005-2007 Report of Hatchery Fish Health (Eagle Creek)

Brood-Year	Date	Species	Location	Findings
2005	1/26/05	Steelhead (adults)	Eagle Creek NFH	Adult winter steelhead spawned and sampled from 1/26/05 to 3/16/05. 149 females and 60 males were examined. ELISA detected very low levels of *Renibacterium salmoninarum* antigen in both males and females. DFAT did not detect *Renibacterium salmoniarum* in the ovarian fluid. Virus and other bacterial pathogens not detected.
	2/8/06	Steelhead (smolts)	Eagle Creek NFH	Pre- release examination of 60 winter steelhead smolts. The fish appeared to be in good health, and no pathogens were detected.
2006	1/18/06	Steelhead (adults)	Eagle Creek NFH	Adult winter steelhead spawned and sampled from 1/18/06 to 3/8/06. 131 females and 60 males were examined. ELISA detected very low levels of *Renibacterium salmoninarum* antigen in both males and females. DFAT did not detect *Renibacterium salmoniarum* in the ovarian fluid. Virus and other bacterial pathogens not detected.
	2/21/07	Steelhead (smolts)	Eagle Creek NFH	Pre- release examination of 60 winter steelhead smolts. The fish appeared to be in good health, and no pathogens were detected.
2007	1/10/07	Steelhead (adults)	Eagle Creek NFH	Adult winter steelhead spawned and sampled from 1/10/07 to 2/21/07. 150 females and 60 males were examined. ELISA detected low levels of *Renibacterium salmoninarum* antigen in both males and females. DFAT did not detect *Renibacterium salmoniarum* in the ovarian fluid. *Ceratomyxa shasta* detected in 6/20 hindguts (low levels). Virus and other bacterial pathogens not detected
	3/24/08	Steelhead (smolts)	Eagle Creek NFH	Pre- release examination of 60 winter steelhead smolts. The fish appeared to be in good health, and no pathogens were detected.

Data collected and analyzed by USFWS Lower Columbia River Fish Health Center, 201 Oklahoma Rd. Willard, WA 98605.