

**Evaluation of the Reproductive Success of Wild and Hatchery Steelhead  
in Hatchery and Natural and Hatchery Environments**

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**Annual Report for 2008**

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**Title:** Evaluate the Reproductive Success of Wild and Hatchery Steelhead in Hatchery and Natural and Hatchery Environments

### **ABSTRACT**

This report summarizes the field, laboratory, and analytical work from December 2007 through November 2008 on a research project that investigates interactions and comparative reproductive success of wild and hatchery origin steelhead (*Oncorhynchus mykiss*) trout in Forks Creek, a tributary of the Willapa River in southwest Washington. First, we continued to successfully sample hatchery and wild (i.e., naturally spawned) adult and wild smolt steelhead at Forks Creek. Second, we revealed microsatellite genotype data for adults and smolts through brood year 2008. Finally, four formal scientific manuscripts were published in 2008 and two are in press, one is in revision and two are in preparations.

## INTRODUCTION TO THE PROJECT

### *Reproductive Success*

Salmon species exist as more-or-less discrete and isolated breeding populations, differing in selectively neutral traits and a variety of adaptive, quantitative traits (Ricker 1972, Taylor 1991; Quinn 1999). This “stock concept” is the cornerstone for the conservation and management of Pacific salmon (*Oncorhynchus* spp.). It is widely recognized that the health of salmon species depends on the maintenance of the complex population structure (Scudder 1989; Riddell 1993; Waples 1995), characterized by great diversity and adaptation to local conditions among populations.

The differential reproductive success (RS) of individuals that also differ in heritable phenotypic traits is a fundamental part of natural selection and the evolution of populations. RS is a function of the success of individuals in obtaining mates and producing viable offspring. Mature adults are subjected to sexual selection (e.g., intra-sexual competition and mate choice), resulting in variation in RS. Mortality agents such as nest disturbance, predation, disease, and abiotic factors like gravel scour or ice may take different proportions of the fish from different families, resulting in further variation in RS among the adults of the previous generation.

A number of traits have been documented or hypothesized to affect RS in salmon. At the breeding stage, males actively compete for access to ripe females, and large males tend to dominate such competition (Gross 1985; Foote 1988; Quinn and Foote 1994). However, small males may adopt alternative reproductive tactics (sneaking rather than fighting) and successfully fertilize some eggs (e.g., Schroder 1981; Chebanov et al. 1983; Maekawa and Onozato 1986; Foote et al. 1997). The success of males using these tactics

changes rapidly over the course of the breeding season as the operational sex ratio (OSR: ratio of sexually active males to sexually active females) increases (Quinn et al. 1996). Behavioral research has tended to focus on variation in RS among males but RS may vary considerably among females as well. Larger females have both more numerous and larger eggs (producing larger fry, with higher survival rates) than smaller females (Beacham and Murray 1993), and they dig deeper (hence less vulnerable) redds (Steen and Quinn 1999), so there could be size-biased variation in female RS. Consistent with this hypothesis, Helle (1989) reported that a chum (*O. keta*) salmon population was much more productive in years when the females were large than when they were small, and that most of this effect was in addition to the projected increase in fecundity. However, Holtby and Healey (1986) questioned the hypothesis that large females are more productive than small females. Much of the high mortality rate for embryos is related to gravel scour, and this may vary greatly among families but be unrelated to female size. In addition to the factors affecting RS in males and females, there is evidence that salmon tend to mate assortatively by size (Hanson and Smith 1967; Foote 1988; Foote and Larkin 1988). That is, large males tend to breed with large females, and smaller males with smaller females, as a result of both competition and choice. Finally, the date of spawning is heritable, hence the progeny of early spawners will be more likely to mature at the same time as their siblings than non-siblings (Siitonen and Gall 1989), leading to genetic isolation by time within populations (Leary et al. 1989; Gharrett and Smoker 1993; Bentzen et al. 2001). This may tend to make them vulnerable to mortality agents affecting certain segments of the population (e.g., flooding during incubation: Thorne and Ames 1987).

Thus the breeding biology of salmonids is characterized by uneven contribution of adults to the next generation (i.e., selection), and this tends to reduce the effective (as opposed to absolute) population size. In addition, a series of connections between juvenile life history events may result in differential survival of sibling groups, magnifying the variance in RS of the parental generation. Juveniles of most species establish feeding territories in streams, and the ability to acquire and hold territories is largely a function of size and prior residence (e.g., coho salmon, *O. kisutch*: Mason and Chapman 1965; Rhodes and Quinn 1998). Egg size controls the initial size of juveniles, and juveniles that emerge early (because they were spawned early in the fall) will have a size advantage when later-emerging juveniles try to compete with them for space. Territoriality is also strongly related to prior residence, hence there may be great variation in territorial possession among families. Studies with Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) indicate the importance of these factors for survival (Brännäs 1995; Einum and Fleming 2000).

In addition to factors related to size and date of emergence, growth rate varies among families (e.g., Beacham 1989). The combination of family-specific variation in fry size, date of emergence, growth, social behavior, and distribution may lead to considerable variation among families in size at the end of their first summer in the stream. Size is positively correlated with survival through the winter in freshwater (e.g., in coho salmon, Quinn and Peterson 1996) and at sea (coho salmon: Holtby et al. 1990; cutthroat trout, *O. clarki*: Tipping and Blankenship 1993; and steelhead trout, *O. mykiss*: Ward et al. 1989). Finally, there is evidence for family-specific variation in survival at sea independent of smolt size in pink salmon (*O. gorbuscha*; Geiger et al. 1997) and chinook salmon (*O. tshawytscha*; Unwin et al 2003).

### ***Artificial Propagation***

Many factors have reduced wild salmon abundance, including impassable or injurious dams, overfishing, land use practices, and variation in marine and freshwater conditions driven by climate (Stouder et al. 1996; National Research Council 1996). To offset the effects of these factors, salmonids have been produced in hatcheries in the Pacific Northwest for over a century. Considerable attention has been focused recently on the benefits and costs of such artificial propagation (e.g., Hilborn 1992). Some of these concerns pertain to the elevated fishing rates on mixed wild and hatchery populations (Wright 1993; Hilborn and Eggers 2000), and possible competitive interactions between populations (Nickelson et al. 1986; Nielsen 1994). These kinds of concerns can be difficult to resolve but are relatively simple to understand. However, the genetic concerns regarding hatcheries are much more intricate and perplexing. Reviews of the subject (e.g., Hindar et al. 1991; Waples 1991; Ryman et al. 1995) identify three major issues: fitness of hatchery populations for post-release survival, interactions between wild and hatchery populations, and reduction in effective population size due to supportive breeding.

Most kinds of animals bred for human consumption (e.g., chickens and pigs) are given no opportunity to interact with wild members of their species (if they even exist). Salmonids are unusual in that we breed them in highly artificial environments but then expect them to range freely on the ocean to feed, grow and return (excepting completely controlled aquaculture operations). A large and growing literature demonstrates that captive rearing induces a number of phenotypic changes in salmonids. Some changes such as color (Maynard et al. 1995) are largely if not exclusively environmentally induced but others may result from both genetic and environmental changes such as

aggression (Berejikian et al. 1996; Rhodes and Quinn 1998), growth (Reisenbichler and McIntyre 1977), and anti-predation responses (Berejikian 1995). Changes in the timing of migration and breeding are largely under genetic control (Flagg et al. 1995; Quinn et al. 2002).

Many studies have indicated that the survival rates of hatchery fish are lower than those of wild conspecifics, but these differences are obviously attributable to many factors. Nevertheless, the genetic changes in hatchery populations are cause for concern, not just for the fitness of the hatchery population but also for wild populations with which they might interbreed. The most convincing studies on this subject, conducted on Kalama River steelhead (Chilcote et al. 1986; Leider et al. 1990), showed that hatchery adults produced markedly fewer offspring, when spawning naturally, than did wild adults. In this case the hatchery stock was derived from another basin so the generality of the finding is not clear. In addition, the hatchery had been operating for many years, so the extent of introgression prior to the study was difficult to ascertain. Finally, the study relied on protein electrophoresis and could assign the fish to population (hatchery or wild) on a probabilistic basis but could not assign them to specific parents. Thus the traits associated with reproductive success (and the poor performance of the hatchery fish) could not be determined. Notwithstanding these drawbacks, the Kalama River work was extremely important and influential, and there is evidence elsewhere of poor performance of hatchery fish in natural environments (e.g., Atlantic salmon: McGinnity et al. 1997; Fleming et al. 2000).

Hatcheries greatly increase egg-smolt survival but often have poor post-release survival. This is largely a result of rearing practices, and there are efforts to improve them (Maynard et al. 1995, 1996a, b), but many phenotypic traits affecting survival have



a genetic basis as well. In some cases there has been deliberate selection for a trait that will facilitate operations in the hatchery (e.g., spawning fish that mature early in the season and discarding those that ripen later: Ayerst 1977; Crawford 1979). More often, though, the selection is inadvertent and less obvious. Since most fish survive in the hatchery, genetic changes probably result from one of two processes. First, hatcheries fail to cull fish of “poor quality” that would be selectively taken by predator or pathogens in nature, and natural processes cull them after release. After eliminating the obviously abnormal fish, most hatchery managers are unwilling to cull large numbers of their fish without a very clear set of criteria. There is very little opportunity in a hatchery to test the vigor of juvenile salmon at a production scale, so this process may be unavoidable.

The second obvious form of selection in hatcheries is the mating system, and this is the main “pressure point” by which we may practically affect salmon evolution in hatcheries. There seems to be little documentary evidence on the specific practices in hatcheries but it is widely acknowledged that staff often tended to select a small number of males for breeding based on size or more subjective criteria related to “quality”. The use of a few males was both a matter of selection and convenience; only a few males are needed to fertilize the eggs of many females. Females tended to be spawned until the capacity of the hatchery was reached. Thus, except for attributes related to timing, there may have been little variation in probability of breeding and reproductive success (other than fecundity) among females but great variation among males.

## OBJECTIVES

### *Objectives for the Project*

Our overall objective is to determine the factors influencing RS in wild and hatchery-origin salmonids in natural and hatchery environments, using steelhead trout as the test species. Our specific objectives are as follows: (1) quantify the breeding pattern (i.e., selection of fish for spawning) in a production hatchery, (2) directly determine the realized RS of the individual hatchery fish by DNA parentage analysis of the adults returning over 2-3 complete generations (3) directly measure the effective population size ( $N_e$ ) of the hatchery population over multiple generations (4) determine whether there was any loss of genetic diversity in the hatchery population over two generations, (5) determine the realized heritability of key life history traits for hatchery fish released to the sea, (6) determine the realized reproductive success of wild and hatchery-wild hybrid parents spawning naturally, and compare these levels to those of the first generation of hatchery steelhead spawning naturally.

### *Goals for 2008*

Our primary goals for 2008 were to 1) continue sampling hatchery and wild (i.e., naturally spawned) adult steelhead and wild smolts at Forks Creek, 2) perform parentage and genetic assignment analysis, 3) examine and analyze data and 4) prepare three scientific manuscripts.

## TASKS AND METHODS

### *Background*

Our study site, Forks Creek, is a tributary of the Willapa River in southwest Washington, with a small wild population of steelhead that generally enters from March through May and spawns from April through June (Mackey et al. 2001). A hatchery, situated just above the creek's confluence with the Willapa River, has been operated by the Washington Department of Fisheries (WDF, now WDFW) to produce coho and chinook salmon since 1895. There had been occasional releases of steelhead in the Willapa River but apparently not in Forks Creek, and there had been no continuous propagation of steelhead in the system. When WDF merged with the Washington Department of Wildlife, a decision was made to produce steelhead as well. Beginning in the winter of 1995-96, the creek received the first adult returns from hatchery-produced steelhead released as smolts in spring of 1994. These fish originated from the Chambers Creek hatchery population but had been propagated at the Bogachiel Hatchery, mixed with unknown proportions of local wild steelhead. The Chambers Creek population is widely released in Washington by the WDFW, and has been artificially selected for early return and spawning timing (generally December through February) to minimize fishery conflicts and interbreeding with wild fish, and to facilitate culture operations. There were additional plants from the Bogachiel Hatchery in 1995 and 1996 but since then the hatchery has produced its own fish without outside influence. We initiated sampling with the first brood year, so this site provides an excellent opportunity to examine the genetics of hatchery steelhead.

Our sampling of naturally spawning steelhead and their progeny as smolts and adults, and adults spawned (or killed as surplus) at the hatchery is designed to take

advantage of a special feature of the history of Forks Creek hatchery operation. In the first two years when hatchery adults returned (1996 and 1997), the decision was made to allow surplus adults to spawn in the river (Mackey et al. 2001). Accordingly, a minimum of 362 hatchery adults were allowed upriver, along with 59 wild fish; 117 hatchery fish were also spawned at the hatchery in those two years. However, the decision was then reversed, to deny hatchery fish access to the spawning grounds. Thus the wild population was exposed to a very strong “pulse” of hatchery influence for two years, followed by an open-ended “recovery” period. This unusual situation allows us to gather critical data on the extent to which a wild population resists hatchery influence once the influence is largely terminated. Some hatchery fish bypass the weir during periods of extremely high water, and they might be considered to be the equivalent of strays.

### ***Sampling***

The hatchery has spawned steelhead since winter 1995-96 (Brood Year [BY] 1996 by our designation) and we have obtained samples (length, weight, scales for age determination and fin clip for DNA, and egg size and fecundity from females) from almost all of them. There is a weir across the creek that guides salmon and steelhead into a concrete fish ladder filled with water from the hatchery leading to a small pond. This weir functions well except under very high water conditions. As per their normal operating procedure, hatchery staff examined captured steelhead once each week. Wild, or unmarked, steelhead were sampled then released upstream of the weir. Hatchery, or marked, steelhead were sampled then either returned to the pond, spawned, or sacrificed depending on whether they were fully mature or not and whether the hatchery’s capacity has been reached. Sampling of hatchery fish took place after the hatchery manager’s

decision was made. We estimated the fecundity of females by weighing the entire mass of eggs, and weighing and counting a subsample of eggs.

We also operated a smolt trap from mid-late April to early June. This fan trap catches a very large fraction of the smolts (essentially all smolts, except during high water events). All steelhead smolts were measured and weighed and a small caudal fin clip was removed; these data were recorded with the date of capture. In addition, many steelhead kelts (outmigrating, post-spawning adults) were caught in the smolt trap, and by angling or tangle net, augmenting our sampling of naturally-spawning migrants. We do not know precisely what fraction of the naturally spawning population was sampled. Plans for improving the weir are still being developed.

### ***Genetic Analysis***

DNA was extracted from fin clippings using DNAeasy columns (Qiagen, Valencia, CA). PCR amplification of eight microsatellite loci were carried out in 10 $\mu$ l reaction volumes using 55 °C (*Oki23*, *Omy77*, *Omy1001UW*, *Omy1011UW*, *One108*), 60 °C (*Ssa85*) and 65°C (*Omy1191UW*, *Omy1212UW*) annealing temperature and a MgCl<sub>2</sub> concentration of 2 (*Ssa85*) or 1 (all others)  $\mu$ M. Amplified products were size separated on a 96 capillary automated DNA sequencer (MegaBace1000, Amersham Biosciences). Allele size was estimated using Genetic Profiler v2.2 (Amersham Biosciences).

## **PROGRESS DURING 2008**

### ***Objective 1) Continued Collection and Processing of Samples***

During the 2008 season (including some samples collected in November and December of 2007 and November 2008 [brood year 2009]) we sampled 677 adult

hatchery-produced steelhead and 64 naturally-spawned adult steelhead (Table 1). The smolt trap, in operation on Forks Creek from April 8 through June 17, caught 331 steelhead smolts (Table 2), as well as other salmonids (*O. clarki*, *O. kisutch*, *O. tshawytscha*, and lamprey, *Lampetra* spp.). The median date of emigration for steelhead smolts was May 13 (Figure 1). Partial or complete genotypes have been generated for many of these samples.

## ***Objective 2) Parentage and Assignment Analysis***

### ***Parentage***

Parentage analysis was carried out based on principles of exclusion. WHICHPARENTS (Banks & Eichert, Bodega Bay Marine Lab, California, unpublished, available at <http://marineresearch.oregonstate.edu/genetics/software.htm>) was used to identify candidate parents that matched offspring at one allele at 6 of 8 loci to allow for genotyping errors and non-Mendelian inheritance of alleles (e.g., null alleles). The genotypes of adults identified by WHICHPARENTS as potential parents were then directly compared to offspring genotypes. After checking both adult and adult offspring genotypes for errors, only adults that matched one allele at all loci were assigned as parents. Because of the possibility of repeat spawning, the lack of age data of returning offspring and incomplete sampling of both parents and offspring in some years, adults from all years previous to the return year of adult offspring were considered potential parents. No assignment was possible for some offspring, likely because of incomplete sampling of parents due to weir inefficiency or because parents spawned before this study was initiated.

In some cases only a single parent was assigned to an offspring. If it was a hatchery fish (i.e., clipped adipose fin) this parent was accepted as true only when it was known that adults of the opposite sex, spawned on the same day as the assigned parent had no genotype available due to missed sampling or sample processing problems. If it was a wild fish (i.e., intact adipose fin) single parent parentage was accepted if the parent was either an unclipped fish, or a clipped fish that had been released above the weir (i.e., naturally spawning). In order to qualitatively examine the veracity of single parent parentage of wild fish we calculated the probability of randomly drawing the genotype composed of a homozygote for the most frequent allele at each locus in the wild population, representing the high end of the range of probabilities. In comparison with assignment results, we used fish with both single and both parents for comparison.

At the time of this writing, parentage analysis was performed for parental brood years 1996 through 2007, but not 2008 for adult and smolt offspring of both wild and hatchery steelhead. Genotyping of individuals from 2008 is nearly complete, but parentage analysis cannot be completed until genotyping is complete. As reported last year, through brood year 2007, 142 adult offspring have been assigned one parent, and 3503 have been assigned both parents. Most parental assignments are to hatchery produced adult steelhead (3496). A total of 740 adults produced these adult offspring. Through brood year 2006, 268 smolt offspring have been assigned one parent, and 15 have been assigned both parents. A total of 271 adults produced these smolt offspring.

### ***Assignment tests***

We evaluated available genetic assignment tests for accuracy and found that the methods of (Rannala, Mountain, 1997) as carried out in GENECLASS II (Piry *et al.*, 2004) made the fewest errors (Hauser *et al.* 2006). Assignment tests were performed using

Bayesian computations, with baseline data. Baseline data consisted of hatchery produced (clipped) and wild (unclipped) adult steelhead returning in brood years 1996-1998. Only assignments of greater than 0.95 probability were accepted as final. Assignments of less than 0.95 probability were classified as 'unknown'.

At the time of this writing, assignment tests were performed for parental brood years 1996 through 2007, but not 2008 for adult and smolt offspring of both wild and hatchery steelhead. Genotyping of individuals from 2008 is nearly complete, but assignment tests cannot be completed until genotyping is complete. As reported last year, through brood year 2007, we determined that 4467 adults were of hatchery ancestry, 524 were of wild ancestry, and 566 of unknown ancestry. Hatchery ancestry has been determined for 794 smolts, wild ancestry for 1243 smolts, and unknown ancestry for 955. We are in the process of conducting further analyses on these data, including comparisons of size and migration date between wild and hatchery fish, trends in the percent of hatchery fish over time, etc.

***Objective 3) Data analysis and Objective 4) report preparation***

Data analysis is ongoing. Selected results will be published in scientific manuscripts in 2009. We dealt with nine manuscripts in 2007-08. One in review at the end of 2007 was published in 2008. Three were written and published in 2008, two are currently in press and one is in revision and two are in preparation (see below for details).

1. Carlson SM, **Seamons TR**, 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. *Evolutionary Applications* 1:222-238.



Below is the abstract from the manuscript.

Salmonine fishes are commonly subjected to strong, novel selective pressures due to anthropogenic activities and global climate change, often resulting in population extinction. Consequently, there is considerable interest in predicting the long-term evolutionary trajectories of extant populations. Knowledge of the genetic architecture of fitness traits is integral to making these predictions. We reviewed the published, peer-reviewed literature for estimates of heritability and genetic correlation for fitness traits in salmonine fishes with two broad goals in mind: summarization of published data and testing for differences among categorical variables (e.g., species, life history type, experimental conditions). Balanced coverage of variables was lacking and estimates for wild populations and behavioral traits were nearly absent. Distributions of heritability estimates were skewed toward low values and distributions of genetic correlations toward large, positive values, suggesting that significant potential for evolution of traits exists. Furthermore, experimental conditions had a direct effect on  $h^2$  estimates, and other variables had more complex effects on  $h^2$  and  $r_G$  estimates, suggesting that available estimates may be insufficient for use in models to predict evolutionary change in wild populations. Given this and other inherent complicating factors, making accurate predictions of the evolutionary trajectories of salmonine fishes will be a difficult task.

2. **Naish KA**, Hard JJ, 2008. Bridging the gap between the genotype and the phenotype: linking genetic variation, selection and adaptation in fishes. *Fish and Fisheries* 9:396-422.

Below is the abstract from the manuscript.

One of the most challenging problems in evolutionary biology is linking the evolution of the phenotype with the underlying genotype, because most phenotypes are encoded by

many genes that interact with each other and with the environment. Further, many phenotypes are correlated and selection on one can affect evolution of the other. This challenge is especially important in fishes, because their evolutionary response to harvest, global warming and conservation actions are among the least understood aspects of their management. Here, we discuss two major genetic approaches to studying the evolution of complex traits, multivariate quantitative genetics and molecular genetics, and examine the increasing interaction between the two fields. These interactions include using pedigree-based methods to study the evolution of multivariate traits in natural populations, comparing neutral and quantitative measures of population structure, and examining the contribution that the two approaches have made to each other. We then explore the major role that quantitative genetics is playing in two key issues in the conservation and management of fish populations: the evolutionary effects of fishing and adaptation to climate change. Throughout, we emphasize that it is important to anticipate the availability of improvements in molecular technology and statistical analyses by creating research populations such as inbred lines and families segregating at fitness traits, developing approaches to measuring the full range of phenotypes related to fitness, and collecting biological material and ecological data in natural populations. These steps will facilitate studies of the evolution of complex traits over informative temporal and spatial scales.

3. **Hauser L**, Carvalho GR, 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish and Fisheries* 9:333-362.

Below is the abstract from the manuscript.

By providing new approaches to the investigation of demographic and evolutionary dynamics of wild populations, molecular genetics has led to fundamental changes in our understanding of marine ecology. In particular, genetic approaches have revolutionized our understanding in three areas: (i) most importantly, they have contributed to the discovery of extensive genetic population structure in many marine species, overturning the notion of large, essentially homogenous marine populations limiting local adaptation and speciation. (ii) Concomitant differences in ecologically important traits now indicate extensive adaptive differentiation and biocomplexity, potentially increasing the resilience to exploitation and disturbance. Evidence for rapid adaptive change in many populations underlies recent concerns about fisheries-induced evolution affecting life-history traits. (iii) A compilation of recent published research shows estimated effective population sizes that are 2–6 orders of magnitude smaller than census sizes, suggesting more complex recruitment dynamics in marine species than previously assumed. Studies on Atlantic cod are used to illustrate these paradigm shifts. In our synthesis, we emphasize the implications of these discoveries for marine ecology and evolution as well as the management and conservation of exploited marine fish populations. An important implication of genetic structuring and the potential for adaptive divergence is that locally adapted populations are unlikely to be replaced through immigration, with potentially detrimental consequences for the resilience to environmental change – a key consideration for sustainable fisheries management.

4. **Hauser L**, Seeb JE, 2008. Advances in molecular technology and their impact on fisheries genetics. *Fish and Fisheries* 9:473-486.

Below is the abstract from the manuscript.

Although genetic approaches to questions in fisheries management have been very useful in the past, they have encountered consistent hurdles despite the development of new marker systems. However, recent technological advances in molecular genetics will help to overcome many of these hurdles and are likely to revolutionize fish and fisheries biology. DNA-sequencing costs have been decreasing exponentially, and recent breakthroughs have led to rapid increase in throughput that allows sequencing the entire expressed genome of a non-model organism with standard project budgets. Increase in screening throughput and number of available markers, reduction in costs and improved insights into gene function and control of gene expression will allow applications that were impossible until recently. Here, we briefly recount the recent history of fisheries genetics, provide an outlook on near-term and long-term developments in genetic technology and consider their applications and implications for fisheries science and education.

5. **Seamons TR, Dauer MB, Sneva J, Quinn TP**, 2009. Use of parentage assignment and DNA genotyping to validate scale analysis for estimating steelhead trout (*Oncorhynchus mykiss*) age and spawning history. North American Journal of Fisheries Management, In Press.

Below is the abstract from the manuscript.

Sound fisheries management depends on understanding life history characteristics such as age at maturity, migration, and spawning history, and hard parts such as scales and otoliths are commonly used to estimate values for these traits. Validation of these techniques is often difficult but critical, as errors in mean values for a population can result in erroneous estimates of sustainable exploitation rates. In this study we compared

information on age and spawning history derived from genetic analysis to that from scale analysis for 1836 individual steelhead trout (*Oncorhynchus mykiss*), representing two life history stages (smolt and mature adult), from hatchery and wild fish from two rivers in Washington State sampled over 19 years. Ageing error rates were < 5% for both wild smolts and hatchery adults but the error rate for wild adults was much higher (13%, 95% confidence interval = 1.82% - 29.22%). Adult scale ages were biased; scale readers typically overestimated the age of younger fish and underestimated the age of older fish. Scale readers misidentified fish that had spawned more than once as virgin spawners 6.5% (95% CI = 0.79% – 20.26%) of the time, in addition to the errors in age estimation. The higher error rates in using scales for ageing and identifying repeat spawning wild adults may in part stem from their more complex life history than smolts and hatchery origin adults, and highlights the need for verification of scale analysis, especially for wild populations of *O. mykiss*.

6. **Dauer MB, Seamons TR, Hauser L, Quinn TP, Naish KA**, 2009. Estimating the ratio of hatchery-produced to wild adult steelhead on the spawning grounds using scale pattern analysis. Transactions of the American Fisheries Society, In Press.

Below is the abstract from the manuscript.

Hatcheries produce Pacific salmon and trout (*Oncorhynchus* spp.) for many purposes, including fishery enhancement. The genetic integrity of wild populations spawning near such hatcheries may depend on the efficacy of their spatial or temporal separation from hatchery fish. We describe a simple, novel approach based on the examination of scales from an iteroparous species, steelhead trout (*O. mykiss*), to evaluate whether the ratio of hatchery-produced adults to wild adults on the spawning grounds met recommended

levels. In this river, migrating steelhead are diverted into the hatchery by a weir. Hatchery-produced fish are manually spawned and killed in the hatchery whereas wild fish are passed over the weir and allowed to spawn naturally upstream of the hatchery. Therefore, in principle, all hatchery-produced adults should be captured at the hatchery on their first spawning migration. However, scales from 8.3% (58 of 699) of female and 2.6% (22 of 844) of male hatchery-produced adults showed evidence of previous spawning migrations. Although lower than the incidence of repeat spawning by wild fish (males: 11.0%, females: 20.3%), these records nevertheless indicated significant reproductive opportunities in the wild for hatchery-produced fish. Combining these frequencies of repeat spawning with estimates of survival and numbers of wild fish, we modeled the ratio of hatchery to wild fish on the spawning grounds using data from eight return years. This ratio exceeded the recommended levels under all reasonable scenarios modeled (14.7 to 89.6% hatchery fish). Combined with evidence of spatial and temporal overlap, our data suggest that unless changes are made to increase the capture efficiency of adults at this and similar hatcheries relying on segregated stocks, risks of genetic introgression and ecological interactions with wild spawning populations will remain high.

**7. Naish, KA, Dauer MB, Seamons TR, Hauser L, Quinn TP.** Inbreeding effects on adult fitness correlates in a steelhead (*Oncorhynchus mykiss*) hatchery population. In Revision at Molecular Ecology.

Below is the abstract from the manuscript.

The likelihood of mating between relatives, or inbreeding, increases when a population is small over several generations, and therefore is of serious concern in captive

breeding, in which the number of breeding adults is often limited by available space, their scarcity, or other constraints. In Pacific salmonids, *Oncorhynchus* spp., such limitations of captive breeding are of particular concern, since offspring may be released in large numbers to supplement wild stocks and to support commercial and recreational fisheries. Inbreeding in captivity may therefore affect both hatchery and wild populations. Using molecular markers, we reconstructed the pedigree of a hatchery steelhead trout (*Oncorhynchus mykiss*) population over three generations since their establishment in 1994. From this pedigree, we calculated the change in average inbreeding ( $\Delta f$ ) in the most recent generation to be 0.64%. The increase in inbreeding was likely due to the small effective population size maintained in this hatchery over three generations (average 103.2  $\pm$  3.32 SD) which we attribute to a small proportion of returning fish spawned each year and high variance in reproductive success. Inbred fish were significantly shorter, weighed less and returned to the hatchery later than outbred fish. This study is one of the first to show that small changes in level of inbreeding affected fitness-related traits in a steelhead hatchery population, and demonstrates the relevance of molecular-based pedigrees to study the effects that inbreeding in captivity and associated inbreeding depression may have on the conservation of wild populations.

8. Quinn TP, Seamons TR, Vøllestad LA. Effects of growth and reproductive history on the egg size - fecundity tradeoff in steelhead trout, *Oncorhynchus mykiss*. In Preparation.

9. Seamons TR, Hauser L, Naish KA, Dauer MB, Quinn TP. Lower survival and production in naturally produced offspring of hatchery produced steelhead (*Oncorhynchus mykiss*). In Preparation.





## REFERENCES

- Ayerst, J.D. 1977. The role of hatcheries in rebuilding steelhead runs of the Columbia River system. Pages 84-88 in E. Schwiebert, editor. Columbia River salmon and steelhead. American Fisheries Society, Special Publication 10. Bethesda, Maryland.
- Beacham, T.D. 1989. Effect of siblings on growth of juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology. 67: 601-605.
- Beacham, T.D., and C.B. Murray. 1993. Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). Journal of Fish Biology. 42:485-508.
- Bentzen, P., J.B Olsen, J. McLean, T.R. Seamons, and T.P Quinn. 2001. Kinship analysis of Pacific salmon: insights into mating, homing and timing of reproduction. Journal of Heredity 92: 127-136.
- Berejikian, B.A. 1995. The effects of hatchery and wild ancestry and experience on the relative ability of steelhead trout fry (*Oncorhynchus mykiss*) to avoid a benthic predator. Canadian Journal of Fisheries and Aquatic Sciences 52: 2476-2482.
- Berejikian, B.A., S.B. Mathews, and T.P. Quinn. 1996. Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behavior in steelhead trout (*Oncorhynchus mykiss*) fry. Canadian Journal of Fisheries and Aquatic Sciences 53: 2004-2014.
- Bernard, R.L. and K.W. Myers. 1996. The performance of quantitative scale pattern analysis in the identification of hatchery and wild steelhead (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences 53: 1727-1735.

- Brännäs, E. 1995. First access to territorial space and exposure to strong predation pressure: a conflict in early emerging Atlantic salmon (*Salmo salar* L.) fry. *Evolutionary Ecology* 9: 411-420.
- Chebanov, N.A., N.V. Varnavskaya, and V.S. Varnavskiy. 1983. Effectiveness of spawning male sockeye salmon, *Oncorhynchus nerka* (Salmonidae), of differing hierarchical rank by means of genetic-biochemical markers. *Journal of Ichthyology* 23: 51-55.
- Chilcote, M.W., S.A. Leider and J.J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. *Transactions of the American Fisheries Society* 115: 726-735.
- Crawford, B.A. 1979. The origin and history of the trout brood stocks of the Washington Department of Game. Washington State Game Department, Fishery Research Report, Olympia.
- Davis, N.D. and J.T. Light. 1985. Steelhead age determination techniques. University of Washington, Fisheries Research Institute FRI-UW-8506.
- Einum, S., and I.A. Fleming. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution* 54:628-639.
- Flagg, T.A., F.W. Waknitz, D.J. Maynard, G.B. Milner, and C.V.W. Mahnken. 1995. The effects of hatcheries on native coho salmon populations in the lower Columbia River. *American Fisheries Society Symposium* 15: 366-375.
- Fleming, I. A., K. Hindar, I.B. Mjølnerød, B. Jonsson, T. Balstad, and A. Lamberg. 2000. Lifetime success and interactions of farm salmon invading a native population. *Proceedings of the Royal Society of London B*. 267:1517-1523.

- Foote, C.J. 1988. Male mate choice dependent on male size in salmon. *Behaviour* 106: 63-80.
- Foote, C.J. and P.A. Larkin. 1988. The role of male choice in assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). *Behaviour* 106: 43-62.
- Foote, C.J., G.S. Brown, and C.C. Wood. 1997. Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. *Canadian Journal of Fisheries and Aquatic Sciences* 54:1785-1795.
- Gharrett, A.J. and W.W. Smoker. 1993. Genetic components in life history traits contribute to population structure. p. 197-202. In J. G. Cloud, and G. H. Thorgaard [eds.], *Genetic conservation of salmonid fishes*. Plenum Press, N.Y.
- Geiger, H.J., W.W. Smoker, L.A. Zhivotovsky, and A.J. Gharrett. 1997. Variability of family size and marine survival in pink salmon (*Oncorhynchus gorbuscha*) has implications for conservation biology and human use. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2684-2690.
- Gross, M.R. 1985. Disruptive selection for alternative life histories in salmon. *Nature* 313: 47-48.
- Hanson, A.J. and H.D. Smith. 1967. Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*) of mixed age-groups. *Journal of the Fisheries Research Board of Canada* 24: 1955-1977.
- Hauser, L., T.R. Seamons, M. Dauer, K.A. Naish and T.P. Quinn. 2006. An empirical verification of population assignment methods by marking and parentage data: hatchery and wild steelhead (*Oncorhynchus mykiss*) in Forks Creek, Washington, USA. *Molecular Ecology* **15**: 3157-3173.

- Helle, J.H. 1989. Relation between size-at-maturity and survival of progeny in chum salmon, *Oncorhynchus keta* (Walbaum). Journal of Fish Biology. 35(Supplement A): 99-107.
- Hilborn, R. 1992. Hatcheries and the future of salmon in the northwest. Fisheries 17: 5-8.
- Hilborn, R., and D. Eggers. 2000. A review of the hatchery programs for pink salmon in Prince William Sound and Kodiak Island, Alaska. Transactions of the American Fisheries Society. 129: 333-350.
- Hindar, K., N. Ryman and F. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48: 945-957.
- Holtby, L.B., B.C. Andersen, and R.K. Kadowaki. 1990. Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 47: 2181-2194.
- Holtby, L.B. and M.C. Healey. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences 43: 1946-1959.
- Leary, R.F., F.W. Allendorf and K.L. Knudsen. 1989. Genetic differences among rainbow trout spawned on different days within a single season. Progressive Fish-Culturist 51: 10-19.
- Leider, S.A., P.L. Hulett, J.J. Loch, and M.W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead through the returning adult stage. Aquaculture 88: 239-252.
- Mackey, G., J.E. McLean and T.P. Quinn. 2001. Comparisons of run timing, spatial distribution, and length of wild and newly-established hatchery populations of

- steelhead in Forks Creek, Washington. North American Journal of Fisheries Management 21: 717-724.
- Maekawa, K. and H. Onozato. 1986. Reproductive tactics and fertilization success of mature male Miyabe charr, *Salvelinus malma miyabei*. Environmental Biology of Fishes 15: 119-129.
- Mason, J.C., and D.W. Chapman. 1965. Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. Journal of the Fisheries Research Board of Canada 22: 173-190.
- Maynard, D.J., T.A. Flagg, and C.V.W. Mahnken. 1995. A review of seminatural culture strategies for enhancing the postrelease survival of anadromous salmonids. American Fisheries Society Symposium 15: 307-314.
- Maynard, D.J., T.A. Flagg, C.V.W. Mahnken, and S.L. Schroder. 1996a. Natural rearing technologies for increasing postrelease survival of hatchery-reared salmon. Bulletin of the National Research Institute of Aquaculture, Supplement 2: 71-77.
- Maynard, D.J., G.C. McDowell, E.P. Tezak, and T.A. Flagg. 1996b. Effect of diets supplemented with live food on the foraging behavior of cultured fall chinook salmon. Progressive Fish-Culturist 58:187-191.
- McGinnity, P., and eight co-authors. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess a freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES Journal of Marine Science 54:998-1008.
- National Research Council. 1996. Upstream: Salmon and Society in the Pacific Northwest. National Academy Press, Washington, D.C.

- Nickelson, T.E., M.F. Solazzi, and S.L. Johnson. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. *Canadian Journal of Fisheries and Aquatic Sciences* 43: 2443-2449.
- Nielsen, J.L. 1994. Invasive cohorts: impacts of hatchery-reared coho salmon on the trophic, developmental, and genetic ecology of wild stocks, p. 361-385. *In*: Theory and application in fish feeding ecology. K. L. Fresh, D. J. Stouder, and R. J. Feller (eds.). University of South Carolina Press, Columbia, S.C.
- Piry, S., A. Alapetite, J-M. Cornuet, D. Paetkau, L. Baudouin and A. Estoup. 2004. GENECLASS2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**: 536-539.
- Quinn, T.P. 1999. Revisiting the stock concept in Pacific salmon: insights from Alaska and New Zealand. *Northwest Science* 73: 312-324.
- Quinn, T.P. and C.J. Foote. 1994. The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. *Animal Behaviour* 48: 751-761.
- Quinn, T.P., M.D. Adkison and M.B. Ward. 1996. Behavioral tactics of male sockeye salmon (*Oncorhynchus nerka*) under varying operational sex ratios. *Ethology* 102: 304-322.
- Quinn, T.P. and N.P. Peterson. 1996. The influence of habitat complexity and fish size on over-winter survival and growth of individually-marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef Creek, Washington. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 1555-1564.
- Quinn, T.P., J. A. Peterson, V. Gallucci, W.K. Hershberger, and E.L. Brannon. 2002. Artificial selection and environmental change: countervailing factors affecting the

- timing of spawning by coho and chinook salmon. Transactions of the American Fisheries Society 131: 591-598.
- Rannala B, J.L. Mountain. 1997 Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Science 94, 9197-9201.
- Reisenbichler, R.R., and J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada 34:123-128.
- Rhodes, J.S. and T.P. Quinn. 1998. Factors affecting the outcome of territorial contests between hatchery and naturally reared coho salmon parr in the laboratory. Journal of Fish Biology 53: 1220-1230.
- Ricker, W.E. 1972. Hereditary and environmental factors affecting certain salmonid populations. pp. 19-160. In: The Stock Concept in Pacific Salmon, R.C. Simon and P.A. Larkin (eds.). H.R. MacMillan Lectures in Fisheries, Univ. of British Columbia, Vancouver, B.C.
- Riddell, B.E. 1993. Spatial organization of Pacific salmon: what to conserve? p. 23-41. In J. G. Cloud, and G. H. Thorgaard [eds.], Genetic conservation of salmonid fishes. Plenum Press, N.Y.
- Ryman N., P.E. Jorde and L. Laikre. 1995. Supportive breeding and variance effective population size. Conservation Biology 9. 1619-1628.
- Schroder, S.L. 1981. The role of sexual selection in determining the overall mating patterns and mate choice in chum salmon. PhD dissertation, Univ. of Washington, Seattle, Washington.

- Scudder, G.G.E. 1989. The adaptive significance of marginal populations: a general perspective. Canadian Special Publication of Fisheries and Aquatic Sciences 105: 180-185.
- Siitonen, L., and G.A.E. Gall. 1989. Response to selection for early spawn date in rainbow trout, *Salmo gairdneri*. Aquaculture 78: 153-161.
- Steen, R.P. and T.P. Quinn. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. Canadian Journal of Zoology 77: 836-841.
- Stouder, D.J., P.A. Bisson and R.J. Naiman (eds.). 1996. Pacific salmon and their ecosystems: status and future options. Chapman and Hall, New York.
- Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98: 185-207.
- Thorne, R.E., and J.J. Ames. 1987. A note on variability of marine survival of sockeye salmon (*Oncorhynchus nerka*) and effects of flooding on spawning success. Canadian Journal of Fisheries and Aquatic Sciences 44:1791-1795.
- Tipping, J.M., and H.L. Blankenship. 1993. Effect of condition factor at release on smolt-to-adult survival of hatchery sea-run cutthroat trout. Progressive Fish-Culturist 55: 184-186.
- Unwin, M.J., M.T. Kinnison, N.C. Boustead and T.P. Quinn. 2003. Genetic control over survival in Pacific salmon (*Oncorhynchus* spp.): experimental evidence between and within populations of New Zealand chinook salmon (*O. tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 60: 1-11.



- Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 48 (Suppl. 1): 124-133.
- Waples, R.S. 1995. Evolutionarily Significant Units and the conservation of biological diversity under the Endangered Species Act. *American Fisheries Society Symposium* 17: 8-27.
- Ward, B.R., P.A. Slaney, A.R. Facchin, and R.W. Land. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1853-1858.
- Wright, S. 1993. Fishery management of wild Pacific salmon stocks to prevent extinctions. *Fisheries* 18: 3-4.

Table 1. Numbers of wild and hatchery-origin adult steelhead sampled at the Forks Creek Hatchery during the course of the research program.

<b>Brood Year</b>	<b>Hatchery</b>		<b>Wild</b>		<b>Total</b>
	<b>Female</b>	<b>Male</b>	<b>Female</b>	<b>Male</b>	
1996	130	102	11	21	264
1997	134	195	10	17	356
1998	23	28	33	36	120
1999	42	102	4	12	160
2000	65	68	51	37	221
2001	56	69	45	43	213
2002	220	255	22	61	558
2003	250	300	29	45	624
2004	327	466	92	77	962
2005	506	578	22	24	1130
2006	434	592	40	78	1144
2007	414	369	44	37	864
2008	311	311	28	36	686
2009	25	30	-	-	55

Table 2. Numbers of naturally-produced steelhead smolts sampled at Forks Creek during the course of the research program.

<b>Steelhead</b>	
<b>Year</b>	<b>Smolts</b>
1998	365
1999	290
2000	285
2001	193
2002	571
2003	192
2004	380
2005	590
2006	553
2007	350
2008	331

Figure 1. Daily counts of steelhead smolts leaving Forks Creek in 2007. The arrow indicates the median (50%) date.

