

**Interactions between wild and hatchery steelhead: Evaluating Key Assumptions**

**Final Report for Hatchery Scientific Review Group  
2000-2001 Research**

**Thomas Quinn, Jennifer McLean and Paul Bentzen**

**School of Aquatic and Fishery Sciences  
Box 355020, University of Washington  
Seattle, WA 98195**

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## **Abstract**

Natural resource agencies are challenged to not only maintain the overall abundance of salmon and steelhead but also to maintain their genetic and ecological diversity. Hatchery production, designed to achieve the first objective, sometimes conflicts with the second. Steelhead in Washington have been bred to return earlier in the year than wild fish and this separation has the potential to minimize genetic, ecological and fisheries interactions between the two stocks minimized (though the selective breeding was initiated for other reasons). The recent establishment of hatchery steelhead at Forks Creek in southwestern Washington presents a unique opportunity to examine the assumptions underlying this innovative approach. HSRG funds were used to supplement an ongoing study, supported by the Weyerhaeuser Foundation and with previous cooperation from Long Live the Kings, to sample hatchery and naturally produced steelhead at discrete juvenile and adult life history stages. We sampled all adults spawned at the hatchery, naturally-produced adults returning to the river to spawn, as well as naturally produced juveniles in the river, smolts leaving the system, and hatchery pre-smolts. Analysis of DNA microsatellites from fin-clip samples to reveal the parentage and origin of juveniles and returning adults is ongoing. From this we will determine the relative production and survival of wild, hatchery, and naturally-spawning hatchery origin steelhead, and the extent of interbreeding between groups. The results will be conveyed to agency management staff, contributing sponsors and regional organizations, and the scientific community. Preliminary results indicate that hatchery fish allowed up the river to spawn naturally were successful, but did poorly compared to native fish. Further analysis will involve specific parentage analysis and examination of traits important for reproductive success in both hatchery and wild individuals.

## **Project Goal**

This project evaluates the current steelhead management approach, embodied in the tendency to breed early-returning, generalized hatchery stock for release in rivers whose wild steelhead differ in timing of migration and spawning. Using Forks Creek as our study site, we are using DNA microsatellites to (1) determine the parentage (wild, hatchery or naturally-spawned hatchery origin) of returning adults to test the hypothesis of equal fitness of the two stocks, and (2) determine the proportion of naturally produced juveniles and smolts of wild and hatchery origin to test the hypothesis of equal per capita productivity rates and to compare size and distribution of the two groups.

## **Project Background**

### **Rationale/Justification**

Although hatcheries were constructed to enhance wild populations or mitigate for habitat losses, concern has been expressed that genetic and ecological interactions with hatchery-produced salmonids have contributed to the decline of wild populations. In Washington, the approach to steelhead propagation has been to breed generic early-returning hatchery stock (e.g., Chambers Creek stock) and release them at a wide range of locations. These early stocks were initially developed to give the juveniles a longer growing season so that they could reach a sufficient size for smolt transformation after one year in the hatchery rather than two, as would be typical of wild steelhead. However, ancillary benefits are that these early-returning fish can be selectively harvested and they may be unlikely to interbreed with wild steelhead or to successfully reproduce in the rivers. Pacific salmon hatcheries, on the other hand, tend to homogenize wild and hatchery fish at a given location because little effort has been made to separate them. Most salmon and steelhead hatchery programs have been on-going for so long that it is difficult to determine how they are affecting wild populations. However, the recent initiation of steelhead production at Forks Creek Hatchery presents a unique opportunity to test the hypotheses underlying the approach to steelhead conservation. Accordingly, we obtained samples from the first hatchery adults that returned in winter 1995-96 (brood year, "BY," 1996), and from fish returning in subsequent years.

Ground-breaking work on this subject has been conducted at the Kalama River but further work is needed, not only to test the generality of those results but also because: 1) the Kalama River work began only after years of hatchery releases, 2) the protein electrophoresis techniques did not allow individuals to be identified and only distinguished wild from hatchery fish on a probabilistic basis, 3) the presence of summer and winter runs complicated genetic analyses. At a time when the two fisheries resource agencies, Wildlife and Fisheries, have

recently merged, it is particularly critical that the relative merits of the steelhead and salmon models of genetic conservation be evaluated.

## **Study Design and Methods**

Forks Creek supports a small (about 50-100 adults) late-winter wild run of steelhead and a recently-established hatchery program with early returning stock. In the spring of 1994, 25 000 steelhead smolts were planted in Forks Creek as the start of a new hatchery-supported population. These smolts were from the Bogachiel Hatchery, whose hatchery run is composed of Chambers Creek stock and native Bogachiel River steelhead. The first adults from this planting returned in BY 1996 and comprised the first cohort of steelhead spawned at Forks Creek Hatchery. Our approach has been to sample all adult steelhead returning to the hatchery, measuring phenotypic traits likely to affect reproductive success such as size, fecundity, egg size, and spawning date, and taking DNA samples for genetic analysis. We subsample their offspring as pre-smolts, and then sample returning adults in subsequent years to determine which of the parents produced offspring. We apply similar methods to the wild steelhead, sampling them as adults at the hatchery's weir and at a smolt fence. We subsample juveniles in the creek and all smolts trapped at the fence. In addition, during the first two years of sampling, large numbers of hatchery-origin adults were allowed to spawn in the creek, and they were also sampled. Given the lifespan of steelhead, this is necessarily a multi-year study. However, we obtained samples from adult hatchery-origin steelhead in the first year that they returned, including fish spawned and killed at the hatchery and those released to spawn in the creek. As of BY 2000, unclipped (i.e., naturally spawned) adults returned whose parents could have been hatchery origin, and only genetic analyses such as ours will be able to distinguish the naturally-spawned offspring of hatchery-origin adults from fully wild fish.

Sampling adults at the hatchery is simple because they are diverted into a holding area by a weir across the creek and are spawned on Mondays each week from about mid-December to mid-February. All fish that matured the previous week are spawned. Sampling continues in this manner until the trap ceases to catch fish. Thus any fish returning through February or early March has a high probability of being caught, though some fish may get upriver unsampled if they arrive at the weir and experience just the right conditions of flow and debris. Given the discharge regime of the watershed, prevailing seasonal rainfall pattern and physical structures in place, this cannot be avoided. After March the hatchery's weir does not operate but a smolt fence, put in place in late April and operated through June or July (previously by Long Live The Kings), enables us to sample naturally spawned smolts and also trap adult steelhead migrating either upstream or downstream. This trap can also be compromised by floods but we have sampled approximately 325 adults in this manner so far.

We obtained samples from all steelhead smolts trapped in the years when their parents would have been sampled (365 in 1998, 295 in 1999, 285 in 2000 and 193 in 2001), and we anticipate similar numbers in the future, depending on escapement and recruitment success. Hatchery pre-smolts are sampled (300 per year) in February prior to release and juveniles produced in the stream are sampled by electrofishing from four index sections of the creek in late September or early October, at the end of the growing season and before high flows inhibit sampling (about 2100 samples obtained through fall 2000).

We are determining the parentage (and other forms of kinship) for steelhead by genotyping microsatellite loci, which are 1-5 base pair (bp) repeats that form tandem arrays <300 bp in length, and exhibit high levels of allelic variation in repeat number. Genetic variation at multiple highly variable microsatellite loci is being assayed by first amplifying them from genomic DNA samples via the polymerase chain reaction (PCR) and then analyzing their allelic states using a Molecular Dynamics MegaBACE (96 well capillary system) automated sequencer operated in "genotyper" mode. Alleles are scored with the assistance of Genetic Profiler software marketed by Molecular Dynamics.

We are using several approaches to determining kinship among steelhead using the microsatellite data. One approach relies on simple exclusion criteria to identify potential parent-offspring triads. The ability to examine partial genotypic matches between offspring and candidate parents will allow us to diagnose any false exclusions that might arise from genotyping errors, null alleles or mutations. Second, we are using the recently released parentage assignment program, Cervus 1.0 to assign parentage. Cervus combines a likelihood-based approach to assigning parentage, along with simulation abilities that allow the statistical confidence of parentage assignments to be determined. Third, we are employing allele sharing and relatedness measures and likelihood procedures to identify likely siblings even in the case where we have not sampled the parents. Fourth, we are using a likelihood-based assignment procedure to determine the probable population affinity (hatchery, wild or hybrid) of naturally spawned Forks Creek fish whose parents are not in our database.

All hatchery-propagated fish are marked by clipping the adipose fin, and hence are easily identifiable. However, an important goal of this study is to compare the reproductive success of hatchery-origin fish spawning in the creek to that of wild native individuals but these fish carry no external marks to indicate their parentage. Likewise, we will wish to identify any hatchery-wild hybrids that occur, and to compare their success to individuals in the parental populations but they too would be unmarked. We may not sample all naturally-reproducing fish in Forks Creek so we must be able to identify unmarked steelhead as hatchery type, wild, or hatchery-wild hybrids in the absence of parental data. We will estimate the relative likelihood that a fish belongs to one population or the other by computing the likelihood of its multilocus genotype in each candidate population, based on allele frequency data from each population, and assuming Hardy-Weinberg equilibrium.

We conducted a preliminary test of our ability to correctly assign Forks Creek fish to 'hatchery' or 'wild' using allele frequency data from the 9 loci we

have examined to date. Using a jackknife procedure in which each fish was removed from the allele frequency database from its population before assigning it, 98% of the known hatchery fish were correctly assigned to 'hatchery', and approximately 86% of the known wild fish were correctly assigned to 'wild'. This preliminary analysis shows promise, but we want to have more and 'better' loci to definitively identify an even higher proportion of fish to population, and particularly to identify probable hybrids.

Having determined either the specific parents of individual fish (either as juveniles in the creek, smolts at the fence, pre-smolts in the hatchery or adults returning) or their origin (wild, hatchery or hybrid) if we had not sampled their parents, we will be able to test the biological hypotheses underlying the policy of temporal isolation of wild and hatchery steelhead. We will be able to determine the per capita reproductive success of wild and hatchery-origin fish spawning in the river, compare the success of hatchery fish spawned in the hatchery to those spawning in the river, determine if wild and hatchery fish interbreed, whether the early spawning hatchery fish produce larger juveniles at the end of the summer, etc.

## **Project Status**

### **Objective 1. Adult sampling**

Adult steelhead returning to Forks Creek in the 2000/2001 season were sampled weekly at the hatchery weir from December 18 to February 26, and daily at the smolt trap from March 28 to June 12. Data taken included some to all of the following: length, weight, egg size and fecundity information, scale samples and fin clips for DNA analysis. All fish had scales and fin clips taken but hatchery fish were sampled more extensively (i.e., weight and fecundity data were taken) than wild fish to limit the amount of handling to which the wild fish were exposed. There was a total of 212 adult steelhead at Forks Creek in the 2000/2001 season.

### **Objective 2. Hatchery pre-smolt, wild smolt and wild juvenile sampling**

Three hundred hatchery pre-smolts were sampled on March 6, 2000, approximately one week before they were released downstream. Length, weight and a fin clip for DNA analysis were taken. Wild smolts were sampled at a trap on the creek where we collected length and weight data, as well as a fin clip from each of 192 steelhead smolts emigrating in 2001.

Naturally-produced juvenile steelhead were sampled upstream of the hatchery on Forks Creek using backpack electrofishing gear on September 20, 2001. Captured fish were sedated using MS-222 and measured for length and weighed. A small (approximately 0.25 cm<sup>2</sup>) section of caudal fin was removed and stored in 95% ethanol for DNA extraction and genetic analysis. Fish were allowed to recover in freshwater and then released back into the stream. We collected samples from 172 fish.

### Objective 3. Genetic analysis

DNA has been extracted from all adult and naturally-produced smolt fin clips except the most recently collected samples (April 2002-present). Juvenile and hatchery-produced smolt samples have not yet been extracted. Microsatellite development and screening is on-going. At present we have a panel of 9 microsatellite markers in 3 multiplexes (Table 1), however, as the study progresses we hope to include up to 20 microsatellite markers in our analysis. All adults and naturally-produced smolts (except 2002 samples) have been run with the 3 multiplexes. Further analysis with computer programs to estimate relatedness between individuals (such as parent-offspring relationships) is contingent on completion of genetic data collection.

Table 1. Microsatellite loci examined and PCR details.

<b>Locus</b>	<b>Reference</b>	<b>Repeat type</b>	<b>MgCl<sub>2</sub></b>	<b>T<sub>m</sub>(°C)</b>
Oki3A	A. Spidle (unpub.)	Tetranucleotide	1	55
Omy77	Morris <i>et al.</i> 1996	Dinucleotide	1	53
Omy1001	P. Bentzen (unpub.)	Tetranucleotide	1	53
Omy1011	P. Bentzen (unpub.)	Tetranucleotide	1	53
Oneu2	Scribner <i>et al.</i> 1996	Dinucleotide	1	55
One108	J.B. Olsen (unpub.)	Tetranucleotide	1	55
Ssa85	O'Reilly <i>et al.</i> 1996	Dinucleotide	2	50
RT191	P. Bentzen (unpub.)	Tetranucleotide	1	66
RT212	P. Bentzen (unpub.)	Tetranucleotide	1	66

### Objective 4. Determination of reproductive success

Determination of individual reproductive success will not be possible until we have completed genetic analysis of all samples, however, population-level reproductive success (i.e., hatchery versus wild reproductive success) has been examined to the smolt stage for the two years in which hatchery adults were allowed upstream to spawn. Assignment to population of origin is based on allele frequency data, and reproductive success is calculated by the number of offspring assigned to the population divided by the number of females in the population in the previous generation. Hatchery females produced an average of 1.07 smolts per capita spawning in 1996 (smolt year 1998) and 1.33 smolts per capita spawning in 1997 (smolt year 1999). Wild females produced an average of 24.50 smolts per capita in 1996 and 18.80 in 1997.

Attached is a manuscript detailing these results, which was recently submitted as part of the 2002 Wakefield Symposium to be published in "The Environmental Biology of Fishes."