Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River

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Executive Summary

Salmon hatchery programs may unintentionally alter demographic characteristics relative to natural origin fish. Differences in demographic characteristics of adult hatchery and naturally produced fish could contribute to differences in reproductive success. Data from Wenatchee spring Chinook salmon was collected at Tumwater Dam, on spawning grounds, and at a hatchery to determine if differences exist. At Tumwater Dam, we found significant differences in run timing, age composition, sex ratios, and size at age between origin and age classes. Data collected during spawning at a hatchery showed that there were no significant differences in fecundity and egg weight between hatchery and naturally produced fish. Comparisons of data collected on carcasses recovered on the spawning grounds revealed no significant difference in egg retention between hatchery and natural origin fish. Preliminary results suggest that the hatchery program is altering certain demographic characteristics of adult spring Chinook salmon.

Hatcheries have been increasingly asked to contribute to conserving natural salmon populations, as well as to continue to produce fish to mitigate for lost harvest opportunities. A key biological uncertainty about the effects of hatchery production on natural populations is the degree to which hatchery produced fish can reproduce in the natural environment. In order to assess the impact (positive or negative) of supplementation of spring Chinook salmon in the Wenatchee River we are using a DNA-based pedigree analysis to (1) directly measure the relative reproductive success of hatchery and natural-origin spring Chinook salmon in the natural environment, (2) determine the degree to which any differences in reproductive success between hatchery and natural Chinook salmon can be explained by measurable biological characteristics such as run timing, morphology, and reproductive behavior, and (3) estimate the relative fitness of fish produced by hatchery-origin adults breeding in the natural environment and that have themselves returned to spawn.

Population genetic and preliminary parentage analyses have been carried out during the second year of monitoring reproductive success of naturally spawning hatchery and natural Spring Chinook salmon in the Wenatchee River. Eleven microsatellites were used to analyze population genetic structure for 2969 adult Spring Chinook entering the Wenatchee River drainage system during 2004. Significant genetic differentiation exists between adult hatchery and wild fish, and between wild adults returning to spawn in the Chiwawa River, Nason Creek, and the White River. Wild and hatchery samples have similar overall levels of genetic diversity, but patterns of diversity within each group differ. The wild samples are characterized by a slight heterozygote deficit (compared to random mating expectations), and generally have low levels of statistical associations among loci. In contrast, the hatchery samples are characterized by a slight heterozygote excess compared to random mating expectations, and have high levels of statistical associations among loci. These patterns probably reflect differences in effective population size or family structure between the two groups.

Preliminary testing of parentage assignment rates of 2004 Wenatchee River Spring Chinook, performed separately for wild and hatchery fish, indicated assignment success

rates (proportion of simulations in which the most likely parent pair was the correct parent pair) were 97.8% and 82.7% for wild and hatchery fish, respectively. When a statistical criterion was used to limit incorrect assignments to no more than 5%, the total assignment rate dropped to 66.1% for the hatchery fish. These results reflect the higher degree of non-independence among loci observed for hatchery compared to wild fish and appear to be a consequence of the low numbers of spawners that produced the 2004 hatchery return. In order to predict the effects of adding additional loci to the analysis, a subset of several hundred of the 2004 adults were genotyped at an additional four loci (for a total of 15 loci). For the 2004 returns (~1800 hatchery origin fish), we predict ~90% of the time the parent pair with the highest likelihood would be the true parents using the 15 locus dataset, compared to 82.7% for the 11 locus set. Increasing the number of microsatellite loci genotyped will therefore be necessary to boost the power of parentage assignment in order to limit incorrect assignments to < 5% for hatchery fish. Even with the 11 locus dataset, we were able to make some inferences about fitness differences between hatchery and wild fish, however. For example, 2 and 3 year old hatchery males made up a large fraction of the male fish sampled at Tumwater Dam, but even after accounting for differences in assignment success, appeared to be very unsuccessful at producing progeny.

Spawning ground surveys in the upper Wenatchee River basin were used to evaluate spawning distribution and redd microhabitat characteristics of hatchery and naturally produced fish. In 2005, the composite population of spring Chinook redds were distributed similarly to that of years past. A total of 818 redds were found upstream of Tumwater Dam, of which the female origin was identified on 335 redds. Based on redd counts, the survival of spring Chinook from Tumwater Dam to the spawning grounds was estimated at 42.4%. After correction for carcass recovery bias, no differences were found in the estimated age composition or the proportion of hatchery and natural origin fish of the estimated spawning population compared to population sampled at Tumwater Dam. Hatchery origin fish tended to spawn in areas near the acclimation site or in relatively low elevation portions of tributaries. No difference in spawn timing of hatchery and natural origin spring Chinook was detected. Microhabitat variables were measured on 137 redds, which included 107 redds and 30 redds constructed by hatchery and natural origin females, respectively. No differences were found in any of the redd characteristics examined.

PIT tag detections were used to determine composition of adult hatchery and natural origin spring Chinook salmon on the spawning grounds. Snorkel surveys were used to determine the origin and abundance of precocious males on redds. The estimated number of precocious males that potentially contributed to natural spawning was 76 (13 hatchery, 50 natural, and 13 unknown origin). The low relative abundance of precocious males observed on the spawning grounds suggests that the majority of the precocious males observed at Tumwater Dam do not successfully migrate to the major spawning areas or die before spawning. The precocity rate for juveniles released from Chiwawa Ponds, that migrated downstream and survived to migrate upstream of Tumwater Dam was calculated as 0.13% in 2005. Assortative pairing analysis was limited in 2005 because hatchery and wild fish could not be distinguished because hatchery fish were not

externally marked. No difference was detected in the mean fork length of males paired with either hatchery or natural origin females.

All data and analyses in this report should be considered preliminary until published in a scientific journal.

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General Introduction

This project will quantitatively evaluate the relative reproductive success of naturally spawning hatchery and natural origin spring Chinook salmon *Oncorhynchus tshawytscha* in the Wenatchee River. Hatcheries are one of the main tools that have been used to mitigate for salmon losses caused by the construction and operation of the Columbia River hydropower system. In addition to harvest augmentation, hatcheries have recently been used in attempts to protect stocks from extinction and to enhance natural production (supplementation). Surprisingly, little is known about how much the investment in hatcheries benefits or harms natural production. Recent technological advances in genetics have enabled the empirical monitoring of the reproductive success of hatchery and natural spring Chinook salmon using a DNA-based pedigree approach. Specifically, this project will (1) directly measure the relative reproductive success of hatchery and natural-origin Chinook salmon in both natural and hatchery settings, (2) determine the degree to which any differences in reproductive success between hatchery and natural Chinook salmon can be explained by measurable biological characteristics such as run timing or size, and (3) estimate the relative fitness of hatchery-lineage Chinook salmon after they have experienced an entire generation in the natural environment. This report contains results from the second year of work on this project. The results from the previous year of work were addressed in Murdoch et al. 2005. The project is intended to last until 2012 in order to evaluate two entire spring Chinook salmon generations.

This project is collaboration between NOAA-Fisheries (Northwest Fisheries Science Center) and the Washington Department of Fish and Wildlife. Results and progress are reported on jointly. This annual report is a joint authored report that has been split into four chapters in order to address important topics of the project. This project is an extension of the Chiwawa spring Chinook salmon supplementation program in the Wenatchee River operated by WDFW and funded by Chelan County Public utility District (CCPUD).

Description of Project Area

Located in north central Washington, the Wenatchee River subbasin drains a portion of the eastern slope on the Cascade Mountains. The watershed is approximately 3,550 km² with 383 rkm of major creeks and rivers (Andonaegui 2001). Originating from Lake Wenatchee, the Wenatchee River flows 86.9 kilometers to its confluence with the Columbia River (rkm 754) near the town of Wenatchee (Figure 1). High mountainous regions of the Cascade crest are encompassed in the watershed, with numerous tributaries draining subalpine regions included in the Alpine Lakes and Glacier Peak Wilderness areas (Andonaegui 2001).

Historical river discharge monitored by the United States Geological Survey (USGS gauging station number 12462500 at river km 9.4) reported a 41-year mean monthly summer low discharge of 23 m^3/s and a mean monthly spring peak discharge of 257 m^3/s. Of the total river discharge, the Little Wenatchee River (15%) and White River (25%) are

the only tributaries that feed Lake Wenatchee (Mullan et al. 1992). Other primary tributaries of the Wenatchee River below the lake are Nason Creek (18%), Chiwawa River (15%) and Icicle Creek (20%; Mullan et al. 1992).

The Wenatchee River basin supports self-sustaining populations of spring and summer Chinook, steelhead *O. mykiss*, and sockeye salmon *O. nerka*. Spring Chinook spawning occurs primarily in the upper Wenatchee River basin (upstream of rkm 57.3), although limited spawning does occur annually in lower elevation tributaries (i.e., Icicle and Peshastin creeks). Spawning subpopulations have been documented in all major tributaries in the upper Wenatchee River basin including the upper Wenatchee, Chiwawa, Nason, White and Little Wenatchee (Mosey and Murphy 2002). Andonaegui (2001) reported natural fish passage barriers, in the form of waterfalls, limit access in the Chiwawa River (53.3 rkm), Nason Creek (27.0 rkm), White River (23.0 rkm), and the Little Wenatchee River (12.6 rkm). Despite these barriers, spawning typically ends before these barriers. Increases in stream gradient and substrate size may limit spawning below barriers (Andonaegui 2001).

History of Artificial Propagation

Over harvest in the lower Columbia River and destruction of spawning habitat had significantly reduced Chinook populations in the Wenatchee River Basin by the 1930's (Craig and Suomeia 1941). As part of the Grand Coulee Fish Maintenance Project (GCFMP) during 1939 – 1943, salmon and steelhead were trapped at Rock Island Dam and redistributed into the Wenatchee, Entiat and Methow rivers (Chapman et al. 1995). As a result, a mixed gene pool of fish originating from the Wenatchee, Entiat, Methow and Columbia River tributaries located upstream of the Grand Coulee Hydroelectric Project was created (Chapman et al. 1995). However, White River spring Chinook are genetically distinct from spring Chinook populations in the Chiwawa River and Nason Creek (Utter et al. 1995; Ford et al. 2001), and a low, but statistically significant level of genetic differentiation between Nason Creek and Chiwawa River populations was observed by Utter et al. (1995). Artificial propagation of spring Chinook in the Wenatchee Basin began in 1941. Leavenworth National Fish Hatchery (LNFH) released juvenile hatchery fish derived from broodstock collected at Rock Island Dam until 1944. Since 1948, hatchery spring Chinook have been released by the LNFH into Icicle Creek. Broodstock was collected in the Icicle River or transferred from other National Fish Hatcheries located in the lower Columbia River FH (Chapman et al. 1995). Currently, the spring Chinook program at LNFH released 1.6 million yearling smolts into the Icicle River, the purpose of which is harvest augmentation as part of the original mitigation for Grand Coulee Dam.

More recently, a supplementation program was initiated in 1989 on the Chiwawa River as part of the Rock Island Migration Agreement between Chelan County Public Utility District and the fishery management parties (RISPA 1989). The program is designed to mitigate for smolt mortality as a result of the operation of Rock Island Hydroelectric Project and has a production level goal of 672,000 yearling smolts. Currently, the

program is operated under the Rock Island Habitat Conservation Plan and has established a goal for the program to increase the abundance of the naturally spawning population while maintaining the genetic integrity and long-term fitness of the stock (CCPUD 2002). However, low escapement to the Chiwawa River has limited smolt production and the mean number of smolts released since 1991 has been 101,843 (1989-2002 brood).

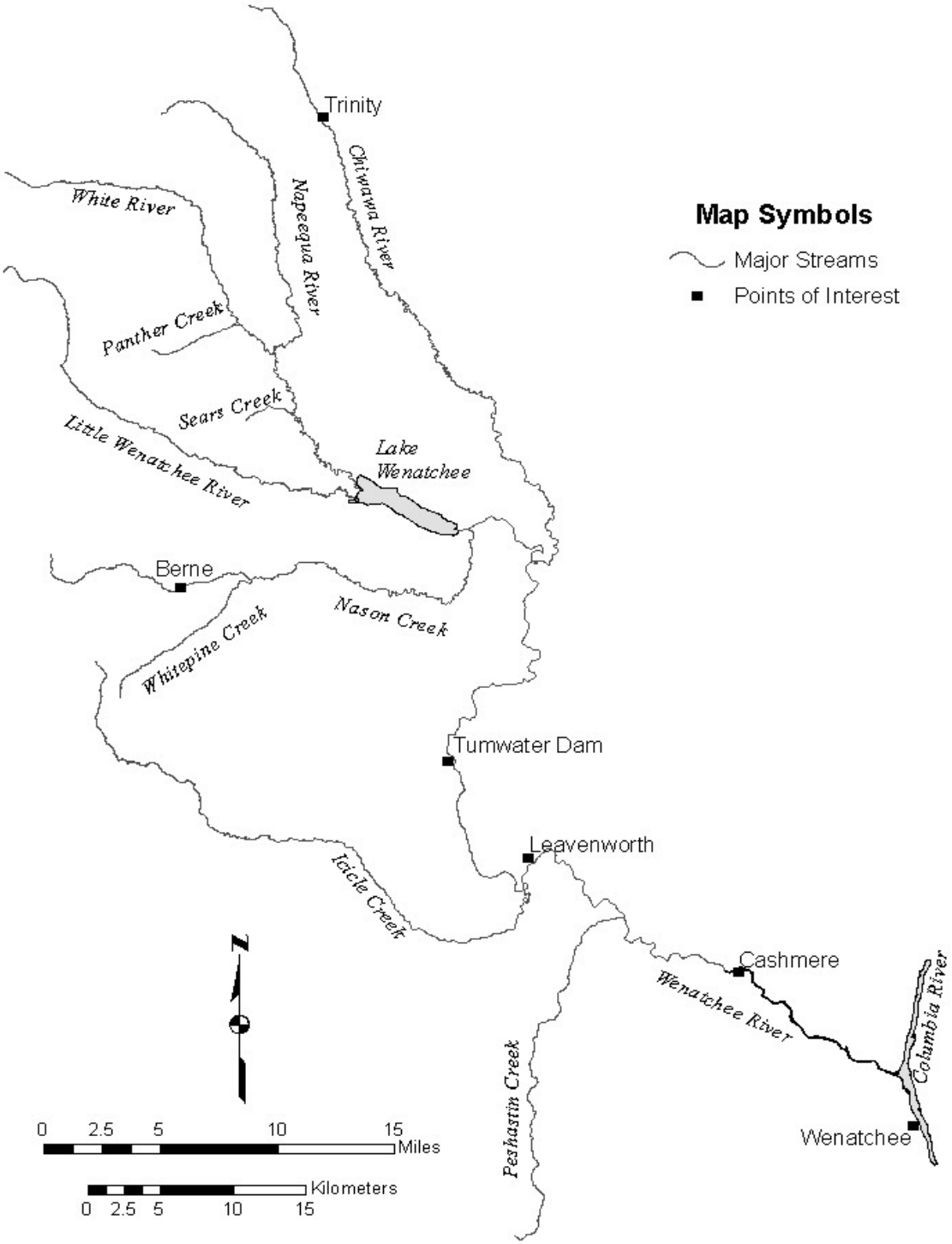

Figure 1. Map of Wenatchee River Basin and spring Chinook spawning tributaries.

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Chapter 1

A comparison of demographic variables of adult hatchery and natural origin spring Chinook in the Wenatchee River Basin

Abstract

Salmon hatchery programs may unintentionally alter demographic characteristics relative to natural origin fish. This is important because differences in demographic characteristics of adult hatchery and naturally produced fish could contribute to differences in reproductive success. Data from Wenatchee spring Chinook salmon were collected at Tumwater Dam, on spawning grounds, and at a hatchery to determine if differences exist. At Tumwater Dam, significant differences were found in the run timing, age composition, sex ratios, and size at age between origin and age classes of hatchery and natural origin spring Chinook. Data collected during spawning at a hatchery showed that there were no significant differences in fecundity and egg weight between hatchery and naturally produced fish. Comparisons of data collected on carcasses recovered on the spawning grounds revealed no significant difference in egg retention between hatchery and natural origin fish. Preliminary results suggest the hatchery program is altering certain demographic characteristics of the spring Chinook salmon population. It is unclear whether these differences are caused by genetic or environmental factors.

Introduction

Hatcheries can change the demographics of salmonid populations (Carmichael and Messmer 1995, Olson et al. 2004, Knudsen et al. in press). These changes may be caused by environmental factors associated with artificial culture or from genetic changes such as loss of within population genetic variation or domestication in the hatchery environment (Busack and Currens 1995). Quantifying differences in phenotypic traits of hatchery and natural origin salmonids can provide explanations for differences that may be observed through genetic analysis of relative reproductive success (Kostow et al. 2003; McLean et al. 2003). Resolving differences, or lack thereof, in phenotypic traits provide a better understanding of the potential causal factors that lead to differences in reproductive success.

This chapter examines some of the demographic variables that influence reproductive success. Specific objectives include examining differences in run timing, sex ratios, length, weight, fecundity, and egg weight. These variables may affect not only the survival of the spawners, but also the progeny. In addition, the proportion of eggs retained in post-spawned females was examined to assess any differences in egg deposition of hatchery and natural origin female spring Chinook.

Methods and Materials

Adult Trapping

Tumwater Dam is located on the Wenatchee River in Tumwater Canyon (rkm 43.7), approximately 30 km below historical spring Chinook spawning habitat (Figure 1). A fish ladder and trapping facility are located on the left bank of the dam. The trapping facility is comprised of four main parts. The first of these is the primary collection chamber (6.7 m × 2.3 m × 2.0 m; 30.8 m^3), which the fish enter after being diverted from the adult fish ladder. Two gravity fed upwells supply the chamber with a constant source of river water. Secondly, at the upstream end of the collection chamber fish must actively swim through a denile. At which time fish can be either diverted back to the river upstream of the dam, into a secondary collection chamber (3.4 m × 1.5 m × 3.4 m; 17.3 m^3), or if fish are to be sampled immediately, into a tank (1.36 m^3) fed by a 5 hp pump. The secondary collection chamber is also fed river water through gravity fed upwells. Located at the bottom of the chamber is a large hopper (1.54 m^3) that is used to hoist fish from the collection chamber and also serves as an anesthetic tank. The final portion of the trapping facility is the recovery tank (1.72 m^3) and return flume, which is supplied with river water from another 5 hp pump. Revived fish are released upstream of the dam.

The fish trap is capable of operating either passively or actively. During periods when fish passage is low (< 20 fish/d) the trap is operated passively and the trap is checked periodically throughout each day as needed. When fish passage is high (> 20 fish/d) the trap is operated actively during the hours of daylight and passively during the night when fish are less likely to migrate. During active trapping, crews sort and divert spring Chinook into the secondary collection chamber using a series of pneumatic gates. Non-target species (i.e., summer Chinook, sockeye and steelhead), if not collected for hatchery broodstock, are immediately diverted back into the river upstream of the dam. The denile is shut down when between 10 and 15 adult spring Chinook have been diverted into the secondary collection chamber. At which time the water level in the secondary collection chamber is lowered and fish are crowded into the hopper. The hopper is hoisted to the work platform and a light concentration of MS-222 (14 ppm) is added before any fish are handled. Spring Chinook are transferred from the hopper into a sampling tank (0.38 m^3) containing a higher concentration of MS-222 (88 ppm). After sampling, fish are then placed either into a recovery tank or tanker truck if being collected as part of the hatchery broodstock. Fish placed in the recovery tank are allowed to fully recover before being released upstream.

Broodstock for the Chiwawa spring Chinook program were collected at Tumwater Dam (only hatchery fish with CWT) or a weir located on the Chiwawa River (both hatchery and natural origin fish) at river kilometer 1.5. The Chiwawa weir was operated 4 days per week and fish were collected weekly in proportion to the run. The broodstock goal for the Chiwawa program was 379 fish. All broodstock were transported to Eastbank FH and held on pathogen free well water until they were spawned.

Biological Sampling

Biological data were collected from all spring Chinook regardless of future disposition, hatchery broodstock or natural spawning. Each fish was identified to gender and scanned for passive integrated transponder (PIT) tags and coded wire tags (CWT). Fork and post orbital to hypural plate (POH) length were measured to the nearest cm and weight to the nearest 0.01 kg. Scale and genetic tissue samples (0.5 cm^2 caudal fin clip) were collected from every spring Chinook. All genetic samples were sent to the NOAA Fisheries, Northwest Fisheries Science Center for analysis (See Chapter 2). The presence or absence of the adipose fin was also recorded. Lastly, a PIT tag was inserted into the dorsal sinus cavity on the left side of the body. In some cases a fish that had been previously sampled (i.e, fallback) was encountered. These fish were confirmed by the presence of caudal fin clips. PIT tag numbers of all fallbacks were recorded and fish were released upstream. All PIT tag data were uploaded to the PTAGIS database on a weekly basis.

Similar biological data were collected on hatchery and naturally produced fish used for hatchery brood stock (i.e., sex, spawn date, fork and POH length, and scales). The fecundity of each female was determined by using an optical egg counter. Before eggs from individual females are counted, the optical counter was calibrated with a known number of eggs. A sample of 100 eggs from each female was also weighed (to the nearest 0.1 g). The mean egg weight of each female was calculated by dividing the sample weight by the number of eggs.

Data Analysis

Non-parametric tests were primarily used to analyze data because most variables were not normally distributed, even after various transformations were applied ($P > 0.05$, Shapiro-Wilk Normality Test). Run timing of hatchery and natural origin fish by age class were compared using a Kruskal-Wallis analysis of variance (KW). Age composition and sex ratios of hatchery and naturally produced adult spring Chinook were compared with a Chi-square test using a Yates (1934) correction for continuity to prevent inflating the probability of committing a Type I error (Zar 1999).

Body length (POH) and weight of hatchery and wild fish by age class and sex were compared using a KW test. Due to small sample sizes of age 3 and 5 fish, only age-4 fish, the dominant age class sampled at Tumwater Dam in 2004 and 2005, were used in the analysis of length (POH) and weight at age. Only natural origin fish collected as broodstock from the Chiwawa weir or sampled during spawning ground surveys in the Chiwawa River were included in the analysis. Comparisons of hatchery fish were limited to only natural origin Chiwawa River spring Chinook because hatchery fish are of Chiwawa River origin. Fecundity and egg weight of hatchery and naturally produced females of the same age were compared using a KW test. A linear regression was performed using fish size (FL) and fecundity for both, hatchery and wild broodstock. The slopes of the regression models were compared using homogeneity of slopes test and

analysis of covariance (ANCOVA). Using the regression models, the estimated fecundity for all females examined for egg retention was calculated and used to determine the proportion of eggs retained. The proportion of eggs retained in hatchery and wild carcasses found on the spawning grounds was compared using a KW test.

Results and Discussion

Trap Operation

The trap was operated between 1 May and 11 August 2005. The trap operated passively from 1 May to 23 May due to low fish passage. During this time period, personnel checked the trap and sampled fish several times daily. Active trapping occurred during the day between 24 May and 11 August, and was passively operated only during night when fish passage was low. Previous trap modifications performed as expected and no mechanical or technical failures occurred throughout the entire time period. No mortality occurred during the trapping period.

A total of 3,827 spring Chinook adults and jacks and 297 precocious males (age-2) were counted at Tumwater Dam, including 3 spring Chinook adults that were counted on videotapes after trapping had ended (Figure 1). Origins of fish were determined by CWT or scales collected at Tumwater Dam, carcasses from the spawning grounds, or broodstock spawned at the hatchery. Of these fish, genetic tissue samples were collected from 3,219 hatchery adults, 570 natural adults, 34 unknown origin, and 295 hatchery precocious males (99.9% of all spring Chinook at Tumwater Dam). Naturally produced spring Chinook were observed (captured or video) at Tumwater Dam between 14 May and 29 August (108 days). Hatchery spring Chinook were captured at Tumwater Dam between 17 May and 09 August (85 days). In addition, hatchery Chinook (94.6%; N=281 adipose clipped and 5.4%; N=16 adipose present) were observed from 30 May to 08 August (Figure 1, Appendix A). No naturally produced precocious males were observed during trapping.

Run timing

Differences in run timing were detected between age classes ($P < 0.001$). Older aged spring Chinook migrate earlier than younger spring Chinook (Table 1). However, within each age class no differences were detected in the passage timing at Tumwater Dam between hatchery and natural origin age-3 ($P = 0.63$) or age-5 ($P = 0.78$) spring Chinook (Figure 2). However, natural origin age-4 fish had significantly later run timing than age-4 hatchery origin fish (Table 2, $P < 0.001$). A comparison of run timing by origin and sex of age-4 spring Chinook detected differences for both male and female spring Chinook ($P < 0.001$). Run timing differences in different ages observed in the Wenatchee Basin are similar to those found in the Yakima Basin. Knudsen et al. (In press) reported that adults had a 19-20 day earlier run timing than jacks. Furthermore,

the run timing of natural origin adult Yakima spring Chinook was not significantly different than adult hatchery origin fish.

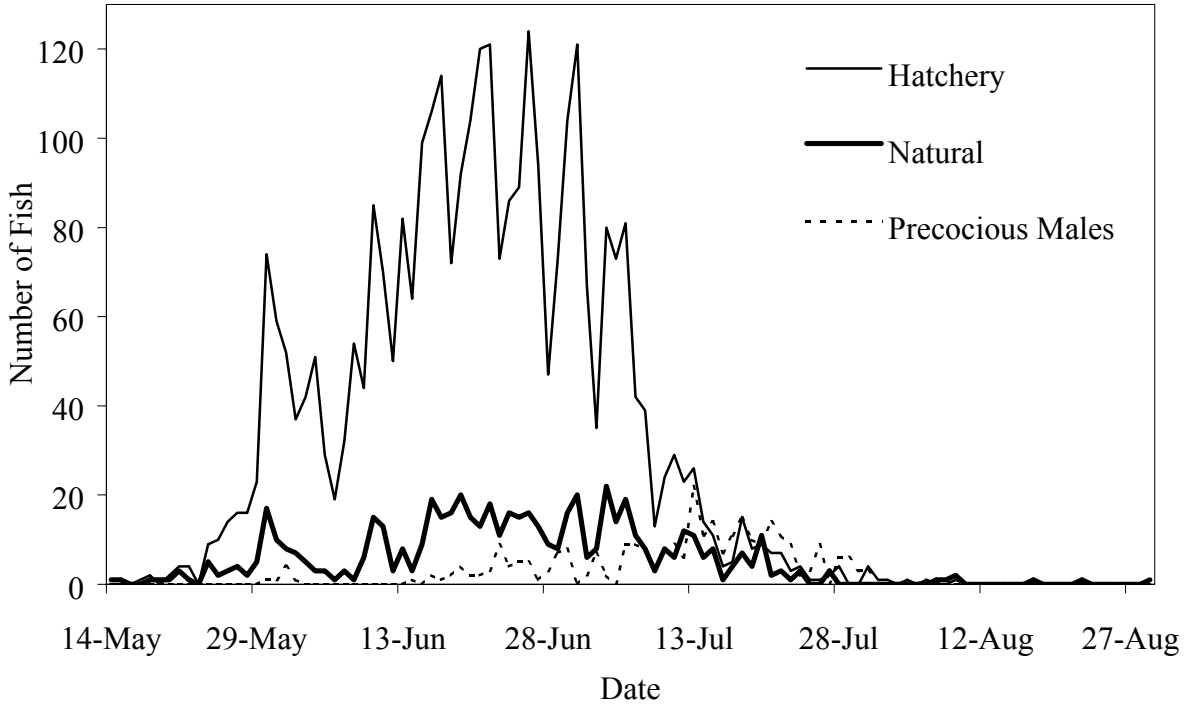

Figure 1. Run Timing of adult hatchery and naturally produced spring Chinook and Chinook sampled at Tumwater Dam in 2005.

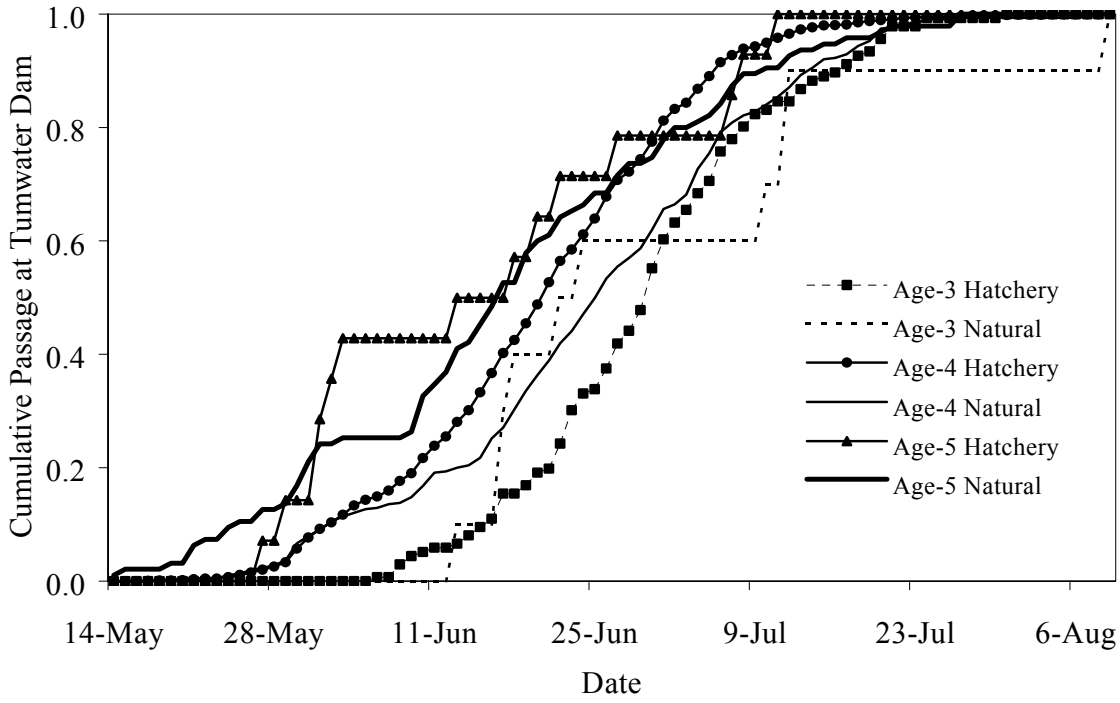

Figure 2. Cumulative passage timing of spring Chinook at Tumwater Dam in 2005.

Table 1. Cumulative passage dates of Wenatchee River spring Chinook sampled at Tumwater Dam in 2004 and 2005.

Origin/Age	Cumulative Run Timing		
	10%	50%	90%
		2004	
Hatchery (All[1])	10-Jun	25-Jun	08-Jul
Age-2	26-Jun	13-Jul	21-Jul
Age-3	13-Jun	27-Jun	09-Jul
Age-4	05-Jun	24-Jun	07-Jul
Age-5	08-Jun	12-Jun	04-Jul
Natural (All)	04-Jun	20-Jun	06-Jul
Age-3	12-Jun	27-Jun	14-Jul
Age-4	03-Jun	20-Jun	05-Jul
Age-5	05-Jun	17-Jun	12-Jul
		2005	
Hatchery (All[1])	02-Jun	21-Jun	06-Jul
Age-2	23-Jun	13-Jul	26-Jul
Age-3	16-Jun	30-Jun	17-Jul
Age-4	02-Jun	21-Jun	06-Jul
Age-5	29-May	13-Jun	08-Jul
Natural (All)	01-Jun	24-Jun	14-Jul
Age-3	13-Jun	22-Jun	12-Jul
Age-4	02-Jun	26-Jun	14-Jul
Age-5	25-May	17-Jun	10-Jul

[1] For comparison age-2 hatchery fish were not included

Table 2. Summary statistics of run timing for hatchery and natural origin spring Chinook at Tumwater Dam in 2004 and 2005 (H = hatchery; N = natural).

Age/Origin	*N*	Mean	Median	Minimum	Maximum	SD (days)
			2004			
2 H	635	Jul 11	Jul 13	Jun 10	Aug 03	9
3 H	826	Jun 26	Jun 27	Jun 04	Jul 26	10
3 N	31	Jun 27	Jun 27	Jun 06	Jul 21	12
4 H	453	Jun 22	Jun 24	May 20	Aug 06	13
4 N	845	Jun 19	Jun 20	May 18	Jul 27	13
5 H	6	Jun 16	Jun 17	Jun 08	Jul 04	10
5 N	12	Jun 19	Jun 17	Jun 03	Jul 13	13
			2005			
2 H	297	Jul 11	Jul 13	May 30	Aug 8	13
3 H	136	Jun 29	Jun 30	Jun 06	Jul 31	11
3 N	10	Jun 30	Jun 23	Jun 13	Jul 21	18
4 H	2,992	Jun 20	Jun 21	May 17	Jul 21	13
4 N	465	Jun 25	Jun 26	May 18	Jul 21	15
5 H	14	Jun 15	Jun 15	May 27	Jul 12	16
5 N	95	Jun 17	Jun 17	May 14	Jul 28	17

Age Composition

Ages were determined through scale samples for 3,142 and 570 hatchery and natural spring Chinook, respectively (Table 3). All 297 hatchery precocious males were scale sampled and determined to be age-2 fish, but were not included in the analysis because the number of natural origin age-2 could not be determined. A significant difference was found in the age composition of hatchery and natural origin fish ($\chi^2 = 3142$, df = 2, $P < 0.001$). Differences in age composition between hatchery and natural origin fish was attributed to the variation in the number of hatchery fish released (range 47,104 – 377,544).

Because of these differences, a comparison of age composition by brood year would demonstrate any real differences in age composition that may be attributed to the hatchery program. Age and sex of the 2000 brood Wenatchee spring Chinook was determined at Tumwater Dam between 2004 and 2005 as part of this study. The number of age-3 spring Chinook in 2003 was determined from videotapes and trapping records from Tumwater Dam (WDFW, unpublished data). Significant differences were detected between hatchery and natural origin fish ($\chi^2 = 26.5$, df = 2, $P < 0.001$). A greater proportion of natural origin fish returned at age-5 than hatchery fish (Table 4). Mean

age-at-maturation was also earlier in the hatchery origin spring chinook salmon (shifting to age 3) than natural origin fish in the Yakima River (Knudsen et al. in press).

Table 3. Age composition of Wenatchee River spring Chinook sampled at Tumwater Dam in 2004 and 2005 (Age-2 fish not included).

Origin	Total Age			*N*
	3	4	5	
		2004		
Hatchery	64.1%	35.4%	0.5%	1,273
Natural	3.5%	95.2%	1.3%	888
All	39.2%	60.0%	0.8%	2,161
		2005		
Hatchery	4.33%	95.22%	0.45%	3,142
Natural	1.75%	81.58%	16.67%	570
All	3.93%	93.13%	2.94%	3,712

Table 4. Age composition of the 2000 brood Wenatchee River spring Chinook sampled at Tumwater Dam in between 2003 and 2005.

Origin	Total Age			*N*
	3	4	5	
Hatchery	7.09%	90.16%	2.76%	508
Natural	7.11%	83.50%	9.39%	1,012
All	7.11%	85.72%	7.17%	1,520

Sex Ratio

Sex determination at Tumwater Dam was based on external morphological characteristics early in the year without secondary sexual characteristics and may not be accurate. A comparison of the sex determined at Tumwater Dam to those fish subsequently recovered on the spawning grounds and during hatchery spawning found that sex determination was correct 86.1 % for female and 80.0% for males. After correction, the male to female ratio of the natural and hatchery fish was 1.0 to 1.0 and 0.78 to 1.0, respectively (Table 5). The overall male to female ratio for the spawning population upstream of Tumwater Dam (broodstock not included) was 0.8 to 1.0. In the future, an ultrasound unit may be used to visualize gonad morphology. This will eliminate error associated with determining gender based solely on external morphological characteristics.

Age-4 hatchery fish had significantly lower proportion of males than age-4 natural origin fish ($\chi^2 = 50.52$, $P < 0.001$). No difference was detected in the sex ratio of age-5 hatchery and natural origin fish ($\chi^2 = 0.14$, $P = 0.71$). A lower proportion of older aged

males suggest that hatchery males may mature at an earlier age than natural origin males. For example, the number of hatchery age-3 males sampled at Tumwater Dam was much greater than natural origin fish. As stated previously, because the number of hatchery fish released was not constant, analysis of sex ratios should be conducted when all age classes from each brood year has been sampled at Tumwater Dam.

Comparisons between gender and origin of the 2000 brood Wenatchee spring Chinook were based on the number of male and female spring Chinook, corrected based on carcass recovery data, sampled at Tumwater Dam between 2003 and 2005. Based on carcass recovery data in 2003, all age-3 fish were determined to be males (WDFW, unpublished data). A significantly greater proportion of the 2000 brood hatchery fish return as females compared to natural origin fish (Table 6; $\chi^2 = 38.94$, df = 1, $P<0.001$). These results are also consistent with differences detected within the run year (e.g., 2005). The overall male to female ratio of hatchery and natural origin fish was 0.42:1 and 0.95:1, respectively. A higher proportion of hatchery females may be attributed to differences in the proportion of males that mature as age-2 (i.e., precocious males). If a greater proportion of hatchery males were sexually mature at age-2, a relatively lower proportion of the returning hatchery adults would be males compared to natural origin fish. These results support the lack of natural origin age-2 fish observed at Tumwater Dam and on the spawning grounds (Chapter 4) in both 2004 and 2005. Sex composition in natural and hatchery origin spring Chinook salmon differed in 3 of 4 brood years in the Yakima Basin (Knudsen et al. in press). This difference was largely attributed to an increase in age 3 jacks, which increased from 38 to 49 percent over time.

Table 5. The estimated number of male and female spring Chinook counted at Tumwater Dam and the corrected number based on carcass recoveries in 2004 and 2005.

Age	Origin	Sex	Tumwater Dam	Corrected Number
		2004		
3	Hatchery	Male	821	823
		Female	5	3
	Natural	Male	31	31
		Female	0	0
	Unknown	Male	1	1
4	Hatchery	Male	115	107
		Female	343	351
	Natural	Male	438	374
		Female	407	471
	Unknown	Male	1	1
	Unknown	Female	0	0
5	Hatchery	Male	2	2
		Female	4	4
	Natural	Male	5	5
		Female	7	7
	Unknown	Male	0	0
	Unknown	Female	0	0
Unknown	Hatchery	Male	27	22
		Female	11	16
Unknown	Unknown	Male	17	14
		Female	13	16
		2005		
3	Hatchery	Male	136	136
		Female	-	-
	Natural	Male	10	10
		Female	-	-
	Unknown	Male	1	1
4	Hatchery	Male	1,243	1,237
		Female	1,749	1,755
	Natural	Male	257	235
		Female	208	230
	Unknown	Female	1	1
5	Hatchery	Male	6	6
		Female	8	8
	Natural	Male	51	46
		Female	44	49
Unknown	Hatchery	Male	29	30
		Female	49	48
Unknown	Unknown	Male	15	14
		Female	17	18

Table 6. Summary of the 2000 brood hatchery and natural origin Spring Chinook by sex and age observed at Tumwater Dam between 2003 and 2005.

Age	Male		Female	
	Hatchery	Natural	Hatchery	Natural
3	7.1%	7.1%	0.0%	0.0%
4	21.0%	37.0%	69.1%	46.5%
5	1.2%	4.6%	1.6%	4.8%

Size-at-Age

In 2005, no difference in POH was detected between age-4 natural origin male and female Chiwawa spring Chinook ($P = 1.0$). However, age-4 hatchery male spring Chinook were significantly larger than hatchery female and both natural male and female spring Chinook (Table 7, $P < 0.05$). A comparison of age-3 spring Chinook in 2004 (i.e., same brood year 2001) also found differences consistent with age-4 fish (two sample t-test, $t = -2.09$, $P < 0.04$). When compared to spring Chinook sampled in 2004, the only difference detected between similar groups (sex and origin) was hatchery males ($P = 0.05$; Figure 3). Age-4 hatchery males were significantly larger in 2005 than 2004. Similarly, the only within or between year differences in weight between hatchery and natural origin Chiwawa spring Chinook were natural origin males in 2005 ($P < 0.001$). Mean lengths and body weights of 3, 4, and 5, year old hatchery spring Chinook salmon in the Yakima Basin were less than those of natural origin fish of the same age (Knudsen et al. in press).

Table 7. Mean fork length (SD) and weight (SD) at age for Wenatchee River spring Chinook sampled at Tumwater Dam in 2004 and 2005.

Origin/Year	Sex	*N*	Age-3	*N*	Age-4	*N*	Age-5
			Fork length (cm)				
Hatchery 2004	Male	821	52.9 (5.9)	115	80.2 (6.6)	2	98.0 (1.4)
	Female	5	62.2 (4.9)	343	79.6 (4.5)	4	82.8 (8.4)
	All	826	53.0 (6.0)	458	79.7 (5.1)	6	87.8 (10.3)
Natural 2004	Male	31	50.7 (5.4)	438	78.5 (6.5)	5	91.6 (4.8)
	Female	0		407	77.9 (4.0)	7	91.3 (5.7)
	All	31	50.7 (5.4)	845	78.3 (5.5)	12	91.4 (5.1)
Hatchery 2005	Male	136	54.8 (4.5)	1,188	82.5 (5.9)	6	90.2 (6.8)
	Female	0		1,804	79.3 (4.0)	8	88.1 (6.4)
	All	136	54.8 (4.5)	2,992	80.6 (5.1)	14	89.0 (6.4)
Natural 2005	Male	10	52.0 (3.7)	231	78.4 (6.6)	44	96.2 (6.5)
	Female	0		234	79.3 (4.8)	51	91.7 (4.1)
	All	10	52.0 (3.7)	465	78.8 (5.8)	95	93.8 (5.7)
			Weight (g)				
Hatchery 2004	Male	821	1.76 (0.66)	115	5.49 (1.40)	2	9.10 (0.42)
	Female	5	2.85 (0.75)	343	5.51 (0.98)	4	6.15 (1.84)
	All	826	1.77 (0.66)	458	5.50 (1.10)	6	7.13 (2.10)
Natural 2004	Male	31	1.52 (0.56)	438	5.33 (1.27)	5	7.86 (1.01)
	Female	0		407	5.29 (0.81)	7	8.22 (1.77)
	All	31	1.52 (0.56)	845	5.31 (1.07)	12	8.08 (1.46)
Hatchery 2005	Male	136	1.84 (0.46)	1,188	6.08 (1.31)	6	8.10 (2.20)
	Female	0		1,804	5.42 (0.87)	8	7.23 (1.41)
	All	136	1.84 (0.46)	2,992	5.68 (1.11)	14	7.60 (1.77)
Natural 2005	Male	10	1.60 (0.29)	231	5.21 (1.28)	44	9.46 (2.21)
	Female	0		234	5.38 (0.96)	51	8.13 (1.31)
	All	10	1.60 (0.29)	465	5.30 (1.13)	95	8.75 (1.90)

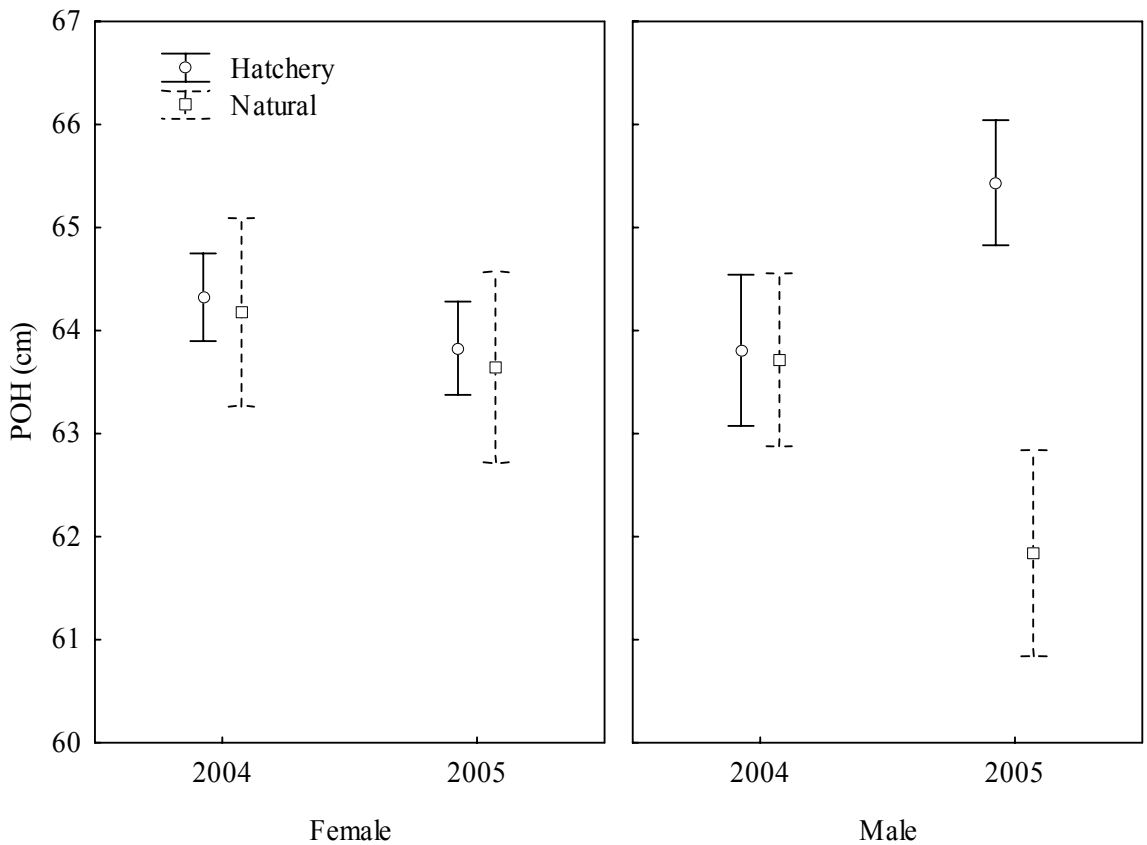

Figure 3. Mean post-orbital to hypural plate length of age-4 Chiwawa spring Chinook sampled on the spawning grounds and as broodstock in 2004 and 2005. Vertical bars denote 95% confidence intervals.

Fecundity and Egg Weight

A total of 283 spring Chinook were collected and held at Eastbank Fish Hatchery for broodstock in 2005. Age and origin was determined through scale analysis and CWT decoding for 183 and 99 hatchery and wild fish, respectively (Table 8). Scales from one fish were unreadable. Fecundity was determined for 89 hatchery and 37 naturally produced age-4 and age-5 female spring Chinook (Table 9). The mean (standard deviation, SD) fecundity of the hatchery and naturally produced females was 4,211 (721) and 4,279 (1,033), respectively. Mean egg weight (SD) of the hatchery fish was 0.23 (0.03) g and 0.23 (0.04) g for the naturally produced fish. No difference was found between the mean fecundity ($P = 0.07$) and egg weight ($P = 0.33$) of hatchery and naturally produced age-4 fish in 2005.

No difference in the slope of the fecundity regression line was detected between years ($P = 0.82$), origin ($P = 0.71$), or the interaction term year x origin ($P = 0.20$). Subsequently, results of the ANCOVA using the same data to examine differences in the intercept of the regression lines detected no difference in origin ($P = 0.57$), but differences were detected between years ($P < 0.001$). These results suggest that age-4 hatchery and natural

Chiwawa spring Chinook have similar fecundity to length relationships within a given year, but both hatchery and natural fish may differ similarly between years (Figure 4). Comparisons between years (2004 and 2005) found significant differences in both fecundity ($P < 0.001$) and egg weight ($P < 0.001$), but no difference was detected among hatchery and natural origin spring Chinook within the same year.

Table 8. Age composition of Chiwawa spring Chinook hatchery broodstock at Eastbank Fish Hatchery in 2004 and 2005.

Origin	Total Age			*N*
	3	4	5	
		2004		
Hatchery	37.3%	62.7%	0.0%	193
Natural	4.3%	92.5%	3.2%	93
All	26.6%	72.4%	1.0%	286
		2005		
Hatchery	4.4%	94.5%	1.1%	183
Natural	1.0%	84.9%	14.1%	99
All	3.2%	91.1%	5.7%	282

Table 9. Summary statistics for Chiwawa spring Chinook broodstock fecundity and egg weights in 2004 and 2005.

Origin	Age	Fecundity			Egg Weight		
		Mean	SD	*N*	Mean	SD	*N*
				2004			
Hatchery	4	4,676	901	83	0.216	0.029	89
Natural	4	4,833	747	37	0.211	0.029	37
Natural	5	4,203	-	1	0.242	-	1
				2005			
Hatchery	4	4,211	721	89	0.228	0.034	91
Natural	4	3,961	637	30	0.225	0.040	31
Natural	5	5,642	1,327	7	0.260	0.039	6

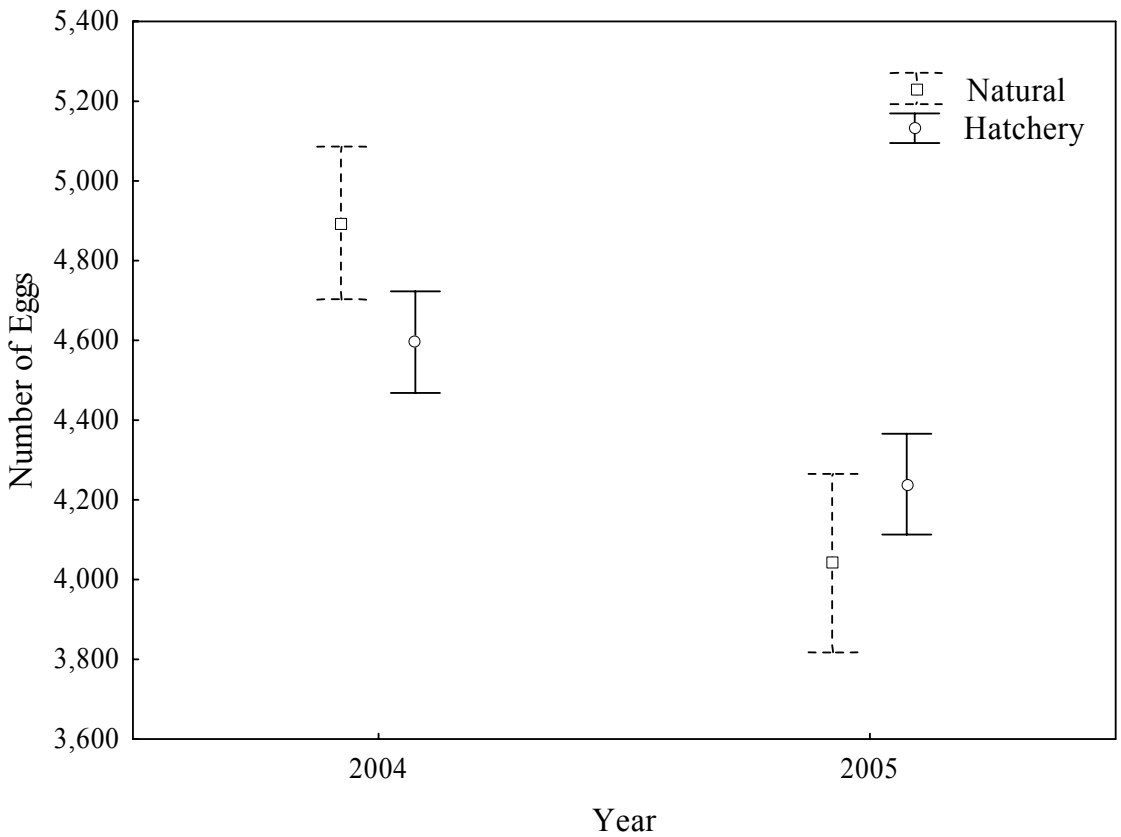

Figure 4. Mean fecundity age-4 hatchery and natural origin Chiwawa spring Chinook. Vertical bars denote 95% confidence intervals.

Egg Retention

A total of 378 hatchery and 79 naturally produced fish were examined to determine the number of eggs retained in the body cavity after spawning (Table 10). The proportion of eggs retained was estimated using fecundity linear regression models for hatchery and natural origin fish derived from hatchery broodstock (Table 11). The estimated mean (SD) percentage of eggs retained for hatchery and naturally produced fish was 0.48 (1.9) and 0.34 (1.2), respectively. A Kruskal-Wallis ANOVA was used to test for differences between origin and spawning location because assumptions of normality and equal variances could not be met. No difference was detected in the proportion of eggs retained between hatchery and naturally produced fish ($P = 0.22$). Significant differences were detected between the Chiwawa River and both the Wenatchee River and Nason Creek. The Wenatchee River was also significantly different from the White River ($P < 0.001$). Further analysis by location and origin found that only the hatchery fish in the Wenatchee River had a significantly higher egg retention rate than both hatchery and wild fish in the Chiwawa River ($P < 0.001$). Overall egg retention rates did not differ between 2004 and 2005 ($P = 0.16$). When between year comparisons were conducted by group (i.e., year, stream, and origin), no significant differences were detected other than those differences found in 2005.

Table 10. Number of female spring Chinook examined and the mean number of eggs retained in the body cavity after spawning in 2004 and 2005.

Stream	Hatchery			Natural		
	N	Mean	SD	*N*	Mean	SD
			2004			
Chiwawa	22	63	255	32	13	53
Nason	14	37	75	56	12	42
Wenatchee	6	10	6	3	2	4
White	2	10	13	5	5	11
Little Wenatchee	0	--	--	1	8	--
			2005			
Chiwawa	179	11	47	35	10	51
Nason	98	31	106	25	21	52
Wenatchee	46	46	107	1	0	
White	32	3	6	7	1	2
Little Wenatchee	23	5	8	11	21	59

Table 11. Estimated mean percentage of eggs retained in the body cavity of female spring Chinook examined on the spawning grounds in 2004 and 2005.

Stream	Hatchery			Natural		
	N	Mean	SD	*N*	Mean	SD
			2004			
Chiwawa	22	1.35	5.40	35	0.27	1.13
Nason	14	0.86	1.79	55	0.26	0.93
Wenatchee	7	0.19	0.17	3	0.04	0.07
White	2	0.28	0.40	5	0.13	0.22
Little Wenatchee	0	-	-	1	0.20	-
			2005			
Chiwawa	179	0.26	1.06	35	0.26	1.35
Nason	98	0.81	2.92	25	0.50	1.30
Wenatchee	46	1.12	2.51	1	0.00	-
White	32	0.07	0.15	7	0.01	0.04
Little Wenatchee	23	0.13	0.22	11	0.46	1.24

Spring Chinook Potential Spawning Population

Based on PIT detections and information collected at Tumwater Dam, Eastbank FH, Chiwawa weir, and other Columbia River dams, the number of spring Chinook remaining upstream of Tumwater Dam that could spawn was 3,475 adults and jacks and 297 hatchery precocious males (Table 12).

Table 12. Distribution of spring Chinook detected at Tumwater Dam in 2004 and 2005. Data includes eight natural and one hatchery origin spring Chinook detected from video counts in 2004 and three natural origin spring Chinook detected from video counts in 2005 (HPM = hatchery precocious males).

Origin	Below Tumwater Dam		Above Tumwater Dam			
	Fallback	Eastbank Hatchery	Prespawn Mortality	Chiwawa Weir	Spawning Grounds	Total
			2004			
Hatchery	11	148	2	48	1,124	1,333
HPM	0	0	0	0	635	635
Natural	0	4	7	93	792	896
Unknown	0	0	0	0	32	32
Total	11	152	9	141	2,583	2,896
			2005			
Hatchery	0	40	54	143	2,983	3,220
HPM	0	0	0	0	297	297
Natural	0	0	10	99	464	573
Unknown	0	0	5	1	28	34
Total	0	40	69	243	3,772	4,124

Summary

In 2005, the natural escapement and hatchery production levels affected the differences in sex ratios and age distribution of hatchery and natural origin fish. Differences in size at return examined over time could prove useful in detecting affects of hatchery fish on size at return of natural origin fish. This was the first year in which within year differences between hatchery and wild age-4 spring Chinook have been detected. Chiwawa adult hatchery spring Chinook have been typically larger than their wild cohorts, but differences were not statistically significant. Size of hatchery origin spring Chinook salmon adults in the Tucannon River were smaller than natural origin spring Chinook salmon during the initial years of hatchery operation but later the differences could not be detected (Gallinat 2004). Similarly, first generation hatchery origin spring Chinook salmon in the upper Yakima River were smaller than natural origin fish (Knudsen et al. in

press). Differences observed in the Wenatchee Basin may be because of the larger size disparity of hatchery and natural origin smolts. In addition, the record high spring Chinook escapement in 2001 may have also affected the size of returning adults. For example, density dependent growth may have caused the small size of smolts that emigrated in 2003. The mean fork length of the 2001 brood Chiwawa spring Chinook smolts in 2003 was 86 mm, the smallest size at emigration detected since monitoring began in 1993 (average between 1993 and 2002 = 96 mm; WDFW unpublished data).

Female hatchery and natural origin spring Chinook have similar length-fecundity relationships. Mean fecundities of both hatchery and natural are also similar within years, but may be different between years. These results suggest that fecundity of both hatchery and natural spring Chinook respond similarly to changes in environmental conditions.

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Chapter 2

Population genetic analyses, pedigree reconstruction and fitness estimation

Abstract

Hatcheries have been increasingly asked to contribute to conserving natural salmon populations, as well as to continue to produce fish to mitigate for lost harvest opportunities. A key biological uncertainty about the effects of hatchery production on natural populations is the degree to which hatchery produced fish can reproduce in the natural environment. In order to assess the impact (positive or negative) of supplementation of spring Chinook salmon in the Wenatchee River we are using a DNA-based pedigree analysis to (1) directly measure the relative reproductive success of hatchery and natural-origin spring Chinook salmon in the natural environment, (2) determine the degree to which any differences in reproductive success between hatchery and natural Chinook salmon can be explained by measurable biological characteristics such as run timing, morphology, and reproductive behavior, and (3) estimate the relative fitness of fish produced by hatchery-origin adults breeding in the natural environment and that have themselves returned to spawn.

Population genetic and preliminary parentage analyses have been carried out during the second year of monitoring reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. Eleven microsatellites were used to analyze population genetic structure for 2969 adult spring Chinook entering the Wenatchee River drainage system during 2004. Significant genetic differentiation exists between adult hatchery and wild fish, and between wild adults returning to spawn in the Chiwawa River, Nason Creek, and the White River. Wild and hatchery samples have similar overall levels of genetic diversity, but patterns of diversity within each group differ. The wild samples are characterized by a slight heterozygote deficit (compared to random mating expectations), and generally have low levels of statistical associations among loci. In contrast, the hatchery samples are characterized by a slight heterozygote excess compared to random mating expectations, and have high levels of statistical associations among loci. These patterns probably reflect differences in effective population size or family structure between the two groups.

Preliminary testing of parentage assignment rates of 2004 Wenatchee River spring Chinook, performed separately for wild and hatchery fish, indicated assignment success rates (proportion of simulations in which the most likely parent pair was the correct parent pair) were 97.8% and 82.7% for wild and hatchery fish, respectively. When a statistical criterion was used to limit incorrect assignments to no more than 5%, the total assignment rate dropped to 66.1% for the hatchery fish. These results reflect the higher degree of non-independence among loci observed for hatchery compared to wild fish and appear to be a consequence of the low numbers of spawners that produced the 2004 hatchery return. In order to predict the effects of adding additional loci to the analysis, a subset of several hundred of the 2004 adults were genotyped at an additional four loci (for a total of 15 loci). For the 2004 returns (~1800 hatchery origin fish), we predict

~90% of the time the parent pair with the highest likelihood would be the true parents using the 15 locus dataset, compared to 82.7% for the 11 locus set. Increasing the number of microsatellite loci genotyped will therefore be necessary to boost the power of parentage assignment in order to limit incorrect assignments to < 5% for hatchery fish. Even with the 11 locus dataset, we were able to make some inferences about fitness differences between hatchery and wild fish, however. For example, 2 and 3 year old hatchery males made up a large fraction of the male fish sampled at Tumwater Dam, but even after accounting for differences in assignment success, appeared to be very unsuccessful at producing progeny.

Introduction

Hatcheries have been increasingly asked to contribute to conserving natural salmon populations, as well as to continue to produce fish to mitigate for lost commercial, recreational, and tribal harvest opportunities. For example, supplementation projects, in which adult hatchery fish are deliberately encouraged to spawn naturally to augment a population's abundance, have become common throughout the Columbia River Basin. However, little direct data are available regarding the beneficial or harmful influence hatchery production has on the natural production of Chinook salmon.

A key biological uncertainty about the effects of hatchery production on natural populations is the degree to which hatchery produced fish can reproduce in the natural environment. Accurately measuring the biological causes of variance in reproductive competence is important not only for determining the benefits of conservation hatcheries, but also for risk assessment of fish that stray from 'production' type hatcheries. For instance, if the relative reproductive success of hatchery fish is low, a supplementation program is unlikely to be successful at increasing natural production. Evaluating relative reproductive success is therefore critical for determining if the considerable investment the region has made in hatchery supplementation programs is actually contributing to (or even impeding) the recovery of salmon populations. Determining the relative reproductive success of hatchery fish that stray from traditional hatchery programs is also important. Stray hatchery fish can often mask the status of natural populations because their reproductive success is unknown, and may lead to reduced short and long-term natural productivity due to genetic deterioration of the natural population as a result of interbreeding between naturally produced fish and some hatchery strays. By directly quantifying the reproductive success of stray hatchery fish in the natural environment relative to that of fish from the natural population, the viability of natural populations receiving substantial stray hatchery fish can be much more accurately evaluated.

This goal of this project is to quantitatively assess the relative reproductive success of naturally spawning hatchery and natural origin spring-run Chinook salmon in the Wenatchee River by employing a molecular genetic pedigree analysis. Specifically, we will (1) directly measure the relative reproductive success of hatchery and natural-origin spring Chinook in the natural environment, (2) determine the degree to which any differences in reproductive success between hatchery and natural Chinook salmon can be

explained by measurable biological characteristics such as run timing, morphology, and reproductive behavior, and (3) estimate the relative fitness of fish produced by hatchery-origin adults breeding in the natural environment and that have themselves returned to spawn.

A baseline genetic data set for Wenatchee River spring Chinook has been developed using data on adult fish collected during 2004. Data for the 2005 adults and juveniles are still being collected. In addition, a preliminary evaluation of parentage assignment has been made using data from subsets of the 2004 adults and 2005 juveniles. In this report, we describe patterns of genetic variation within and among spawning populations of spring Chinook salmon in the Wenatchee River and within and between hatchery and natural origin fish. We also describe the results from our preliminary parentage analyses, using both simulated and real progeny from the 2004 parents sampled at Tumwater Dam.

Methods

Sampling - Washington Department of Fish and Wildlife (WDFW) personnel obtained fin and scale samples from 2969 and 4098 adult spring-run Chinook as they were being passed over the Tumwater Dam fish weir from May to August 2004 and 2005, respectively. A higher number of 2004 spring Chinook adults are reported on here (compared to 2896 fish reported on in the first annual report) due to subsequent reanalysis of individuals that did not initially produce data. These samples represent adult spring-run fish returning to spawn in the major tributaries (primarily the Chiwawa River, Nason Creek, and the White River) of the Wenatchee River. Other data collected during sampling included fork length, weight, secondary sexual characteristics, and presence of adipose fin. The age as well as hatchery origin of fish was evaluated by scale growth pattern analysis (John Sneva, WDFW, personal communication). All sampled spring-run Chinook salmon were also PIT tagged at Tumwater Dam (Chapter 1). Individuals re-sampled on the spawning grounds as carcasses were evaluated for the presence of a coded wire tag (CWT) or PIT tag. WDFW also provided dried fin-clip samples from 350 Leavenworth National Fish Hatchery (Carson stock) spring-run Chinook adults that had been out-planted from Peshastin Creek in 2004,192 Wenatchee River summer-run Chinook adults collected during 2004, and 48 Chiwawa River hatchery spring Chinook collected in 1994 (Ford et al. 2004).

We also took advantage of another study in which juvenile spring Chinook salmon were temporally detained in rotary screw traps as they migrated down stream in the Chiwawa River, Nason Creek, and White River. Fin-clips were sampled from 1210 juveniles during 2005, but only ~196 of these have been genotyped to date.

Microsatellite genotyping - Genomic DNA was extracted from fin clips using a QIAgen DNA tissue extraction kit, eluted into a 96-well sample plate, and quantified using a FLX 800 Microplate Fluorescence reader (Bio-Tek Instruments, Winooski, Vermont). All original DNA extractions as well as the working stocks of DNA were stored at -20°C until needed. Unused portions of fin-clips have been appropriately cataloged and stored.

Individuals were genotyped at 11 previously developed di- and tetranucleotide repeat microsatellite loci: Ots3, Ots104, Ots201b, Ots211, Ots213, Ots2M, Ots10M, OtsD9, Oke4, Ogo4, and Ssa408 (references provided in Table 1). A subset of 384 adults and 192 juveniles collected during 2004 and 2005, respectively, were genotyped at four additional tetranucleotide repeat microsatellite loci: Ogo2, Oki23MMBL, Omy1011, and Ots208b (references provided in Table 1). The growth hormone pseudogene locus (GH-Ψ) (Du et al. 1993) was used to estimate the sex of each individual. Microsatellite alleles were amplified by Polymerase Chain Reaction (PCR) assays using 15 ng of genomic DNA, 1.75 or 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM of each PCR primer, 0.25 Units of T_{aq} DNA polymerase (Promega Biosciences, San Luis Obispo, California), 20 mM Tris (pH 8.5) and 50 mM KCl in 10 μl volumes. The forward primer of each PCR primer pair was labeled with a fluorescent phosphoamidite (FAM, NED, PET, or VIC). Tetrad thermal cyclers (MJ Research, San Francisco, CA) were programmed with the conditions, shown in Table 1, which permitted pairs of loci to be co-amplified (duplexed) into single PCR reactions. Each set of PCR conditions (Table 1) included a lengthy final extension cycle used to "fill-in" the +A nucleotide additions Taq DNA polymerase creates at the 3'-end of each synthesized DNA strand thereby permitting more consistent and accurate scoring of PCR products. PCR products and in-lane size standards (GeneScan 500) were resolved using an ABI3100 capillary electrophoresis system (Applied Biosystems, Inc., Foster City, California). Individual genotypes were scored using Genotyper software (Applied Biosystems, Inc., Foster City, CA). Prior to assigning genotypes to individual samples, the raw, un-binned data for every allele detected was plotted on a locus by locus basis. This pre-screen of the data set was performed in order to ascertain whether or not shifts in allele mobility occurred during the period of data collection. Genotyping error rate per locus (Table 1) was determined by re-amplifying and re-scoring microsatellite loci for a subset of individuals, and calculating the number of alleles mis-scored over the total number of alleles observed at each locus. Genotypic sex, according to GH-Ψ (Du et al. 1993), and phenotypic sex were compared for 240 spring-run Chinook adults collected as brood stock during the 2004 sampling period.

Table 1 -- Thermocycler conditions, genotyping error rate, and references for 15 microsatellite loci and one sex-specific locus (GH-Ψ) used to evaluate the 2004 Wenatchee River Spring-Run Chinook adults. Thermocycler conditions for each pair of loci simultaneously amplified (duplexed) in a single PCR reaction include: one denaturation cycle at 95 °C for 150 seconds, amplification cycles of 95 °C for 40s, X °C annealling temperature (T_m °C) for 40s, 72 °C for 40s, and a final extension cycle of 60 °C for 45 min.

Locus Name	$MgCl_2$ (mM)	T_m (°C)	Genotyping % Error Rate	References
Oke4	1.75	54	1.43	Buchholz et al. 1999
Oki23MMBL	1.75	54	NDa	Spidel et al., unpublished
Ogo2	1.75	60	NDa	Olsen, Bentzen, and Seeb 1998
Ogo4	1.75	60	1.53	Olsen, Bentzen, and Seeb 1998
Omy1011	1.75	54	NDa	Bentzen et al. 2001
Ots2M	2.00	60	1.39	Greig and Banks 1999
Ots3	1.75	48	0.60	Banks et al. 1999
Ots10M	1.75	54	0.95	Greig and Banks 1999
OtsD9 (Ots519NWFSC)	1.75	54	1.43	Naish and Park, 2002
Ots104	1.75	48	1.46	Nelson and Beacham 1999
Ots201b	2.00	60	1.55	none
Ots208b	1.75	60	NDa	Grieg, Jacobson, and Banks 2003
Ots211	1.75	60	0.68	Grieg, Jacobson, and Banks 2003
Ots213	1.75	54	1.30	Grieg, Jacobson, and Banks 2003
Ssa408	2.00	60	1.22	Cairney, Taggert, and Hoyheim 2000
Growth Hormone psuedogene	2.00	60	2.50	Du et al. 1993

[a] Genotyping error rate not determined for locus.

Identifying Summer-Run and stray Spring-Run hatchery fish- The putative Wenatchee River spring Chinook data set was evaluated for admixture with summer-run fish. Putative spring Chinook were assigned to either a spring- or summer–run baseline population using the software program Genetic Mixture Analysis (GMA) (Kalinowski 2003). The first 574 (out of 2969) spring-run Chinook adults collected at the Tumwater Dam weir and 192 summer-run Chinook adults collected from the Wenatchee River in 2004 were used as spring- and summer-run Chinook baseline population data, respectively. Spring- and summer-run Chinook included in the baselines were selected on the basis of non-overlapping migration time. The sample data set of putative spring Chinook adults contained the remaining 2395 individuals. Jack-Knife analyses of baseline populations were performed with WHICHRUN V.4.1 (Banks and Eichert 2000) to characterize the robustness of assignments to the spring- and summer-run populations. Log of odds (LOD) scores, the log of the ratio of the probability that an individual's genotype occurs in one population compared to another population, were calculated for each individual. Individuals with LOD scores >2 (100X more likely to be assigned to one population than another population) assigned to specific populations; individuals with LOD <2 were considered ambiguous. Putative "spring" Chinook with genotypes that assigned to the summer-run baseline, had an ambiguous assignment, had an origin that could not be ascertained, or that carried a LNFH CWT were not used for population genetic analyses. All fish that were not positively identified as summer Chinook were used in the parentage assignment analyses.

For population structure analyses, the positively identified Wenatchee River spring Chinook (N=2823) were grouped according to hatchery (N=1947) or wild (N=876) origin. Hatchery fish were initially identified as having an adipose fin-clip and/or CWT. Wild fish were initially identified by the presence of an adipose fin. To confirm these assignments, scale growth pattern analysis (John Sneva, WDFW, personal communication) was used to positively discriminate hatchery vs. wild origin. When sample sizes were sufficiently large to provide meaningful comparisons (n>~50), hatchery and wild fish were analyzed separately by carcass recovery location (Chiwawa River, Nason Creek, or White River) and age.

The numbers of spring Chinook adult carcasses collected on the White River in 2004 and 2005 (N= 11 and 29, respectively) were too low to provide an adequate representative sample of the White River population in each year alone. However, combining the adult carcass recovery samples from both years with a subset of juvenile fish sampled in the White River during 2005 provided a reasonable sample size. To avoid over representation of only a few families in the available 2005 White River juvenile sample (N=70), pair-wise relatedness values for all possible combinations of juveniles were calculated using the software program Pedigree v.2.0 (provided by Christophe Herbinger, Dalhousie University). Pedigree v.2.0 uses the pair-wise relatedness score approach described in Smith et al. (2001) to partition individuals into full sib families without parental information. The size and membership of full-sib groups were stable over a wide variety of Monte Carlo Markov-Chain simulation parameters. By selecting only one individual from each full sib group or doublet and all other juveniles not partitioned to a group, non-related juveniles (N=27) collected during 2005 were added to the sample of 2004 and 2005 adults to achieve an overall larger sample size (N=67) for the White River population.

The presence of stray Leavenworth National Fish Hatchery (LNFH) spring-run fish within the 2004 Wenatchee River hatchery and wild spring Chinook datasets was investigated prior to quantifying population genetic statistics and performing cluster analyses. One of the adipose fin clipped adult spring Chinook sampled at the Tumwater Dam weir carried a Leavenworth CWT. This fish was used as a reference to help determine which fish, if any, in the 2004 Wenatchee River spring Chinook samples might originate from the LNFH. Putative assignment of Wenatchee River spring Chinook to either the LNFH or Wenatchee River spring-run baseline population was carried out using WHICHRUN V.4.1 (Banks and Eichert 2000). Baseline datasets included the 2004 Peshastin Creek Hatchery spring adult out-plants (N=350) used as a proxy for LNFH spring-run, 2004 Wenatchee River summer-un adults (N=192), and the 2004 Wenatchee River spring-run wild adults (N=876). Individual assignment of Wenatchee River spring-run Chinook hatchery fish to the LNFH baseline population was carried out using a LOD score criterion of 2. The hatchery fish sample set included 1948 fish, including the single LNFH CWT recovery. Because of the relatively large genetic differences between spring- and summer–run fish, the robustness of assignment of Wenatchee River hatchery spring Chinook to the LNFH baseline population was tested by re-performing assignment testing after removal of the summer Chinook sample from the of baseline dataset.

Characterization of microsatellite loci – Microsatellite loci were characterized separately in hatchery and wild Chinook populations, as well as in hatchery fish grouped by age, and wild fish grouped by carcass recovery location. Allele frequencies, the total number of observed alleles, expected heterozygosity (H_e) under Hardy-Weinberg equilibrium (HWE), observed heterozygosity (H_o), and F_{IS} values (and their 95% confidence intervals) for the first 11 microsatellite loci were calculated using the program GENETIX version 4.05 (Belkhir et al. 2000, available at http://www.University-montp2.fr/~genetix/genetix.htm). Pair-wise comparisons of loci for linkage disequilibrium were made by estimation of exact P-values by the Markov chain method (Guo and Thompson 1992) as implemented in GENEPOP (dememorization steps 1000; 50 batches; 1000 iterations per batch). Sequential Bonferroni adjustments to α were applied, where appropriate, for simultaneous tests to decrease the chance of erroneously rejecting null hypotheses (Rice 1989).

Characterization of Spring Chinook population structure – Pair-wise F_{ST} matrices were calculated using GENETIX V4.05 (Belkir et al. 2003). The Peshastin Creek Hatchery spring-run outplant and Wenatchee River summer-run samples were included in the analysis of Wenatchee River spring Chinook. Significance ($\alpha < 0.05$) of pair-wise F_{ST} values was assessed using 1000 bootstrap replicates of the entire data set. Unrooted Neighbor-Joining phenograms, based on Cavalli-Sforza (1967) cord distance units, were created with PHYLIP (Felsenstein 1989). The phenograms were constructed using data from the first 11 microsatellites, 1000 boot-strap replicates of the data set, and were 're-rooted' through the 2004 Wenatchee River summer-Run Chinook out group.

Parentage assignment – Our first planned sampling of progeny for this project will occur in spring of 2006, when smolts produced from the 2004 spawning year will be captured in the lower Wenatchee River near Monitor. However, we took advantage of another ongoing sampling program in the Wenatchee River tributaries to obtain samples from 2004 brood year parr collected from the Chiwawa River, Nason Creek, and White River in fall/winter of 2005. We conducted preliminary parentage analyses of 196 of these parr, all collected from the Chiwawa River.

Parentage assignments were made using the likelihood methods of Meagher and Thompson (1986) and Gerber et al. (2000) as implemented in the program FAMOZ (Gerber et al. 2003). Each individual in a sample of progeny was tested against all potential pairs of parents (discarding information on parent sex) and a log of odds (LOD) score was calculated for each potential parent pair/offspring triplet as the log of the ratio of the probability of a parent pair/offspring relationship compared to the probability they were drawn randomly from the population. The most likely pair of parents was compared to the second most likely and the difference in LOD scores (ΔLOD) was calculated. The simulation function of the FAMOZ program was used to generate expected distributions of ΔLOD scores for correct and incorrect assignments.

Simulations and actual parental assignments were conducted assuming a genotyping error rate of 1.5% per locus, and an analysis error rate of 0.01% per locus (i.e., the rate at which errors were produced in the simulations was 1.5% per locus, but the error rate

assumed in the analysis of the simulated and real data was 0.01% per locus). The 1.5% error rate is approximately equal to what we have observed in our laboratory, and the 0.01% analysis error rate was used because it produced a higher fraction of correct assignments in the simulations than did an error rate of either 1.5% or 0. In general, the highest fraction of correct assignments were obtained with a non-zero but small error rate, similar to what has been reported previously (Gerber et al. 2000; Sancristobal and Chevalet 1997).

Results

Genotyping error rates – The overall genotyping error rate for the microsatellite loci was 1.23% and ranged from 0.60% (Ots3) to 1.55% (Ots201b). Out of the 240 Spring-Run brood stock examined six (2.5%) had a GH-Ψ genotype that was incongruent with its gonad phenotype. Inconsistencies included phenotypic male fish lacking the male-specific PCR fragment (~274 bp), and phenotypic females that were positive for the male-specific PCR fragment. The percentage of mismatches between genotypic and phenotypic sex on the Wenatchee R appears to be similar to that observed in other Columbia River Basin spring Chinook populations (Devlin et al. 2005) and smaller than that observed in some fall Chinook populations (Chowden and Nagler 2005).

Differentiation between spring and summer run Chinook -- Assignment testing of 2395 Wenatchee River putative spring-run fish to either the spring- or summer–run baseline data revealed 97 summer-run and three ambiguously assigned individuals. All 100 of these fish, another 41 individuals whose origin could not be determined and five individuals carrying LNFH CWTs were removed from the spring Chinook dataset prior to further population genetic analyses. Jack-Knife analyses of assignment to the spring- and summer–run baseline populations indicated a very high percentage of correct assignments (99.5% and 100%, respectively). The remaining 2823 (2249 assigned to Wenatchee spring-run plus the first 574 fish collected at Tumwater Dam used as a 'pure' spring baseline population) individuals in the Wenatchee River spring Chinook dataset were grouped into 1947 hatchery and 876 wild origin fish. These 1947 hatchery spring Chinook include putative LNFH strays.

A total of 57 hatchery and zero wild spring Chinook were identified as potential LNFH strays based on their genotypes (data not shown). The known Leavenworth CWT individual was assigned to the Peshastin Creek Hatchery baseline population with a LOD score of 2.44.

Table 2 -- Jack-Knife analyses of reassignment of individuals back to their original baseline (critical) population using WHICHRUN V.4.1 (Banks and Eichert 2000) and a LOD threshold of 2.0. The number of individuals per critical population (N) and the number of individuals correctly reassigned back to their own baseline population are shown. Numbers of incorrectly assigned individuals and the baseline population to which they were mis-assigned are presented in the last three columns.

Critical Population	N	# Correctly Reassigned to Crit. Pop.	# Incorrectly Assigned		
			Peshastin Outplants	Spring Hatchery	Spring Wild
2004 Summer-Run adults	192	188	0	0	0
Peshastin Crk. Spring-Run outplants	350	85	--	1	4
2004 Spring Hatchery adults	1947	162	0	--	
2004 Spring Wild adults	876	28	0	2	--

An additional four LNFH CWT fish were recovered as carcasses on Nason Creek (Travis Maitland, WDFW, personal communication). Only one out of these four LNFH CWT bearing fish was assigned (LOD>3) to the LNFH proxy baseline. The poor ability to discriminate LNFH CWT bearing fish within the 2004 Wenatchee River spring Chinook sample suggests that some un-tagged LNFH origin fish are present in the sample of Wenatchee River spring Chinook Table 2). Because we did not feel confident in our ability to assign individual spring-run Chinook salmon to the LNFH or Wenatchee River populations, only the five LNFH CWT fish were removed from the dataset prior to conducting population genetic analyses. All spring-run Chinook salmon, including the five with LNFH tags, were used in the parentage analyses, however.

Summary of variation -- Basic population genetic statistics were calculated for 1947 hatchery and 876 wild Wenatchee River spring Chinook (Table 3A and 3B, respectively). For the combined 2004 hatchery spring Chinook (Table 3A) the number of observed microsatellite alleles ranged from 6 (Ots10M) to 46 (Ots104). A small excess of heterozygous genotypes was indicated by negative F_{IS} values for five out of nine loci significantly (α=0.0045) different from HW expectations (Table 3A). Out of 55 pair-wise comparisons of loci, 48 (87%) had nonrandom (significant α=0.0045) allelic associations (Linkage Disequilibrium, LD). Too few hatchery spring-run fish could be grouped based on carcass recovery location to perform statistically relevant analyses. Hatchery spring Chinook were grouped according to age and the analyses repeated. Similar results (not shown) were obtained as for the combined hatchery fish data. In contrast, the sample of 1994 Chiwawa River hatchery spring Chinook showed no significant (α=0.0045) deviations from HW equilibrium at the eight microsatellite loci examined and no LD (Table 3C). Since the sample size (N=48) of the 1994 hatchery fish is much lower than that examined in 2004 (N=1947), the observed number of alleles/locus in the 1994 sample is lower as well.

Table 3 -- Population genetic statistics of 11 microsatellite loci for the 2004 Wenatchee River combined hatchery (A) and wild (B) Spring-Run Chinook, and 8 microsatellite loci for the 1994 Chiwawa R. Hatchery sample (C). Observed number of alleles (n), expected and observed heterozygosities (H_e and H_o, respectively), and Hardy-Weinberg equilibrium (F_{IS}, Weir & Cockerham 1984) are shown. The 95% confidence intervals for F_{IS} values were calculated by bootstrapping 500 times using the software package GENETIX4.05 (Belkhir et al. 2000). The p-values for were calculated using the software package GenePop3.4 (Raymond and Rousset 1995). * F_{IS} values statistically significant at $\alpha = 0.0045$.

A – 2004 Hatchery fish

Msat Locus	Obs. # Alleles	H_e	H_o	F_{IS} value	95% CI	p-value
Ogo4	13	0.81	0.81	-0.007*	(-0.031 - 0.014)	<0.001
Ots10M	6	0.53	0.51	0.046	(0.001 - 0.090)	0.029
Ots211	27	0.94	0.93	0.005*	(-0.006 - 0.016)	<0.001
Ots213	32	0.91	0.93	-0.022*	(-0.035 - -0.010)	<0.001
Ots2M	14	0.54	0.56	-0.042	(-0.081 - 0.005)	0.018
Oke4	7	0.62	0.61	0.014*	(-0.015 - 0.045)	<0.001
Ots104	57	0.95	0.96	-0.012*	(-0.021 - -0.003)	<0.001
Ots201b	31	0.93	0.95	-0.022*	(-0.033 - -0.011)	<0.001
Ots3	13	0.64	0.66	-0.038*	(-0.067 - -0.009)	<0.001
OtsD9	9	0.70	0.66	0.059*	(0.029 - 0.090)	<0.001
Ssa408	29	0.89	0.86	0.031*	(0.015 - 0.048)	<0.001
All loci		0.77	0.77	<0.001*	(-0.006 - 0.006)	<0.001

total # individs used = 1947

B – 2004 Wild fish

Msat Locus	Obs. # Alleles	H_e	H_o	F_{IS} value	95% CI	p-value
Ogo4	13	0.81	0.79	0.024	(-0.004 - 0.060)	0.278
Ots10M	5	0.54	0.52	0.038	(-0.019 - 0.093)	0.614
Ots211	28	0.93	0.94	-0.011*	(-0.027 - 0.005)	<0.001
Ots213	33	0.91	0.90	0.010	(-0.009 - 0.031)	0.005
Ots2M	14	0.56	0.53	0.054	(-0.002 - 0.108)	0.448
Oke4	7	0.60	0.55	0.094	(0.048 - 0.142)	0.010
Ots104	49	0.95	0.92	0.027*	(0.012 - 0.043)	<0.001
Ots201b	34	0.93	0.92	0.012*	(-0.005 - 0.030)	<0.001
Ots3	8	0.55	0.53	0.032*	(-0.017 - 0.076)	0.001
OtsD9	5	0.70	0.72	-0.034*	(-0.080 - 0.009)	<0.001
Ssa408	24	0.90	0.86	0.043	(0.017 - 0.069)	0.076
All loci		0.76	0.76	0.023*	(0.012 - 0.034)	<0.001

total # individs used = 876

C. – 1994 Chiwawa River Hatchery fish

Msat Locus	Obs. # Alleles	H_e	H_o	F_{IS} value	95% CI	p-value
Ogo4	9	0.78	0.80	-0.017	(-0.156 - 0.111)	0.770
Ots10M	4	0.54	0.43	0.220	(-0.015 - 0.486)	0.261
--	--	--	--	--	--	--
--	--	--	--	--	--	--
Ots2M	4	0.55	0.39	0.304	(0.029 - 0.541)	0.027
Oke4	5	0.55	0.57	-0.011	(-0.272 - 0.268)	0.183
Ots104	28	0.94	0.96	-0.006	(-0.066 - 0.056)	0.732
--	--	--	--	--	--	--
Ots3	6	0.63	0.67	-0.042	(-0.217 - 0.096)	0.083
OtsD9	4	0.65	0.59	0.108	(-0.081 - 0.336)	0.373
Ssa408	15	0.87	0.85	0.035	(-0.087 - 0.135)	0.936
All Loci		0.69	0.66	0.061	(-0.008 - 0.097)	0.160

total # individs used = 48

For the combined 2004 wild spring Chinook population (Table 3B) the number of observed microsatellite alleles ranged from 5 (Ots10M and OtsD9) to 49 (Ots104). Three loci (Ots104, Ots201b, and Ots3) had slightly fewer heterozygous genotypes (indicated by significantly (α=0.0045) positive F_{IS} values) than expected under HW equilibrium (Table 3B). Over all 11 loci combined, the wild population had about 2.3% fewer heterozygotes than expected under random mating assumptions (F_{IS} = 0.023, p<0.001). Analysis of Linkage Disequilibrium indicated 14 of 55 (25%) pair-wise comparisons of loci had significant (α=0.0045) allelic associations.

Population genetic statistics were also calculated for subsets of wild fish recovered as carcasses on the Chiwawa River (N=106), Nason Creek (N=85), and the White River (N=67) Table 4A, B, and C, respectively). The multilocus F_{IS} values for the Chiwawa River (F_{IS} = 0.022, p=0.002) and Nason Creek (F_{IS} = 0.012, p=0.002) wild Spring Chinook were significantly different from HW equilibrium. The White River spring Chinook population, which included adults collected during 2004, as well as adults and juveniles collected during 2005, did not deviate significantly from HW equilibrium after correction for multiple tests, but the estimate of F_{IS} was numerically similar to the other populations (F_{IS} = 0.020, p=0.036). Sub-grouping wild fish based on carcass recovery location resulted in fewer individual loci within the Chiwawa River, Nason Creek, and White River wild spring-run populations (0, 2, and 0, respectively) deviating from HW equilibrium, and the percentage (4%, 2%, and 2%, respectively) of pair-wise comparisons of loci in LD decreased. Most (96%) of the 876 wild spring Chinook sampled were 4 year-old fish. Accordingly, statistically relevant analyses based on the age of wild fish could not be performed.

Table 4 -- Population genetic statistics of 11 microsatellite loci for the 2004 wild Chiwawa R. (A), Nason Creek (B), and White R. (C) Spring-Run Chinook. The White R. sample includes adults collected during 2004-5 and juveniles collected during 2005. Observed number of alleles (n), expected and observed heterozygosities (H_e and H_o, respectively), and Hardy-Weinberg equilibrium (F_{IS}, Weir & Cockerham 1984) are shown. The 95% confidence intervals for F_{IS} values were calculated by bootstrapping 500 times using the software package GENETIX4.05 (Belkhir et al. 2000). The p-values for were calculated using the software package GenePop3.4 (Raymond and Rousset 1995). * F_{IS} values statistically significant at α = 0.0045.

A. – Chiwawa River wild

Msat Locus	Obs. # Alleles	H_e	H_o	F_{IS} value	95% CI	p-value
Ogo4	10	0.81	0.69	0.151	(0.058 - 0.260)	0.008
Ots10M	4	0.55	0.57	-0.030	(-0.209 - 0.109)	0.054
Ots211	19	0.92	0.94	-0.020	(-0.068 - 0.029)	0.516
Ots213	24	0.89	0.88	0.014	(-0.050 - 0.079)	0.179
Ots2M	6	0.55	0.60	-0.093	(-0.039 - 0.104)	0.752
Oke4	6	0.60	0.48	0.192	(0.057 - 0.316)	0.009
Ots104	31	0.93	0.87	0.068	(0.009 - 0.126)	0.131
Ots201b	22	0.92	0.94	-0.022	(-0.070 - 0.026)	0.034
Ots3	7	0.59	0.60	-0.018	(-0.157 - 0.112)	0.598
OtsD9	4	0.69	0.73	-0.045	(-0.156 - 0.076)	0.894
Ssa408	17	0.89	0.88	0.019	(-0.049 - 0.082)	0.157
All Loci		0.76	0.74	0.022*	(-0.012 - 0.046)	0.002

total # individs used = 106

B – Nason Creek wild

Msat Locus	Obs. # Alleles	H_e	H_o	F_{IS} value	95% CI	p-value
Ogo4	11	0.79	0.83	-0.036	(-0.128 - 0.047)	0.824
Ots10M	4	0.53	0.52	0.036	(-0.055 - 0.189)	0.813
Ots211	22	0.94	0.94	0.002	(-0.052 - 0.064)	0.576
Ots213	24	0.90	0.83	0.086*	(0.009 - 0.170)	0.004
Ots2M	6	0.50	0.54	-0.070	(-0.281 - 0.100)	0.460
Oke4	6	0.57	0.45	0.211	(0.018 - 0.373)	0.020
Ots104	33	0.95	0.96	-0.007	(-0.046 - 0.031)	0.550
Ots201b	22	0.92	0.94	0.019	(-0.0172 - 0.038)	0.776
Ots3	5	0.40	0.42	-0.040	(-0.194 - 0.149)	0.279
OtsD9	5	0.72	0.78	-0.077*	(-0.205 - 0.069)	0.001
Ssa408	19	0.88	0.85	0.043	(-0.042 - 0.116)	0.031
All Loci		0.74	0.73	0.012*	(-0.028 - 0.039)	0.002

total # individs used = 85

C – White River Wild

Msat Locus	Obs. # Alleles	H_e	H_o	F_{IS} value	95% CI	p-value
Ogo4	10	0.77	0.79	-0.015	(-0.142 - 0.094)	0.083
Ots10M	3	0.56	0.54	0.048	(-0.145 - 0.250)	0.592
Ots211	24	0.92	0.88	0.052	(-0.029 - 0.122)	0.120
Ots213	20	0.91	0.92	-0.004	(-0.074 - 0.059)	0.021
Ots2M	4	0.54	0.55	-0.015	(-0.228 - 0.178)	0.890
Oke4	6	0.66	0.70	-0.042	(-0.193 - 0.114)	0.588
Ots104	34	0.94	0.94	0.010	(-0.050 - 0.069)	0.022
Ots201b	20	0.93	0.88	0.053	(-0.033 - 0.129)	0.294
Ots3	6	0.55	0.54	0.016	(-0.166 - 0.178)	0.484
OtsD9	6	0.64	0.61	0.066	(-0.100 - 0.205)	0.220
Ssa408	15	0.89	0.86	0.037	(-0.052 - 0.135)	0.503
All Loci		0.76	0.75	0.020	(-0.025 - 0.050)	0.036

total # individs used = 67

Differences among spawning tributaries -- Analyses of population subdivision using F-statistics indicated almost all pair-wise F_{ST} comparisons were significant (Table 5; α=0.05). The sole exception was the comparison between the 2004 wild Chiwawa River and 1994 Chiwawa River hatchery spring-run populations. Both the LNFH out-planted spring Chinook as well as the Wenatchee River summer Chinook were well differentiated from the wild and hatchery spring-run fish (Table 5).

Table 5 -- Matrix of pair-wise F_{ST} (Weir and Cockerham 1984) values for 2004 Wenatchee R. Spring- and Summer-Run fish grouped according to origin (Hatchery vs. Wild). Significance of pair-wise F_{ST} values was assessed by using 1000 bootstrap replicates of an 11 microsatellite dataset. * F_{ST} values statistically significant at α = 0.05. Fish identified as summer-run were removed from the dataset prior to analysis. Location was based solely on carcass recovery on tributaries (Chiwawa R., Nason Crk., and White R.).

	Spring-Run								
	Wild			Chiwawa R. Hatchery				LNFH	Summer-
Groups	Chiwawa	Nason	Remainder	2 Yr. old	3 Yr. old	4 Yr. old	1994	Outplants	Run
Wild White R.	0.012*	0.017*	0.009*	0.018*	0.013*	0.022*	0.005*	0.012*	0.083*
Wild Chiwawa R.		0.016*	0.001*	0.019*	0.013*	0.008*	0.003	0.016*	0.084*
Wild Nason Crk.			0.009*	0.037*	0.017*	0.018*	0.011*	0.018*	0.091*
Wild Remainder				0.017*	0.010*	0.009*	0.003*	0.012*	0.088*
Hatchery 2 Yr. old					0.023*	0.030*	0.025*	0.019*	0.092*
Hatchery 3 Yr. old						0.013*	0.008*	0.013*	0.084*
Hatchery 4 Yr. old							0.010*	0.021*	0.099*
1994 Chiwawa Hatchery								0.018*	0.110*
LNFH Spring Outplants									0.079*

Combining all 2004 Wenatchee River hatchery spring-run fish into a single group while performing cluster analysis with the Wenatchee River wild spring-run populations indicated strong bootstrap support for the nodes separating LNFH (100%), and White River wild spring-run (89%) fish from all other wild populations (Figure 1). Moderate

bootstrap support (~70%) was obtained for the nodes separating the combined group of hatchery fish and wild Nason Creek fish from all remaining spring populations. A second cluster analysis was performed that included a sample of 1994 Chiwawa River hatchery spring Chinook (Figure 2). Wenatchee River hatchery and wild spring-run fish were grouped according to age and carcass recovery location, respectively. The second cluster analysis was based on 8 loci since the 1994 hatchery fish sample had been genotyped at only 8 of 11 loci. Strong bootstrap support (96%) for the node separating LNFH and Wenatchee River spring Chinook mirrored that obtained in Figure 1. Moderate bootstrap support (62%) was obtained for the node separating the two year old hatchery fish and the cluster containing all other Wenatchee River spring Chinook. Moderate bootstrap support (71%) was obtained for the node separating the cluster of the 4 year-old hatchery and 2004 Chiwawa River wild spring fish, as was for the node (61%) separating the cluster of the 2004 Nason Creek and remaining wild spring fish. The 1994 Chiwawa River hatchery sample clustered with the 2004-2005 White River wild spring fish population, however, bootstrap support for this cluster was very low (39%).

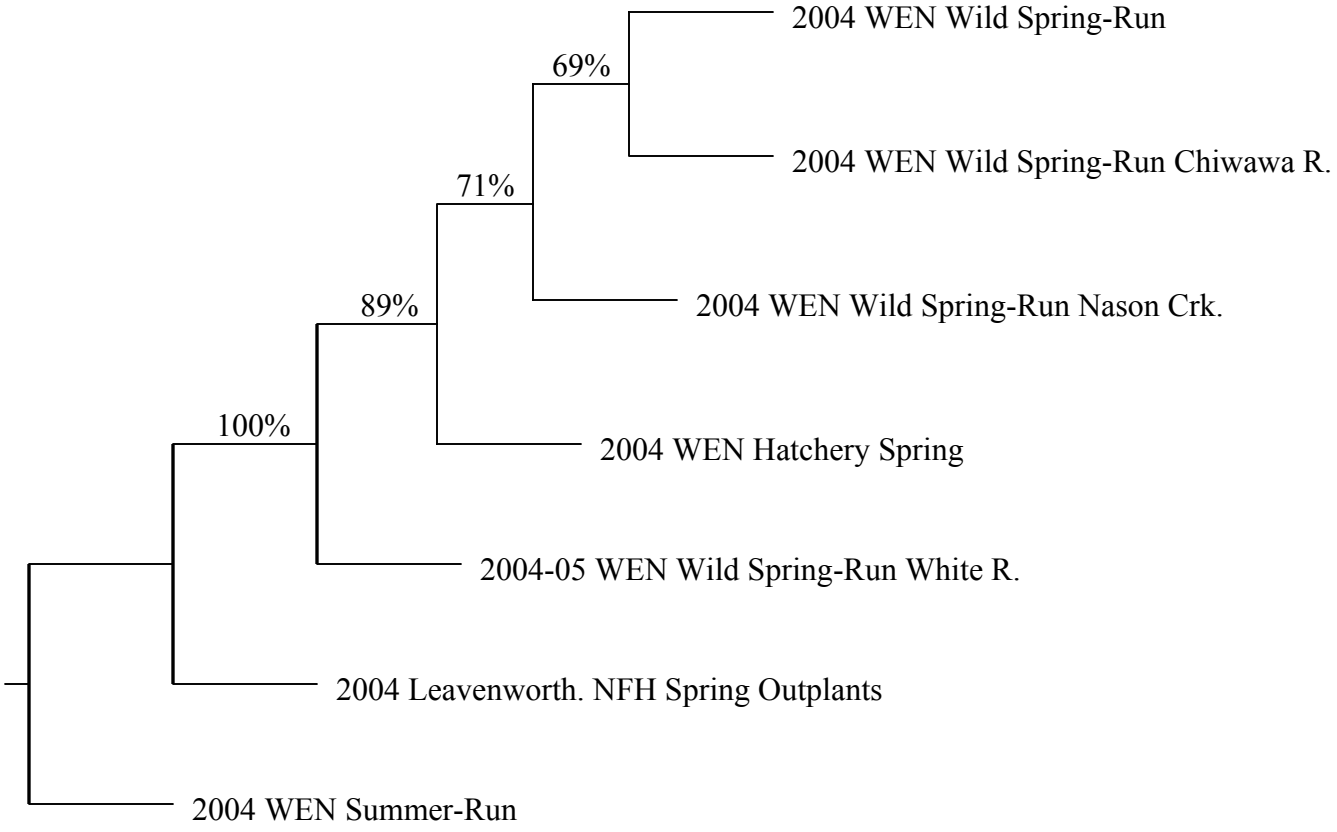

Figure 1-- Unrooted Neighbor-joining phenogram based on Cavalli-Sforza (1967) cord distance units among the 2004 Spring- and Summer-Run Wenatchee R. (WEN) Chinook, and the Spring adults outplanted from Peshastin Creek Hatchery. Note that this unrooted phenogram is merely "re-rooted" by the Summer-Run outgroup. The phenogram was constructed using data from 11 microsatellite loci with PHYLIP (Felsenstein 1989). For 1000 boot-strap replicates, node values of 69% and greater are given. Individuals were grouped as hatchery (CWT, adipose fin-clip, or scale pattern), or wild as well as by carcass recovery location on Chiwawa R., or Nason Crk. Fish identified as Summer-Run were removed prior to constructing the phenogram.

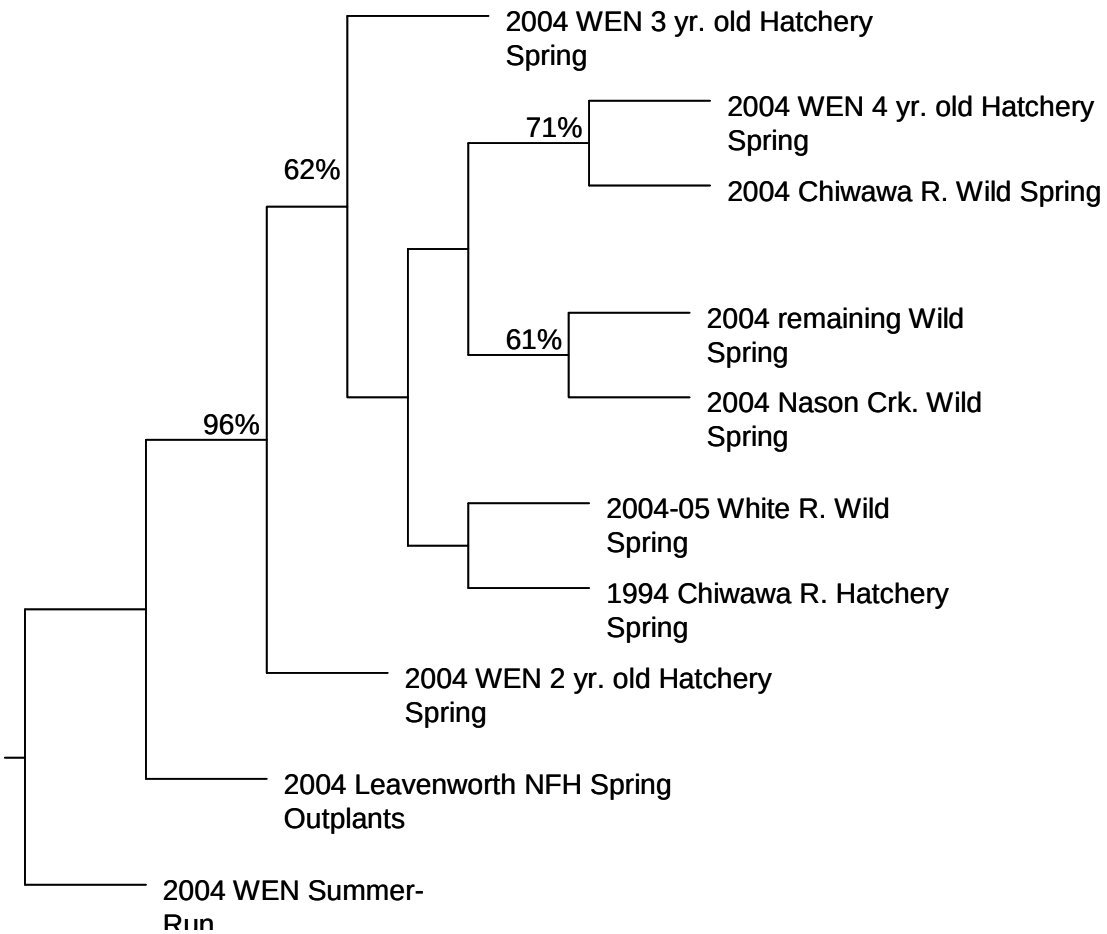

Figure 2 -- Unrooted Neighbor-joining phenogram based on Cavalli-Sforza (1967) cord distance units among the 2004 Spring- and Summer-Run Wenatchee River Chinook, Leavenworth Spring adults out-planted on Peshastin Creek, and 1994 Chiwawa R. Hatchery Spring adults. Note that this unrooted phenogram is merely "re-rooted" by the Summer-Run outgroup. The phenogram was constructed using data from 8 microsatellite loci with PHYLIP (Felsenstein 1989). For 1000 boot-strap replicates, node values of 60% and greater are given. Individuals were grouped as 2, 3 or 4 year old hatchery (CWT, adipose fin-clip, or scale pattern), or wild origin (according to carcass recovery location). Fish identified as Summer-Run were removed from each group prior to constructing the phenogram.

Parentage assignment – Of the 196 Chiwawa River parr genotyped, we attempted to identify parents for the 166 that had complete genotypes (no missing data). The pool of potential parents analyzed consisted of the spring Chinook salmon sampled at Tumwater Dam, except for those fish that were subsequently used for hatchery broodstock. Only potential parents with complete genotypes (no missing data) were used, resulting in 2432 potential parents analyzed out of 2617 total spring Chinook salmon actually on the spawning grounds (i.e., 93% of the potential parents were used in the analysis).

We used the simulation function of the FAMOZ program to determine a critical value, x, for Δ LOD scores such that if only parents with a Δ LOD > x were assigned as "true"

parents we would expect no more than 5% of the assignments would be incorrect. When 1000 simulated progeny were generated from the 2432 potential parents, in only 80% of the time was the most likely pair of parents the true pair of parents. In order to limit false assignments to <5%, a Δ LOD criteria of 1.44 was used to screen assignments, which resulted in assignments for only 65% of the simulated progeny.

This low assignment rate was surprising, as earlier simulations and the calculated exclusion probabilities for the loci used in the analysis indicated that >99% of the progeny should have been assigned correctly (see 2005 annual report). We determined that the discrepancy resulted from a difference in how the simulations were performed. The simulations conducted earlier in the study involved first simulating parents from the observed allele frequencies, simulating progeny from the simulated parents, and then testing how well the simulated progeny could be assigned to the simulated parents. This represents an "ideal" case, in that the parents in question were created assuming they were all unrelated and drawn from an infinitely large population. This results in ideal conditions for parentage assignment and therefore high assignment rates. The FAMOZ simulator, in comparison, uses the observed parents themselves to create simulated offspring. Any non-ideal genotypic distributions in the parents are therefore preserved. For example, if the potential parents consist of many close relatives, these relationships will be maintained by the FAMOZ simulations. The parental genotypic distributions are indeed "non-ideal", particularly for hatchery fish (see Table 3), and this apparently accounts for the lower than expected rate of successful assignment.

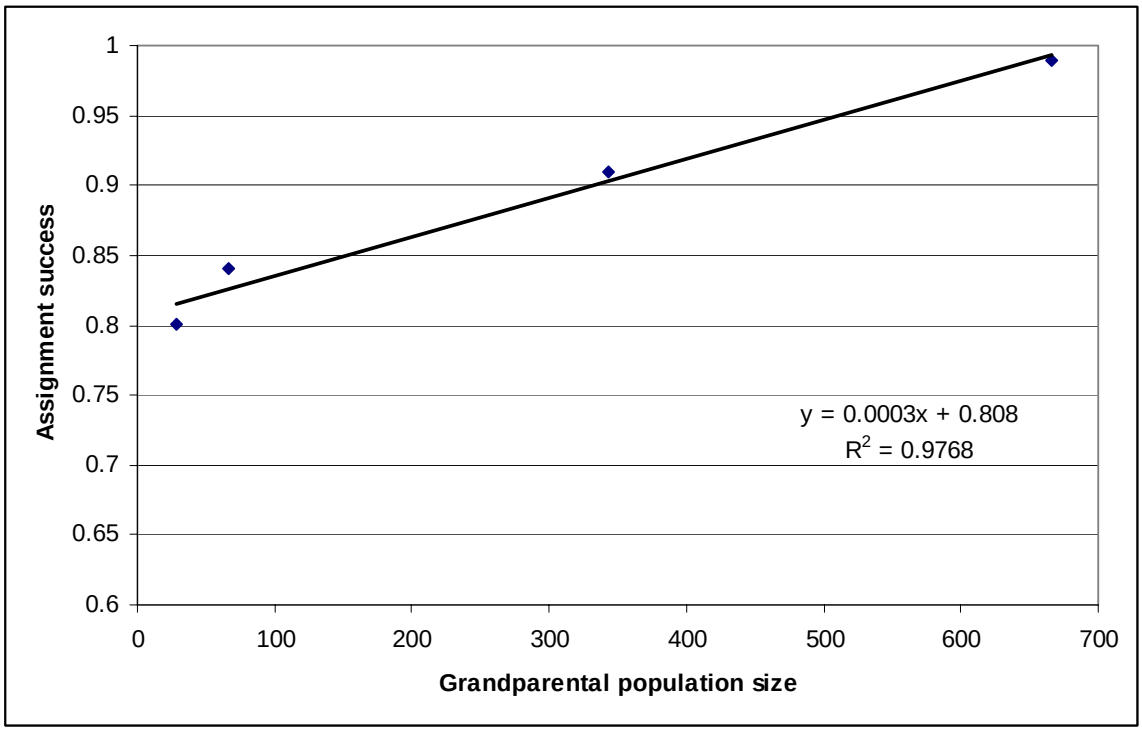

Figure 3 -- Relationship between simulated assignment success rate and the size of the parental populations that produced the spawners returning in 2004 (data from Table 6)

We performed several additional sets of simulations to further examine exactly why the assignment rates were lower than expected. In particular, we wanted to determine if there were differences in assignment rates between hatchery and wild fish. First, we conducted an additional set of simulations (using FAMOZ) for hatchery and wild parents separately. The parents in these simulation consisted of the 840 wild fish in the set of potential parents, and 840 hatchery fish drawn randomly from 1851 (Wenatchee River spring hatchery and LNFH spring out-plants) total potential hatchery parents. Raw assignment success rates (proportion of time the most likely parent pair was the correct parent pair) for hatchery and wild fish were very different from each other: 82.7% and 97.8% for hatchery and wild fish, respectively. When a Δ LOD criterion was used to limit incorrect assignments to ≤ 5%, the total assignment rate dropped to 66.1% for the hatchery fish.

At present, we do not know why parentage assignment success for hatchery fish is lower than that for wild fish, although the proximate cause is likely to be the greater degree of non-independence among loci (linkage disequilibrium) we observed for hatchery fish compared to wild fish. The high correlations of alleles among loci for hatchery fish mean that, in effect, the hatchery fish are being scored for fewer independent loci than are the wild fish. The degree of linkage disequilibrium in a population is affected by the population's effective population size, such that smaller effective population sizes lead to higher levels of linkage disequilibrium. We found a clear relationship between our statistical ability to assign offspring to parents and the spawning population size of the populations that produced the potential parents (Table 6, Figure 3). Regardless of the cause of the difference in assignment rate between hatchery and wild origin fish, we believe it will be necessary to genotype additional loci in order to increase our rate of successful assignment for progeny of hatchery fish.

Table 6 -- Spawning population sizes for cohorts that produced returns to the natural spawning grounds in 2004

Hatchery origin fish

		Parental spawning population size				
Age	Proportion of run in 2004 [3]	females	males	total	Ne [1]	Assignment rate[2]
2	0.36	43	27	70	66	0.84
3	0.43	241	133	374	343	0.91
4	0.20	11	19	30	28	0.80

Natural origin fish

		Parental spawning population size				
Age	Proportion of run in 2004	females	males	total	Ne	Assignment rate
2	0.00					
3	0.04					
4	0.96	282	406	688	666	0.99

[1] Effective population size (Ne) estimated as 4 (Nmales) (Nfemales) / (Nmales + Nfemales). This estimate of Ne includes only effects of unequal number of males and females. Actual Ne will be smaller due to variance in reproductive success within each sex.

[2] Proportion of simulated assignments in which the most likely parent pair was the true parent pair. Simulations were performed using a random subset of 200 from each age-origin group.

[3] A small number of five year old fish were also present in the run, but not included in this table.

In order to evaluate the effect of adding additional loci, we genotyped 223 of the hatchery fish for an additional four loci, for a total of 15 loci altogether. We then conducted parentage simulations using random subsets drawn from these individuals, with sample sizes ranging from 10 to the full 223. Linear regression was then used to examine the relationship between the number of potential (hatchery) parents and the proportion of correct assignments. From this, we estimate that with 15 loci we would be able to achieve ~90% correct assignments if there ~1800 potential hatchery parents with genotypic distributions like those we observed in 2004 (Figure 4, Figure 5). The difference between hatchery and natural fish in assignment rate continued for the 15 locus set (Table 7).

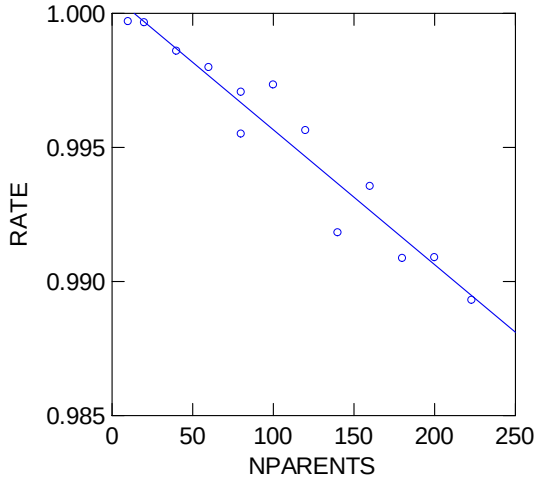

Figure 4 -- Relationship between number of potential parents (hatchery fish only) and the proportion of correct assignments (simulated offspring)

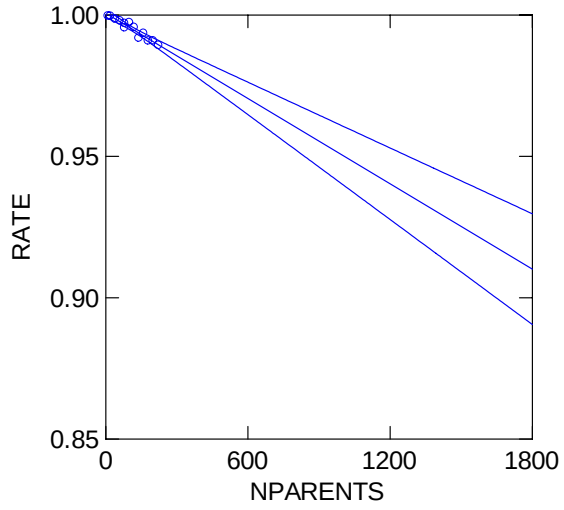

Figure 5 -- Same relationship as plotted in Figure 4, but extrapolated out to 1800 potential parents, with 95% confidence intervals.

Finally, we evaluated the consequences of the difference in assignment rate between hatchery and natural fish to estimation of fitness differences between these two groups. To do this, we used the 2004 Wenatchee spring Chinook parents to simulate 1000 offspring. Offspring were simulated by randomly drawing one male and one female from the parental file and then using Mendelian rules of inheritance (random segregation and independent assortment) to simulate offspring. After offspring were simulated, errors were randomly generated in both the parents at a rate of 1.5% per locus. The resulting data (real potential parents with simulated offspring) were run through the program FAMOZ, which assigns offspring to their most likely parents. After checking the assignments against the known relationships (from the simulations), the assignments were used to generate the mean number of offspring assigned to either hatchery or wild parents, broken down by age and sex. Parents with no progeny assigned were assumed to have zero offspring.

Table 7 -- Parentage assignment success rates for hatchery and wild Wenatchee R. Spring Chinook.

#		assignment succes rate (%)	
Loci	**N**	**Hatchery**	**Wild**
11	840	82.7 (66.1)*	97.8
11	223[R]	95.1	99.4
15	223	98.8	--

* LOD criterion of 1.39 used to limit incorrect assignments to ≤ %5.
[R] randomly chosen subset of adults used to make a direct comparison to the 15 microsatellite dataset (Table 1).

Using an assignment threshold designed to limit the false assignment rate to ~5%, 715 (71.5%) of the offspring were assigned to a set of parents. This is close to the expected assignment rate of 69% (from the FAMOZ simulations). Of the 715 assignments, 38 (5%) were incorrect when compared against the true relationships. This suggests indicates that the basic assignment algorithm is working as expected.

The mean number of assigned progeny from the simulations differed substantially among age and origin classes (Table 8). For females, the dominant age class for both hatchery and wild fish was 1.2, and within this age class wild fish had significantly more simulated progeny assigned than did hatchery fish. For males, the dominant classes were 1.0, 1.1, and 1.2, and hatchery and wild differed significantly in the age distributions. Within the 1.2 age class, wild fish again had significantly more progeny assignments than did hatchery fish. Within the 1.1 age class, however, wild and hatchery fish did not differ significantly in simulated progeny number. When both the 1.1 and 1.2 age classes were combined, wild fish had a significantly higher progeny numbers assigned. The 1.0 age class is limited to hatchery fish, and had a significantly lower progeny numbers assigned than the wild 1.2 age fish. Based on these results, it appears that the non-random assignment among age and origin groups can lead to the appearance of fitness differences among groups, even though the fitness of the groups is in fact the same because the progeny were simulated without reference to age or origin.

Table 8 -- Simulated and actual fitness estimates (mean progeny numbers) by origin, sex, and age
Simulated results from 1000 progeny

		wild				hatchery		
		N	mean	SD	H/W [1]	N	mean	SD
males	age							
	2	0				594	0.30	0.59
	3	27	0.37	0.56	1.08	705	0.40	0.66
	4	387	0.55	0.75	0.55**	90	0.30	0.55
	all	41741	0.54	0.74	0.65**	1415	0.35	0.63
females	4	346	1.35	1.19	0.70**	236	0.94	1.03

Actual results from 73 Chiwawa assignments

		wild					hatchery		
		N	mean	SD	H/W	H/W c[2]	N	mean	SD
males	age								
	2						594	0.002	0.04
	3	27	0.037	0.19	0.38	0.35	705	0.014	0.13
	4	387	0.121	0.42	1.01	1.85	90	0.122	0.52
	all	417	0.12	0.41	0.13	0.21	1415	0.016	0.17
females	4	346	0.15	0.46	0.62	0.89	236	0.093	0.31

[1] Hatchery/wild fitness (mean progeny number of hatchery fish / mean progeny number of wild fish).
[2] Hatchery/wild fitness, corrected by dividing by simulated H/W ratio.
** $p<0.0001$

Of the 166 completely genotyped Chiwawa River parr, we were able to confidently assign 73 (44%) to single pairs of parents. An additional 19 (11%) met the Δ LOD criterion for assignment, but we chose not to assign because they had more than a single incompatible locus. Thirty-five (48%) of the inferred matings involved at least one parent whose carcass was recovered on the Chiwawa River. One inferred mating involved a parent whose carcass was recovered on Nason Creek. The mean number of progeny differed among age and origin classes (Table 8). For males, the parr sample produced far fewer inferred hatchery parents than wild parents. This was entirely due to low progeny numbers for age 2 and 3 fish, age classes which were common for hatchery fish but rare or absent for wild fish (Table 8). After correcting for bias in assignment rates, hatchery females had ~90% as many estimated progeny as wild females (both groups predominantly age 4), and age 4 hatchery males had ~1.8X as many estimated progeny as wild males (Table 8). Note that these results are very preliminary and are unlikely to be statistically meaningful due both to the small number of progeny analyzed and uncertainty about the most appropriate way to analyze the data given the difference in ability to assign parents between the two groups.

We continued to explore the effect of hatchery or wild origin on parental assignment success for the 166 Chiwawa parr. To do this, for each parr, we identified the set of parents whose Δ LOD scores were less than the 5% cutoff criteria. We then divided these groups up into those that contained only potential hatchery parents and those that contained only potential wild parents. For the groups that contained only wild parents, the mean number of likely parents per offspring was 2.15 (Figure 6), suggesting that those progeny that were the products of wild x wild matings were readily assigned to a

single set of potential parents. In contrast, in those groups that contained only hatchery parents, the mean number of parents per progeny was 4.07 (Figure 7), suggesting that progeny produced by hatchery x hatchery matings are more difficult to correctly assign to a single set of parents. These results also suggest the possibility that many of the potential hatchery parents may be close relatives, a hypothesis we are in the process of testing.

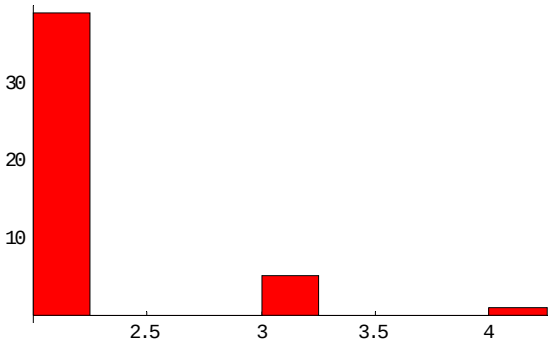

Figure 6 -- Distribution of numbers of high likelihood wild parents

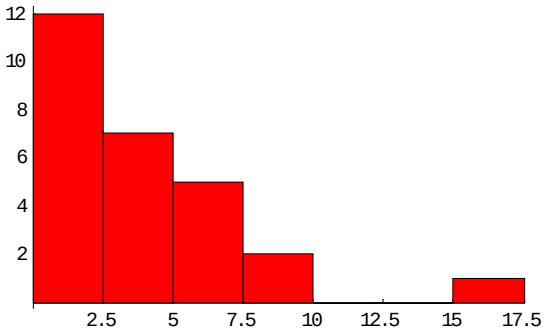

Figure 7 -- Distribution of numbers of high likelihood hatchery parents

Discussion

Population genetic structure appears to exist within Wenatchee River wild spring-run Chinook. In particular, moderate bootstrap support for nodes separating wild populations in the cluster analyses (Figure 1, Figure 2) and the low, but statistically significant pair-wise F_{ST} comparisons between all wild populations (Table 5) indicate that there are significant allele frequencies differences between samples from these three tributaries.

Hatchery and wild spring Chinook also are somewhat genetically distinct from one another. Moderate boot strap support for the node separating two year old hatchery fish from wild fish as well as the separate grouping of the 3 year old hatchery fish (Figure 2) and the low, but statistically significant pair-wise F_{ST} comparisons between hatchery and wild populations (Table 5) indicate a low degree of genetic differentiation between hatchery and wild spring Chinook.

Interestingly, both pair-wise F_{ST} comparisons (Table 5) and cluster analysis using hatchery fish grouped by age (Figure 2) also indicate varying degrees of genetic differentiation between separate cohorts of hatchery and the wild spring Chinook. Genetic differentiation between hatchery and wild fish appears to be greater for younger cohorts of hatchery fish (i.e.- two year old hatchery fish are more genetically distinct from the wild populations compared to either the three or four year old hatchery fish).

At a population-level scale there is statistically significant genetic differentiation between the 2004 Wenatchee River hatchery and wild spring-Run fish (average $F_{ST} = 0.017$) as well as between those two groups and the LNFH Spring-Run fish out-planted in Peshastin Creek (average $F_{ST} = 0.018$, and 0.015, respectively) (Table 5). However, at the level of the individual, the ability to assign a given spring Chinook of unknown origin to the Wenatchee River hatchery or wild population was poor (Table 2). Similarly, individual assignment to either the Wenatchee River or LNFH hatchery baseline populations was poor (Table 2). This reflects the generally low level of genetic distinctiveness of hatchery spring Chinook within the upper Columbia River Basin (Ford et al. 2001). Individual assignment between spring and summer runs was excellent, however.

An earlier report by Ford et al. (2001) using data collected by WDFW at 44 allozyme loci indicated little evidence of genetic distinctiveness between Chiwawa River and Nason Creek spring Chinook. Their Neighbor-Joining phenogram (not shown) of locality by brood year samples based on Nei's unbiased genetic distance (Nei 1987) had, in general, very low bootstrap support for the nodes separating either tributary or for the even and odd brood year Chiwawa River samples. The only strong support (98%) obtained was for the node separating the White River populations from all other populations. Within the Wenatchee River system, contingency analysis (Weir 1996, p. 163) revealed significant differences in allele frequency distributions between only the Chiwawa and White River samples, but not between the 1988 Chiwawa River and Nason Creek samples. While stray hatchery fish were removed from the previous analyses, the hatchery and wild populations were considered to be effectively part of the same

population (Ford et al. 2001). The disparate conclusions reached by this and the previous study are not entirely surprising. First, allozymes are less polymorphic than microsatellites and are less powerful for detecting small differences among populations. Second, the small sample sizes used for Chiwawa River and Nason Creek in the earlier study (average N = 30 and 23, respectively) may have contributed to the inability to resolve their already close genetic relationship. Third, Chiwawa River hatchery adults stray within the Wenatchee River Basin. For instance, in 1997, 33% of the adults sampled on Nason Creek had Chiwawa River hatchery tags (Ford et al. 2001). Genetic diversity between the two tributaries would be low and thus difficult to detect due to gene flow mediated by stray Chiwawa River hatchery fish.

A large percentage of hatchery and wild Chinook (91% and 76%, respectively) evaluated were not recovered as carcasses on the spawning grounds. Increased capacity to detect passive integrated transponder (PIT) tagged hatchery and wild adults on the spawning grounds will increase the fraction of fish that can be grouped by tributary. Potential benefits of doing so include increased resolution of genetic distinctiveness within hatchery and wild populations, and increased likelihood of discriminating potential differences in reproductive fitness between hatchery and wild Chinook that are associated with spawning habitat usage within the Wenatchee River Basin.

Wenatchee River hatchery spring Chinook had somewhat different patterns of within-sample variation than were observed for the wild fish samples. The high proportion of microsatellite loci with significantly negative F_{IS} values compared to that expected under HWE (Table 3A) and the very high level of linkage disequilibrium (48 of 55 pair-wise comparisons of loci) for hatchery fish may reflect differences in population size between the hatchery and wild spawning populations in previous years (Figure 3, Table 6).

One surprising result of our initial parentage analysis was the finding that, at least for the 2004 spawners, there was a significant difference in our ability to identify unique pairs of hatchery fish as parents compared to wild fish. The ability to successfully assign fish as parents appears to be correlated with the size of the spawning populations that produced the potential parents in 2004 (Figure 3). The hatchery fish returning in 2004 came from relatively small groups of spawners compared to the wild fish returning in 2004, and this appears to have an influence on our ability to statistically assign offspring to hatchery-origin parents. If this effect is not accounted for, it would seriously bias the estimates of relative fitness of hatchery and wild fish. For example, in simulations in which fitness was expected to be, on average, the same between hatchery and wild fish, our estimates of fitness between the two groups differed significantly due to differences in "assignability" between hatchery and natural fish (Table 8). This effect is also evident in the actual assignments of Chiwawa River parr (Figure 6, Figure 7).

There are two potential ways in which we can approach the problem of differences in parentage assignment rates between hatchery and wild fish. First, we could increase the number of loci scored. Based on the results reported here, we believe we would need to score at least an additional four, and perhaps more, loci in order to bring the successful assignment rate to >95% for hatchery fish. Another potential approach is use partial

assignment methods (e.g., Morgan and Conner 2001). These methods assign offspring fractionally to parents in proportion to their likelihoods, and would have the effect of correcting for differences in assignability between groups. The disadvantage of these methods is that they do not actually estimate a single pedigree, so there is no ability to track lineages through multiple generations. Our plan is to therefore increase the number of loci scored in order to improve assignment success for hatchery origin fish. We will also continue to explore methods for correcting the bias introduced by differences in assignability.

Even with the 11 locus data set and the very small number of progeny sampled, some differences in fitness between hatchery and natural fish are readily apparent. In particular, the large numbers of age 2 and age 3 hatchery males appeared to have very low fitness based on the current progeny sample (Table 8), a result consistent with the spawning ground observation data (see Chapter 4).

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Chapter 3

Spawning distribution and redd characterization of hatchery and natural origin spring Chinook in the Wenatchee River Basin

Abstract

Spawning ground surveys in the upper Wenatchee River Basin were used to evaluate spawn timing and distribution, redd microhabitat characteristics, and prespawn survival of hatchery and naturally produced fish. In 2005, the composite population of spring Chinook redds were distributed similarly to that of years past. A total of 818 redds were found upstream of Tumwater Dam, of which the female origin was identified on 335 redds. Based on redd counts, the survival of spring Chinook from Tumwater Dam to the spawning grounds was estimated at 42.4%. After correction for carcass recovery bias, no differences were found in the estimated age composition or the proportion of hatchery and natural origin fish of the estimated spawning population compared to population sampled at Tumwater Dam. Hatchery origin fish spawned in significantly lower elevations of the Chiwawa River and Nason Creek than natural origin fish. No difference in spawning timing of hatchery and natural origin spring Chinook was detected. Microhabitat variables were measured on 137 redds, which included 107 and 30 constructed by hatchery and natural origin females, respectively. No differences were found in any of the redd characteristics examined.

Introduction

Hatchery fish may not produce as many progeny as natural fish in natural environments for a variety of reasons. For example, hatchery fish may select inappropriate areas to spawn (e.g., poor water flows or depths), spawn at inappropriate times (Chandler and Bjornn 1988; Leider et al. 1984; Nickelson et al. 1986), construct redds inappropriately (e.g., dig redds that are too shallow to withstand flooding), and die before gametes can be released. Non-representative broodstock selection can skew run timing. Collecting, holding, and spawning salmon broodstock can remove selection pressures (e.g., competing for mates, digging deep redds, maintaining energy stores and other factors) for spawning in the natural environment. Any deviation from naturally produced fish can be assumed to be maladaptive in natural environments.

The reproductive success of hatchery origin fish may be lower than natural origin fish if hatchery origin fish spawn in suboptimal locations. For example, hatchery fish may spawn in unproductive tributaries, portions of tributaries that are suboptimal, or at microhabitats that are suboptimal. If acclimation ponds are located in suboptimal spawning locations and fish home back to these locations, then the reproductive success of hatchery origin fish may be compromised. In short, reproductive success of hatchery origin fish could be compromised even if they are genetically and behaviorally identical to natural origin fish.

The objective of this Chapter is to determine if differences in spawn timing, spawning distribution between and within tributaries, micro site selection, and redd morphologies exist in the upper Wenatchee Basin. Using information collected during spawning ground surveys the relative survival of hatchery and natural origin fish to spawning will be calculated. This information will be used in conjunction with the demographic and genetic data to examine the relative reproductive success of hatchery and natural origin fish spawning naturally in the upper Wenatchee Basin.

Methods and Materials

Spawning ground surveys

All spring Chinook spawning habitat (Mosey and Murphy 2002) in the Upper Wenatchee River (29 rkm), Chiwawa River (49.7 rkm), White River (24.5 rkm), Little Wenatchee River (37.9 rkm) and Nason Creek (24.1 km) was surveyed a minimum of once a week by raft or foot. Rafting was conducted on larger streams (Upper Wenatchee River) or reaches where the flow was too great for foot surveys to be conducted safely (lower Chiwawa River). During periods of peak spawning, one and two person crews surveyed each stream reach a minimum of twice a week. Two or three person crews surveyed reaches, which were selected for redd microhabitat measurements. Historical spring Chinook spawning ground reaches were surveyed to maintain consistency with previous surveys (Appendix C).

When new redds were found, the origin and fork length of the female was determined by live PIT tag detection. Post spawned females guarding redds were scanned for PIT tags using an underwater antenna mounted on an extension handle. Using this technique, we were able to identify an individual fish and correlate the PIT tag with biological data collected at Tumwater Dam. Each redd was assigned a unique GPS waypoint, marked with surveyors flagging attached to nearby vegetation, and recorded in a field notebook. Each flag was labeled with the appropriate reach and redd number, date, redd location, and the surveyor's initials. In addition, a blue flag was used to indicate if the origin of the female was successfully determined. Redd microhabitat variables would later be measured only on completed redds which the female origin was known.

Carcass surveys

Biological data was recorded from all spring Chinook carcasses encountered during spawning ground surveys. Surveys for carcasses continued after spawning was completed until no live fish were observed within the reach. A unique GPS waypoint was assigned to every carcass and the PIT tag code of each carcass was recorded. A genetic tissue sample was collected from those carcasses without a PIT tag (i.e., lost tag before spawning). In addition, the fork and POH length (to the nearest cm), scales, and snouts from all fish were collected. Snouts may contain coded wire tags and due to a low

mark rate of age-4 hatchery fish (i.e., not adipose fin clipped) all snouts were collected and the presence of a CWT would be determined at a later date. The number of eggs retained in the body cavity was counted for females with an intact body cavity. Finally, each carcass was mark sampled by removing the caudal fin to prevent double sampling.

Redd microhabitat data

Microhabitat characteristics of redds were measured in selected reaches of the Chiwawa River and Nason Creek. Based on data collected in 2004, these reaches were selected because of the greater probability that hatchery and natural origin redds would be created in these reaches. Microhabitat characteristics were measured for redds of known female origin. The maximum length and width of the redd was recorded to the nearest 0.1 m. Water depth measurements (nearest cm) were taken at the upstream side of the bowl, the deepest point within the bowl, the upstream side of the tail, the shallowest point of the tail, the downstream side of the tail, and left and right side of the redd (Figure 1).

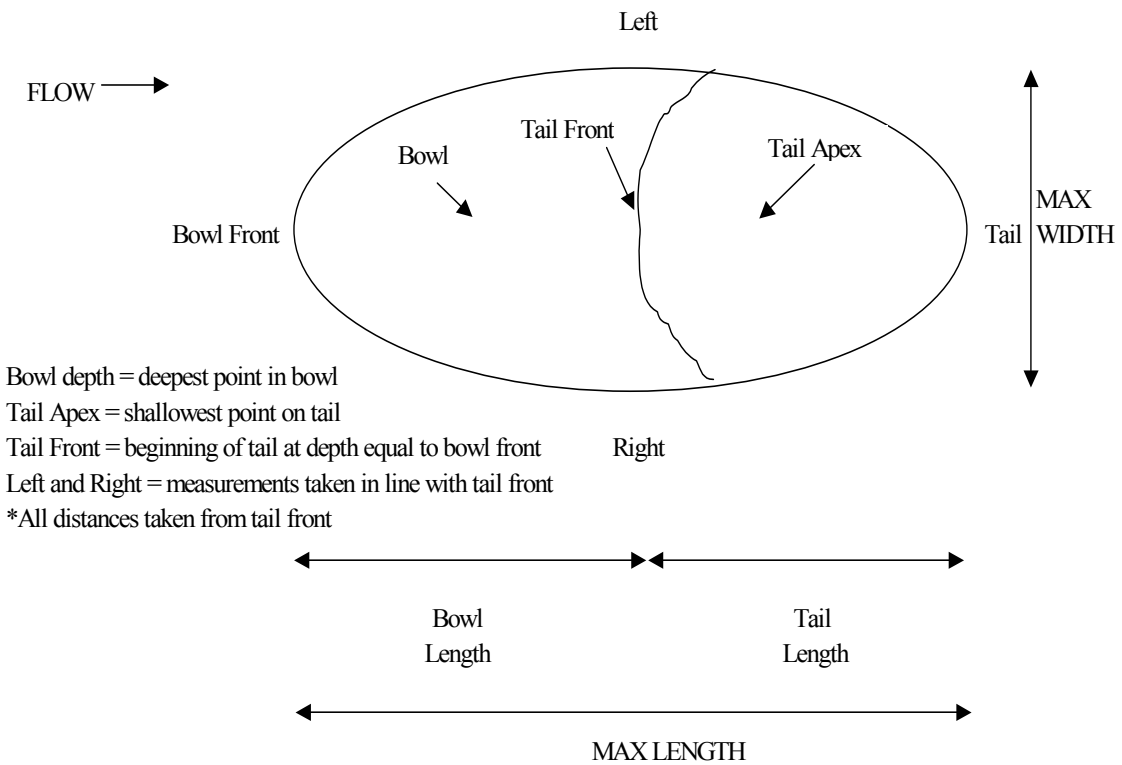

Figure 1. Locations of redd microhabitat characteristic measurements.

Water velocity (m/s) was measured using a Marsh McBirney Model 2000 or Swoffer Model 2100 flow meter. Water velocity was recorded at the upstream side of the bowl (60% depth), maximum depth of the bowl (60% depth), upstream side of the tail (60% depth, surface, bottom), downstream side of the tail (60% depth), and the left and right side of the redd (60% depth). Average redd water depth was the calculated from water depth measurements recorded at the left and right side of the redd and the upstream side of the bowl. Bowl depth was calculated by subtracting the average depth from the maximum depth of the bowl. Average tail depth was calculated by subtracting the depth

at the tail apex for the average depth measured on the right and left side of the redd. The distance to the nearest redd (m) and nearest cover type (i.e., riffle, pool, large woody debris, boulder, vegetation or bank) was also measured. Substrate composition (i.e., sand, gravel, cobble, or boulder) was visually estimated for the bowl and tail. Temperature (C°) was also recorded during microhabitat measurements or later downloaded from temperature probes.

Data Analysis

Non-parametric statistical tests were used when assumptions of parametric tests could not be met. A Chi-square test was used to test for any differences in prespawn mortality of hatchery and naturally produced spring Chinook by comparing the proportion of hatchery and naturally produced fish observed at Tumwater Dam to the spawning population. A Chi-square test was also used to examine the age compositions of hatchery and natural fish at Tumwater Dam and spawning grounds.

Hatchery fish destined for the spawning grounds upstream of Tumwater Dam should return to the Chiwawa River. Unfortunately, freezing conditions in the Chiwawa River during the winter force the use of Wenatchee River water at the Chiwawa acclimation ponds during the month of December through February. As a result, returning adults have poor homing fidelity and spawn throughout the basin. Spawning distribution of hatchery and natural origin spring Chinook was analyzed using carcass recovery location (rkm) as the dependent variable. Differences in the spatial distribution of hatchery and naturally produced fish recovered on the spawning grounds were tested using a Kruskal-Wallis ANOVA (KW). Significant differences in spawning distribution were analyzed using a multiple comparison of ranks test to determine the source of differences.

Based on historical data, specific reaches were selected in both the Chiwawa River and Nason Creek that had the highest probability of containing the greatest number of both hatchery and natural origin spawners. Data collected from these reaches were used in the analysis of spawn timing and redd microhabitat. Spawning timing was assessed at the hatchery during routine spawning operations and on the spawning grounds. A KW test was used to compare the spawn timing of hatchery and natural origin female spring Chinook for both redds and carcasses because statistical assumptions of data normality and equal variances could not be met. Relationships between run timing and spawn timing were examined with a Pearson Product moment correlation statistic.

Microhabitat characteristics of redds constructed by hatchery and natural origin fish were compared using ANOVA. Several variables (i.e., redd depth, bowl front depth, redd area, bowl length and female fork length) were log-transformed to meet assumptions of data normality and equal variances. Correlation analysis was performed to examine the relationship between fish size and redd microhabitat characteristics. All statistical tests were performed at a significance level (α) of 0.05.

Spawning Ground Surveys

Chiwawa River

A total of 332 redds were found in the Chiwawa River basin in 2005. Of those redds, 330 redds (99.4%) were found in the Chiwawa River, while only 2 redds (0.60%) were found in tributaries (i.e., Chikamin and Rock creeks). Redds were constructed first in the higher elevation reaches and progressively downstream as the spawning season ended (Table 1). Spawning began the first week of August and continued until third week of September, with peak spawning occurring during the fourth week of August (Appendix B). The origin of the female constructing the redd was determined for 117 redds (35.2%). Of which, 92 redds were hatchery and 25 redds were naturally produced.

Table 1. Number of spring Chinook redds located within historical reaches during spawning ground surveys on the Chiwawa River in 2004 and 2005.

Survey Week	Historical Reach (rkm)						Totals redds
	0-20	20-32	32-37	37-43	43-45	45-51	
				2004			
07/25	0	0	0	0	0	0	0
08/01	0	3	2	0	0	3	8
08/08	0	20	2	14	1	0	37
08/15	2	10	1	10	4	0	27
08/22	1	33	1	11	11	10	67
08/29	10	40	0	12	5	6	73
09/05	19	5	0	1	0	0	25
09/12	4	0	0	0	0	0	4
09/19	0	0	0	0	0	0	0
09/26	0	0	0	0	0	0	0
Total	36	111	6	48	21	19	241
				2005			
07/31	0	0	0	0	0	0	0
08/07	0	1	0	1	2	1	5
08/14	0	7	0	7	4	5	23
08/21	2	34	1	6	5	8	56
08/28	19	80	5	15	5	5	129
09/04	18	42	1	6	1	2	70
09/11	15	12	0	0	0	0	27
09/18	21	1	0	0	0	0	22
09/25	0	0	0	0	0	0	0
Total	75	177	7	35	17	21	332

Nason Creek

During surveys on Nason Creek a total 193 redds were found in 2005. The temporal distribution of redds was similar to that observed on the Chiwawa River. Spawning began earliest in the uppermost reaches and progressively downstream later (Table 2). Spawning activity began during the fourth week of July and continued until the third week of September, with peak spawning occurring in the second week of September (Appendix B). The origin of the female constructing the redd was determined for 106 redds (54.9%). Of those redds, 82 were hatchery and 24 were naturally produced.

Table 2. Number of spring Chinook redds located within historical reaches during spawning ground surveys on Nason Creek in 2004 and 2005.

Week	Historical reach (km)				Total redds
	0-7	7-14	14-22	22-26	
			2004		
07/25	0	0	0	0	0
08/01	0	0	0	2	2
08/08	0	0	2	2	4
08/15	0	0	8	6	14
08/22	0	1	7	5	13
08/29	5	11	31	13	60
09/05	35	16	1	0	52
09/12	10	2	0	0	12
09/19	3	4	5	0	12
09/26	0	0	0	0	0
Total	53	34	54	28	169
			2005		
07/31	0	0	0	1	1
08/07	0	0	0	1	1
08/14	0	0	1	0	1
08/21	0	0	5	6	11
08/28	4	3	18	6	31
09/04	32	17	13	4	66
09/11	71	3	0	0	74
09/18	1	7	0	0	8
09/25	0	0	0	0	0
Total	108	30	37	18	193

Upper Wenatchee River

A total of 143 redds were located by raft or on foot on the upper Wenatchee River in 2005. Only the two highest elevation reaches were surveyed based on historical spring Chinook spawning ground surveys. The temporal distribution of redds was confined to the upper most reach, with no redds found in the lower reach (Table 3). Spawning began the fourth week of August and continued until the third week of September, with peak spawning occurring during the third week of September (Appendix B). Female origin was determined for 6 redds (4.2%). Of those redds, all were constructed by hatchery females.

Table 3. Number of spring Chinook redds located within historical reaches during spawning ground surveys on the Wenatchee River in 2004 and 2005.

Survey Week	Historical Reach (rkm)		Totals Redds
	59-81	81-90	
	2004		
08/22	0	0	0
08/29	0	0	0
09/05	0	11	11
09/12	0	26	26
09/19	1	8	9
09/26	0	0	0
Total	1	45	46
	2005		
08/21	0	0	0
08/28	0	1	1
09/04	0	34	34
09/11	0	20	20
09/18	0	88	88
09/25	0	0	0
Total	0	143	143

White River

Survey crews found a total of 86 redds in the White River basin in 2005. Of those, 83 redds (96.5%) were found in the White River, while 3 redds (3.5%) were found in the Napeequa River. Redds were distributed primarily in the mid elevation reach (Table 4). Spawning activity started during the second week of August and continued until the third week of September, with peak spawning occurring in the fourth week of August

(Appendix B). The origin of the female was determined for 71 redds (82.6%). Of the redds in which origin was determined, 55 were hatchery and 16 were naturally produced.

Table 4. Number of spring Chinook redds found within historical reaches during spawning ground surveys on the White River in 2004 and 2005. Three redds were found between rkm 11- 18 in the Napeaqua River in 2005.

Survey	Historical Reach (rkm)			Totals
Week	11-18	18-22	22-24	redds
		2004		
08/08	0	0	0	0
08/15	0	0	0	0
08/22	0	5	0	5
08/29	0	5	0	5
09/05	0	7	1	8
09/12	0	3	1	4
09/19	0	0	0	0
09/26	0	0	0	0
Total	0	20	2	22
		2005		
07/31	0	0	0	0
08/07	0	0	0	0
08/14	0	1	0	1
08/21	0	14	1	15
08/28	3	33	0	36
09/04	3	13	0	16
09/11	2	15	0	17
09/18	0	1	0	0
09/25	0	0	0	1
Total	8	77	1	86

Little Wenatchee River

A total of 64 redds were found during spawning on the Little Wenatchee River in 2005. The temporal distribution of redds began at the higher elevation reach and progressed into the lower reach (Table 5). Active spawning began the second week of August and continued until the third week of September, with peak spawning occurring during the fourth week of August (Appendix B). Female origin was determined for 35 redds (54.7%). Of those redds, it was determined that 23 were hatchery and 12 were naturally produced.

Table 5. Number of spring Chinook redds located within historical reaches during spawning ground surveys on the Little Wenatchee River in 2004 and 2005.

Survey Week	Historical Reach (rkm)			Totals redds
	5-9	9-15	15-21	
		2004		
08/08	0	0	0	0
08/15	0	4	0	4
08/22	0	2	0	2
08/29	1	4	0	5
09/05	1	1	0	2
09/12	0	0	0	0
09/19	0	0	0	0
09/26	0	0	0	0
Total	2	11	0	13
		2005		
07/31	0	0	0	0
08/07	0	0	0	0
08/14	0	1	0	1
08/21	0	3	0	3
08/28	10	10	0	23
09/04	7	14	0	21
09/11	7	8	0	12
09/18	1	3	0	4
09/25	0	0	0	0
Total	25	39	0	64

Carcass Surveys

Chiwawa River

Of the 391 carcasses sampled throughout the Chiwawa River basin in 2005, scale analysis determined the proportion of hatchery and naturally produced fish was 83% (N = 321) and 17% (N = 64), respectively. Based on a male to female ratio derived from the broodstock of 0.8 to 1 (i.e., 1.8 fish per redd), spawning escapement was estimated to be 496 hatchery and 102 naturally produced fish. All snouts were collected and sent to the WDFW CWT lab in Olympia to determine if CWTs were present and then decoded. The abundance of hatchery carcasses was highest in the lowest reach (rkm 0.0-20.0), which was near the acclimation pond, while the naturally produced carcass distribution was

more similar to the redd distribution (Table 6). Presumably, the higher abundance of hatchery fish in the lower reaches was influenced by the location of the acclimation pond (See Spawning Distribution).

Table 6. Proportion of redds and carcasses by reach in the Chiwawa River in 2004 and 2005.

River (km)	2004				2005			
	Redds	Carcasses			Redds	Carcasses		
		Hatchery	Natural	Total		Hatchery	Natural	Total
0-20.0	0.15	0.27	0.14	0.41	0.23	0.44	0.04	0.48
20.0-32.0	0.46	0.13	0.29	0.41	0.53	0.34	0.09	0.43
32.0-37.0	0.02	0.01	0.01	0.02	0.02	0.02	0.01	0.03
37.0-43.0	0.20	0.00	0.07	0.07	0.11	0.02	0.01	0.03
43.0-45.0	0.09	0.01	0.04	0.05	0.05	0.01	0.01	0.02
45.0-51.0	0.08	0.02	0.02	0.04	0.06	0.01	0.01	0.01

Nason Creek

A total of 217 carcasses were recovered in Nason Creek during 2005. Scale analysis determined the proportion of hatchery and naturally produced fish was 81% (N =172) and 19% (N = 40), respectively. All carcass snouts were collected and sent to the WDFW CWT lab to extract and decode potential CWTs. All hatchery fish in Nason Creek were considered strays because hatchery programs are currently not releasing fish into Nason Creek. An estimated 281 hatchery and 66 naturally produced fish spawned in Nason Creek during 2005. The largest proportion of hatchery carcasses were recovered in the lowest reach, while naturally produced carcasses were more evenly distributed (Table 7).

Table 7. Proportion of redds and carcasses by reach in the Nason Creek in 2004 and 2005.

River (km)	2004				2005			
	Redds	Carcasses			Redds	Carcasses		
		Hatchery	Natural	Total		Hatchery	Natural	Total
0-7.0	0.31	0.22	0.15	0.37	0.56	0.61	0.08	0.69
7.0-14.0	0.20	0.13	0.19	0.32	0.16	0.08	0.02	0.10
14.0-22.0	0.32	0.06	0.10	0.16	0.19	0.10	0.05	0.16
22.0-26.0	0.17	0.02	0.13	0.15	0.09	0.02	0.03	0.05

Upper Wenatchee River

In the upper Wenatchee River a total of 120 carcasses were recovered during spawning ground surveys in 2005. Scale analysis determined the proportion of hatchery and natural origin fish recovered was 97% (*N* = 113) and 3% (*N* = 3), respectively. All snouts potentially containing CWTs were recovered and sent to the WDFW CWT lab in Olympia to be extracted and decoded. The number and composition of the spawning population was estimated at 249 hatchery and 8 natural origin fish. Carcass distribution of both hatchery and naturally produced fish was similar to redd distribution (Table 8).

Table 8. Proportion of redds and carcasses by reach in the Upper Wenatchee River in 2004 and 2005.

River (km)	2004				2005			
	Redds	Carcasses			Redds	Carcasses		
		Hatchery	Natural	Total		Hatchery	Natural	Total
60.0-81.0	0.02	0.00	0.06	0.06	0.00	0.03	0.00	0.03
81.0-90.0	0.98	0.67	0.28	0.94	1.00	0.95	0.03	0.97

White River

Of the 52 carcasses recovered in the White River during 2005, scale analysis determined the proportion of hatchery and natural origin fish was 78% (*N*=38) and 22% (*N*=11) respectively. All carcass snouts were collected and sent to the WDFW CWT lab to extract and decode potential CWTs. Spawning ground surveys in the White River were conducted at a greater frequency (twice a week) in collaboration with a captive broodstock program funded by Grant County PUD. As a result, the proportion of unique PIT tag recaptures (*N* = 102; 66%) was greater than the number of carcasses recovered (34%). Based on the proportion of hatchery (69%) and natural fish (31%) detected on the spawning grounds, the number of fish on the spawning grounds was 107 and 48, respectively. Hatchery carcass distribution occurred primarily within the reach where a majority of the redds were located (Table 9).

Table 9. Proportion of redds and carcasses by reach in the White River in 2004 and 2005.

River (km)	2004				2005			
	Redds	Carcasses			Redds	Carcasses		
		Hatchery	Natural	Total		Hatchery	Natural	Total
11.0-18.0	0.00	0.00	0.10	0.10	0.09	0.06	0.02	0.08
18.0-22.0	0.91	0.10	0.80	0.90	0.90	0.71	0.20	0.92
22.0-24.0	0.09	0.00	0.00	0.00	0.01	0.00	0.00	0.00

Little Wenatchee River

Of the 48 carcasses recovered in the Little Wenatchee River during 2005, scale analysis indicated that the proportion of hatchery and natural origin fish was 64% (*N*=30) and 36% (*N*=17), respectively (Table 10). The estimated spawning population was 74 and 41 hatchery and naturally produced fish, respectively.

Table 10. Proportion of redds and carcasses by reach in the Little Wenatchee River in 2004 and 2005.

River (km)	2004				2005			
	Redds	Carcasses			Redds	Carcasses		
		Hatchery	Natural	Total		Hatchery	Natural	Total
5.0-9.0	0.15	0.00	0.00	0.00	0.39	0.21	0.09	0.30
9.0-15.0	0.85	0.00	1.00	1.00	0.61	0.43	0.28	0.70
15.0-21.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

PIT Tag Retention and Detectability

PIT tag retention by adult spring Chinook was higher than in 2005 (93%) than observed in 2004 (85%). Additional training on proper technique and placement conducted early in the field season likely contributed to the higher retention rates. In 2005, both 20 mm and 12 mm PIT tags were inserted in adults, while only 12 mm PIT tag were inserted into jacks and precocious males. Larger PIT tags should result in higher detections rates on the spawning grounds, however potentially lower retention rates may negate any potential benefits. The 20 mm and 12 mm PIT tags were inserted in adult spring Chinook systematically throughout the run (i.e., every 25 fish) in order to compare detection and retention rates on the spawning grounds. No difference was detected in detection rates on the spawning grounds between the 20 mm and 12 mm PIT tags ($\chi^2 = 2.83$, df = 1, P = 0.09). Furthermore, no difference in PIT tag retention was detected in either age-4 (χ^2 = 0.20, df = 1, P = 0.65) or age-5 ($\chi^2 = 0.005$, df = 1, P = 0.79) spring Chinook. New generation 12 mm PIT tag are now available that have a greater range in detection and should result in a higher detection rate on the spawning grounds.

Spring Chinook Spawning Ground Surveys Downstream of Tumwater Dam

Spring Chinook spawn in limited numbers downstream of Tumwater Dam. Smolts produced from Peshastin Creek and the Icicle River may be captured during smolt sampling in 2006. Therefore, it is important to include potential production from these streams in the future sampling designs. Chelan County Public Utility District (CCPUD) personnel conducted the spawning ground surveys and sampled carcasses recovered during surveys using similar methodologies previously described.

Icicle Creek

A total of 8 redds were found during spawning ground surveys in 2005. Historically, fish recovered on the Icicle River originate from the Leavenworth National Fish Hatchery (LNFH), which is also located on the Icicle River. Of the 2 carcasses sampled, scale analysis determined that both were hatchery origin (100%). The spawning population was estimated at 14 hatchery fish. Of the hatchery fish sampled, one snout containing a CWT was sent to the WDFW CWT lab in Olympia to be extracted and decoded.

Peshastin Creek

CCPUD personnel found 3 redds in Peshastin Creek and Ingalls Creek. However, no carcasses were recovered in 2005. No hatchery adults were expected to return to Peshastin Creek in 2005 (i.e., no hatchery releases). Therefore, the spawning population was assumed to be natural origin fish ($N = 5$).

Spawning Ground Summary

Composition of fish on the spawning grounds for each stream was calculated based on the number of redds multiplied by the fish per redd values. The proportion of hatchery and natural origin fish was calculated by multiplying the proportion of carcasses recovered within each reach. The composition of the spawning population upstream of Tumwater Dam was 80% hatchery and 20% naturally produced (Table 11). Sampling at Tumwater Dam indicated the proportion of hatchery and natural origin fish available for spawning upstream of Tumwater Dam was 87% and 13%, respectively. The estimated composition of the spawning population upstream of Tumwater Dam was significantly different than the population sampled at Tumwater Dam ($\chi^2 = 11.53$, df = 1, $P<0.001$). In 2005, 99% of the spring Chinook redds were found upstream of Tumwater Dam. Based on the number of potential spawners at Tumwater Dam ($N = 3{,}475$) and the estimated spawning population, the survival to spawning was 42.4% (Table 11).

Table 11. Number of redds, proportion of population recovered as carcasses, and the estimated number of hatchery and natural origin fish, based on scale samples from carcasses or PIT tag recaptures that spawned in the upper Wenatchee River Basin in 2004 and 2005.

River	Number of redds	Sample Rate	Number of fish		
			Hatchery	Natural	Total
		2004			
		Upper Wenatchee Basin			
Chiwawa	241	0.2086	371	487	858
Nason	169	0.3669	217	290	507
Little Wenatchee	13	0.0256	0	39	39
White	22	0.1969	7	59	66
Wenatchee	46	0.1667	97	41	138
Subtotal	491		692	916	1,608
		Lower Wenatchee Basin			
Icicle	30	0.2963	50	4	54
Peshastin	55	0.4590	99	0	99
Subtotal	94		149	4	153
Wenatchee Basin Total	585		841	920	1,761
		2005			
		Upper Wenatchee Basin			
Chiwawa	332	0.6109	463	135	598
Nason	193	0.6182	270	78	348
Little Wenatchee	64	0.4138	75	41	116
White	86	0.3333	119	34	153
Wenatchee	143	0.4615	251	7	258
Subtotal	818		1,178	295	1,473
		Lower Wenatchee Basin			
Icicle	8	0.1429	14	0	14
Peshastin	3	0.0000	0	5	5
Subtotal	11		14	5	19
Wenatchee Basin Total	829		1,221	270	1,491

Differences in the expected and observed composition of spawners may be attributed to either differential mortality or biases in the carcasses recovered on the spawning grounds. Carcasses were recovered in similar proportions to the spawning populations (See Carcass Recovery Section in this Chapter). The age composition of the hatchery and natural spring Chinook was different (See Chapter 1). If age composition of hatchery and natural fish is different and carcass recovery probability unequal, the estimated proportion of hatchery and natural fish on the spawning grounds would be biased towards the group of fish with the greater proportion of larger or older fish. Zhou (2002) reported that the probability of carcass recovery was size dependent and the abundance of smaller fish (i.e., age-3) was negatively biased by 21.1% and larger fish (i.e., age-5) was positively biased by 16.2%. In that study age-4 fish, the dominant age class in the Wenatchee Basin, was positively biased only 1.4%. These results support the observed differences in age distribution between Tumwater Dam and carcasses recovered on the spawning ground.

In the Wenatchee Basin, the proportion of carcasses recovered in each age class was also size dependent (i.e., age-2 = 0.0; age-3 = 0.182; age-4 = 0.438; age-5 = 0.495) and the expected and observed age composition of carcasses recovered on the spawning grounds was significantly different than that observed at Tumwater Dam ($\chi^2 = 149.6$, df = 3, $P < 0.001$). Excluding age-2 fish from the analysis (i.e., recovery probability of age-2 fish was zero) did not influence the results ($\chi^2 = 21.2$, df = 2, $P < 0.001$). The mean carcass recovery probability was calculated using the formula provided in Zhou (2002), except the length measurement used was post-orbital to hypural plate (POH) instead of mid-eye to posterior scale (MEPS). Because carcass recovery probabilities were calculated for each age class and not individual fish, the difference in POH and MEPS should not affect the results. The estimated age composition of the spawning population was calculated by dividing the number of carcasses by the mean recovery probability (Table 12). No difference was found between the age composition of fish at Tumwater Dam and the estimated age composition of hatchery spawners ($\chi^2 = 5.3$, df = 2, $P = 0.07$), natural origin spawners ($\chi^2 = 2.4$, df = 2, $P = 0.30$), or when combined ($\chi^2 = 1.2$, df = 2, $P = 0.56$). These results suggest that there is no differential survival of hatchery and natural origin fish from Tumwater Dam to the spawning grounds. However, mortality may be quite high between the time that fish are sampled at Tumwater Dam and when spawning occurs.

Table 12. Age composition of spring Chinook at Tumwater Dam destined for the spawning grounds and the age composition of the carcasses recovered from the spawning grounds. The estimated proportion of fish on the spawning grounds was calculated from the number of carcasses recovered and the recovery probability.

	Tumwater Dam		Carcasses		Recovery Probability	Estimated Proportion
	N	%	*N*	%		
			2004			
Age-3	771	0.412	92	0.245	0.064	0.434
Age-4	1,086	0.581	279	0.744	0.150	0.561
Age-5	13	0.007	4	0.011	0.218	0.006
			2005			
Age-3	137	0.040	25	0.017	0.063	0.043
Age-4	3,200	0.933	1,401	0.952	0.161	0.934
Age-5	93	0.027	46	0.031	0.213	0.023

Spawning Distribution

Differences were detected in the distribution of hatchery and natural origin female spring Chinook in both the Chiwawa River (Figure 2; df = 3, H = 26.3, $P < 0.001$) and Nason Creek (Figure 3; df = 3, H = 24.5, $P < 0.001$). The spawning distribution of both male and female hatchery spring Chinook was more constrained than that of natural origin fish. Natural origin male spring Chinook exhibited the greatest distribution of all groups. Natural origin female spring Chinook spawned with a greater proportion of natural origin spring Chinook. No differences in spawning distribution were found in the Little Wenatchee River or White River, probably due to the limited length of stream with suitable spawning habitat. The spawning distribution in the upper Wenatchee River was not analyzed because only three natural origin carcasses (2.7% of the estimated spawning population) were recovered.

Figure 2. Mean carcass recovery locations of hatchery and natural origin spring Chinook in the Chiwawa River in 2005. Vertical bars denote 95% confidence intervals.

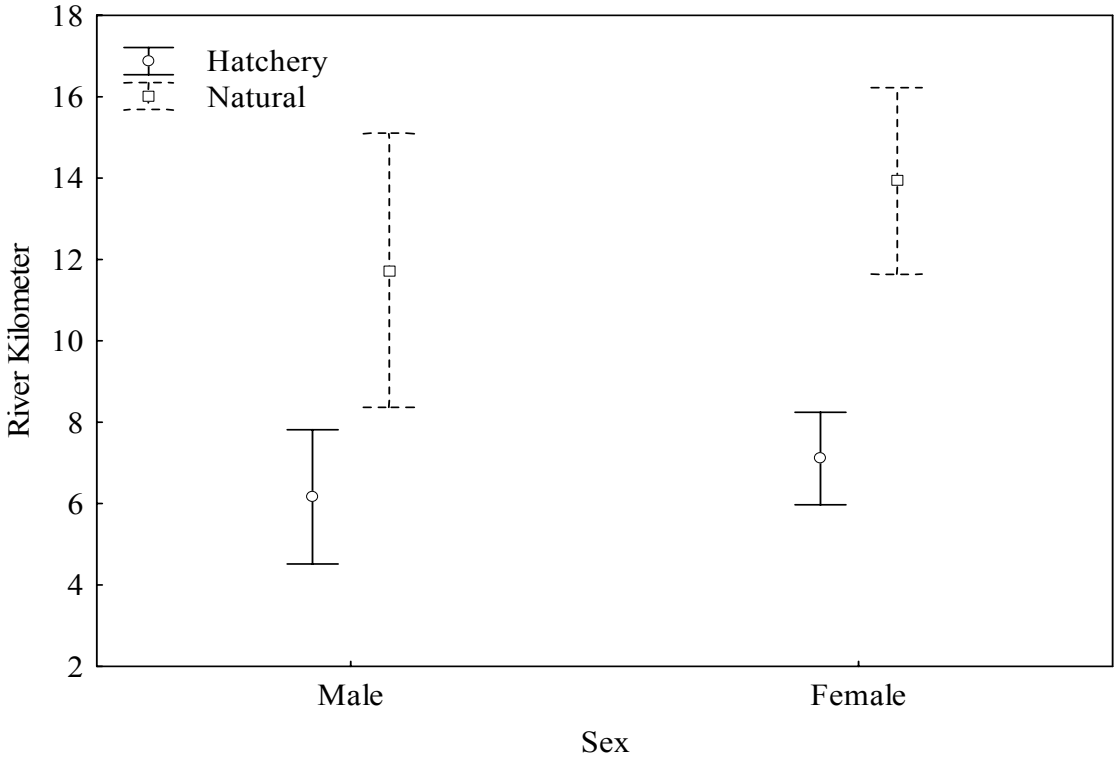

Figure 3. Mean carcass recovery locations of hatchery and natural origin spring Chinook in the Nason Creek in 2005. Vertical bars denote 95% confidence intervals.

Spawn Timing

Passage timing and spawning timing for hatchery and natural origin fish collected as broodstock were not significantly correlated for hatchery ($r = 0.01$, $P > 0.05$) or natural origin fish ($r = 0.11$, $P > 0.05$). A similar lack of correlation between passage and spawn timing was also reported in the Yakima Basin (Knudsen et al. In Press). During spawning at the hatchery, no difference in spawn timing was detected between hatchery and natural origin fish (two sample t-test, t = 1.28, df = 122, $P = 0.20$).

Passage date at Tumwater Dam and the spawn date of the female that was observed spawning (See Redd Microhabitat Characteristics) was also not significantly correlated ($r = 0.03$, $P = 0.85$). Spawning in the natural environment begins at the higher elevations and progresses to lower elevations (See this Chapter). Spawn timing on the spawning grounds was assessed using the date redds were constructed and the date carcasses were recovered (females only). As previously discussed, the spatial distribution of hatchery and natural origin fish in the Chiwawa River and Nason Creek were different. The difference in spatial distribution and subsequently the elevation of spawning locations required that the influence of elevation be controlled in the analysis. The same reaches used in the redd microhabitat analysis (Chiwawa N = 1; Nason N = 2) were used to test for differences in spawn timing (Table 13).

No difference in spawn timing ($P > 0.05$) was detected within reaches using either redds (Figure 4) or carcasses (Figure 5). However, differences were detected between reaches ($P < 0.05$). Differences between reaches were attributed to significant differences ($P < 0.01$) in elevation between reaches. Knudsen et al. (2005) reported that Yakima hatchery spring Chinook spawned earlier at the hatchery, but using carcasses recovered on the spawning grounds no consistent difference was found.

Table 13. Summary of spawn timing analysis for spawning clusters in the Chiwawa River and Nason Creek in 2004 and 2005.

Stream/method	Spawning cluster elevation (m)		Sample size	
	Lower	Upper	Hatchery	Natural
		2004		
Chiwawa/Redds	729	739	13	17
	775	814	16	80
Chiwawa/Carcass	607	610	16	7
	668	673	15	4
	727	737	27	40
	775	804	11	40
Nason/Redds	606	613	13	22
	665	686	8	41
Nason/Carcass	605	615	45	36
	663	680	27	40
	730	746	3	23
		2005		
Chiwawa/Redds	660	810	48	14
Chiwawa/Carcass	711	810	87	27
Nason/Redds	566	618	51	5
	630	684	14	8
Nason/Carcass	564	622	69	10
	635	694	13	9

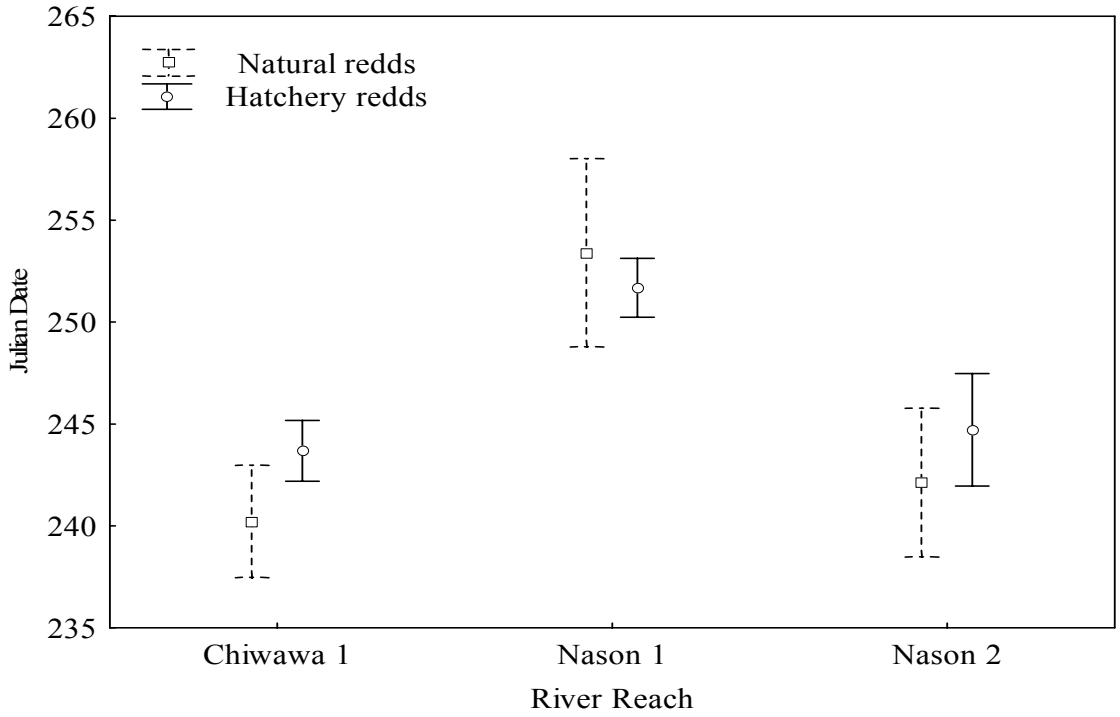

Figure 4. Mean date redds were constructed by female hatchery and natural origin spring Chinook fish spawning in selected reaches of the Chiwawa River and Nason Creek in 2005. Vertical bars denote 95% confidence interval.

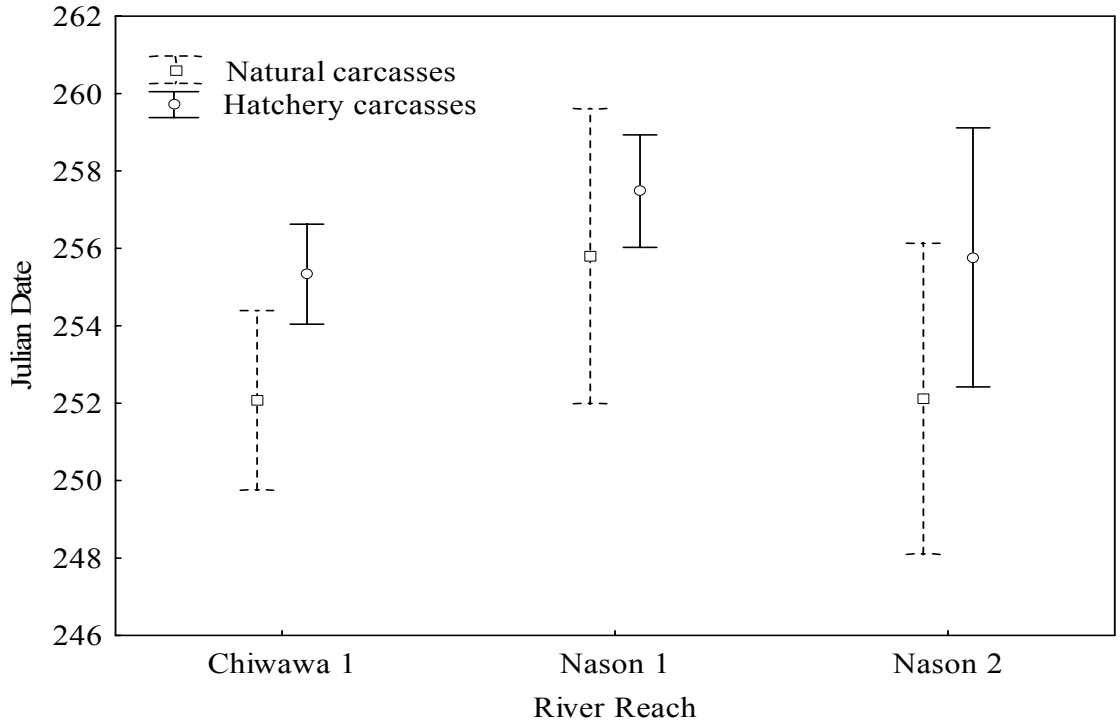

Figure 5. Mean date female spring Chinook carcasses were recovered in selected reaches of the Chiwawa River and Nason Creek in 2005. Vertical bars denote 95% confidence interval.

Survival to Spawning

In 2005, the Wenatchee River Basin experienced severe drought conditions. The proportion of spring Chinook that migrated upstream of Tumwater Dam and subsequently accounted for on the spawning grounds was 42.4%. Differences between run and spawning escapement estimates may be the result of fall back, undetected spawning, or inaccurate redd expansion values. Fallback at Tumwater Dam has not been a significant factor. No PIT tag recaptures were reported at any hydroelectric dam or at LNFH. Furthermore, the number of redds found downstream of Tumwater Dam does not account of the differences observed upstream of Tumwater Dam. Due to record low discharge observed in 2005, it is unlikely that any spawning was undetected. The use of sex ratios as a redd expansion factor does assume each female construct only one redd and males spawn with only one female. Hence, if these assumptions are not valid, the estimated spawning escapement would be an overestimate and the actual difference between run and spawning escapement estimates would be greater.

Poor survival was presumably attributed to extreme environmental conditions prior to and during spawning as a result of record low discharges in the Wenatchee Basin. The estimated number of fish by origin and age was calculated from carcasses recovered during spawning ground surveys and using the estimated age compositions derived from carcass probabilities. The number of hatchery and natural origin spring Chinook in each age class were calculated from carcass recoveries (Table 14). Differences were detected between the proportion of hatchery and natural origin fish at Tumwater and on the spawning grounds ($\chi^2 = 6.01$, df = 1, $P < 0.02$). However, no difference was detected between Tumwater Dam and the estimated spawning population ($\chi^2 = 3.27$, df = 1, $P = 0.07$). Differences observed on the spawning grounds are due to differences in the age composition of hatchery and natural origin spring Chinook (See Chapter 1) and the subsequent size related bias in carcass recoveries. After the bias was corrected using carcass recovery probabilities, no difference was detected in the proportion of hatchery and natural spring Chinook on the spawning grounds. These results are consistent with comparisons between the age composition of hatchery and natural origin spring Chinook (See Spawning Ground Summary) and suggest the survival of hatchery and natural origin spring Chinook from Tumwater Dam to the spawning ground was not different. In the future, a basin specific recovery probability model will be developed.

Table 14. Age and origin of Wenatchee Basin spring Chinook at Tumwater Dam, estimated from carcasses on the spawning grounds, and the estimated number derived using carcass recovery probabilities (H= hatchery; N = natural).

Source	Age-3		Age-4		Age-5		Number of fish		Proportion	
	H	N	H	N	H	N	H	N	H	N
					2004					
Tumwater Dam	745	28	331	755	5	8	1,081	789	0.56	0.44
Spawning grounds	382	13	309	887	4	13	695	913	0.43	0.57
Estimated number	674	23	233	669	2	7	909	699	0.57	0.43
					2005					
Tumwater Dam	128	9	2,819	381	12	81	2,959	471	0.86	0.14
Spawning grounds	23	2	1,198	203	0	46	1,221	251	0.83	0.17
Estimated number	58	5	1,176	199	0	34	1,234	238	0.84	0.16

Redd Microhabitat Characteristics

Spring Chinook redd microhabitat variables were measured on 68 redds in the Chiwawa River and 69 redds in Nason Creek (Appendix D). Redd microhabitat characteristics may be influenced by habitat availability (spatial distribution) and discharge (temporal distribution). In order to reduce the natural variation in the analysis, only those redd which overlapped in elevations and the difference in discharge (i.e., between the day the redd was constructed and microhabitat measurements were recorded) did not exceed 10% were included in the analysis. All tributaries, except Panther Creek a tributary of the White River, have discharge gauging stations. The change in river discharge for all redds was calculated using the mean daily discharge on the day a redd was constructed and the day when the redd was measured.

Although differences in microhabitat characteristics were detected between streams ($F = 2.25$, df = 55, $P < 0.03$), no differences was detected for redds constructed by hatchery and natural origin female spring Chinook in the Chiwawa River or Nason Creek ($F = 0.54$, df = 55, $P = 0.87$). Comparisons between years were not conducted because of poor sample size (Table 15). In the Chiwawa River, no significant correlations were found between female fork length and the variables examined. Weak significant correlations were found between female fork length and redd depth ($r = 0.45$, $P < 0.05$), bowl depth ($r = 0.51$, $P < 0.05$), and tail depth ($r = 0.42$, $P < 0.05$) in Nason Creek.

Table 15. Summary of spring Chinook redd microhabitat variables measured in the Wenatchee River Basin in 2005.

Stream	Variable	Hatchery			Natural		
		Mean	SD	*N*	Mean	SD	*N*
		2004					
Chiwawa	Female FL (cm)	76.9	4.0	10	77.8	4.5	25
	Mean water depth (m)	0.38	0.10	10	0.43	0.10	25
	Bowl depth (m)	0.13	0.04	10	0.12	0.05	25
	Tail depth (m)	0.15	0.05	10	0.23	0.10	25
	Bowl front depth (m)	0.46	0.06	10	0.50	0.12	25
	Redd length (m)	5.8	1.3	10	5.8	1.3	25
	Redd width (m)	3.2	0.9	10	4.7	0.9	25
	Redd area (m^2)	18.8	7.9	10	21.5	7.9	25
	Bowl front velocity (m/s)	0.57	0.16	10	0.48	0.16	25
	Tail front velocity (m/s)	0.61	0.17	10	0.56	0.17	25
Nason	Female FL (cm)	71.4	2.7	7	71.9	4.8	31
	Mean water depth (m)	0.39	0.10	7	0.38	0.10	31
	Bowl depth (m)	0.09	0.04	7	0.10	0.03	31
	Tail depth (m)	0.11	0.04	7	0.14	0.07	31
	Bowl front depth (m)	0.41	0.10	7	0.42	0.12	31
	Redd length (m)	7.9	2.2	7	6.0	1.2	31
	Redd width (m)	3.7	1.0	7	3.5	1.0	31
	Redd area (m^2)	28.9	1.5	7	21.4	9.1	31
	Bowl front velocity (m/s)	0.72	0.20	7	0.48	0.17	31
	Tail front velocity (m/s)	0.63	0.11	7	0.50	0.17	31
		2005					
Chiwawa	Female FL (cm)	79.9	4.3	19	79.3	6.1	9
	Mean water depth (m)	0.3	0.1	19	0.3	0.1	9
	Bowl depth (m)	0.1	0.1	19	0.1	0.1	9
	Tail depth (m)	0.2	0.1	19	0.1	0.1	9
	Bowl front depth (m)	0.4	0.1	19	0.4	0.1	9
	Redd length (m)	6.4	2.0	19	7.2	1.9	9
	Redd width (m)	3.8	0.9	19	4.5	1.8	9
	Redd area (m^2)	25.4	10.4	19	33.6	16.6	9
	Bowl front velocity (m/s)	0.31	0.14	19	0.31	0.12	9
	Tail front velocity (m/s)	0.33	0.14	19	0.36	0.14	9
Nason	Female FL (cm)	79.6	4.9	30	83.5	6.4	10
	Mean water depth (m)	0.3	0.1	30	0.3	0.1	10
	Bowl depth (m)	0.1	0.1	30	0.1	0.0	10
	Tail depth (m)	0.2	0.0	30	0.2	0.1	10
	Bowl front depth (m)	0.3	0.1	30	0.4	0.2	10
	Redd length (m)	6.4	1.6	30	5.9	1.2	10
	Redd width (m)	4.2	1.1	30	4.1	0.8	10
	Redd area (m^2)	27.5	11.2	30	24.3	7.7	10
	Bowl front velocity (m/s)	0.38	0.16	30	0.36	0.13	10
	Tail front velocity (m/s)	0.37	0.12	30	0.35	0.13	10

Summary

Spring Chinook survival from Tumwater to the spawning grounds was the lowest observed in the last seven years (WDFW, unpublished data). However, no difference in survival between hatchery and natural origin fish was detected. Poor survival was attributed to the drought condition that existed before and during spawning. The spawning distribution between tributaries of the upper Wenatchee River Basin spring Chinook population was similar to that observed in previous years (Mosey and Murphy 2002). Hatchery female and male spring Chinook spawned in the lower reaches of the Nason Creek and the Chiwawa River, while natural origin female spring Chinook spawned in the upper reaches. Differences in reproductive success of hatchery and natural origin fish may differ because of differences in spawning location.
No difference in spawn timing was found in the natural or hatchery environment. On the spawning grounds, spawn timing comparisons using the date redds were constructed or the date female spring Chinook carcasses were recovered had similar results. The use of carcass recovery data as a surrogate for spawning timing was used successfully in both 2004 and 2005. Should results be consistent across years, greater statistical power may be achieved simply be using all carcasses in the analysis.

No differences were detected in any of the redd microhabitat variables examined. The selection of specific spawning reaches minimized the temporal and spatial variation in the naturally spawning population and will be used in future years. The relatively low abundance of natural origin spring Chinook in 2005 limited the sample sizes used in the analysis. However, these data may be pooled across years and provide greater statistical power in our final report.

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Chapter 4

Assortative pairing of adult hatchery and natural origin spring Chinook on the spawning grounds and incidence of precocious males in the Wenatchee River Basin

Abstract

PIT tag detections were used to determine composition of adult hatchery and natural origin spring Chinook salmon on individual redds. Snorkel surveys were used to determine the origin and abundance of precocious males on redds. The estimated number of precocious males that potentially contributed to natural spawning was 106 (19 hatchery, 68 natural, and 19 unknown origin). The low relative abundance of precocious males observed on the spawning grounds suggests that the majority of the precocious males observed at Tumwater Dam do not successfully migrate to the major spawning areas or die before spawning. In 2005, the precocity rate was calculated as 0.13% for juveniles released from Chiwawa Ponds, migrated downstream, and survived to pass upstream of Tumwater Dam. Assortative pairing analysis was limited in 2005 because of the lack of externally marked hatchery fish. No difference was detected in the mean fork length of males paired with either hatchery or natural origin females.

Introduction

Salmon are known to select mates based on factors such as competitive dominance and fish size. Selection of mates that are similar to each other (e.g., large size) is termed assortative mating. We are aware of few studies that have investigated assortative pairing of hatchery and natural origin salmon in the natural environment. Assortative pairing by origin (e.g., hatchery or natural) may be a detriment to integrated hatchery populations because the goal is to have hatchery and wild fish interbreed. Hatchery origin fish may pair with other hatchery fish because they are larger, migrate at a certain time, or look different (e.g. adipose fin absent). Some have observed pairs of fish migrating upstream and have speculated that fish pair up prior to reaching the spawning grounds. In this study, we compare the composition and characteristics of hatchery and natural origin fish at Tumwater Dam (potential spawners) with the pairing of fish on redds to determine if assortative pairing occurs.

The number of age 1+ precociously mature salmon on the spawning grounds may be significantly increased by hatchery programs (Reviewed by Mullan et al. 1992) and these fish have the potential to breed with anadromous females. Hatcheries may enhance precocious maturation of males by the kinds of diets that are fed to fish (e.g., high fats) or the types of growth schedules that fish are placed on. For example, approximately, 40% of the males produced by the Yakima Klickitat Fisheries Project (YKFP) spring Chinook supplementation hatchery are precocious males and some of these fish are observed on the spawning grounds approximately four months after they are released from acclimation sites (Larsen et al. 2004). Preliminary results from the YKFP indicate that

precocious males sired a significant number of offspring in an experimental spawning channel that contained anadromous males and females (Schroder et al. 2005). Age 1+ precocious males may migrate downstream, but generally do not reach the ocean. These fish are undesirable because of the potential for negative ecological and genetic impacts to natural fish, and because they are an undesirable fishery product. For example, a high incidence of precociously maturing males will lead to direct ecological interactions with native conspecifics and other non-target species of concern. Also, age structure, sex ratio and, potentially, other phenotypic characters of the spawning population will be altered. Precocity and other forms of residualism in hatchery fish is an expression of the genotype x environment interaction. To the extent that the phenomenon has in part a genetic basis and is coupled with changes in the reproductive potential of individuals within the hatchery population as a whole, high precocity or residualism is a source of domestication selection. In this study, we will examine if hatchery precocious males are (1) produced by the hatcheries in question, (2) observed on the spawning grounds, and (3) contribute genetic material to future generations (i.e., progeny attributed to unknown male parentage).

Methods and Materials

Spawning Ground and Snorkel Surveys

During spawning ground surveys active redds were snorkeled to count the number of precociously maturing fish associated with each redd. Active redds were defined as new redds with anadromous fish present. A single snorkeler began approximately 10 m downstream of the active redd and slowly moved upstream. The origin of all spring Chinook observed and the number of precocious fish was recorded. The mean number of precocious fish per redd were calculated for each stream by dividing the number of fish observed while snorkeling by the number of redds snorkeled. The proportion of redds with precocious fish and the mean number and origin of precocious fish per redd was calculated for each stream.

Chinook salmon that are on or associated with active redds were counted and identified to sex and size while snorkeling. Similar information was collected from redds using PIT tag detections (i.e., not snorkeled). Surveys were conducted weekly and lasted throughout the spawning season. Active redds (the presence of an anadromous fish) were found by floating downstream in an inflatable raft or by walking. When a salmon redd was observed and adult salmon were present, then a snorkeler entered the water. A snorkeler began 5-10 meters downstream of the redd and snorkeled upstream, counting all spring Chinook encountered. Fish were categorized as either being on the redd (in the bowl), or associated with the redd (within 5 meters). Hatchery fish were distinguished from natural fish by the presence (natural) or absence (hatchery) of an adipose fin or in the case of adipose fin present hatchery fish through PIT tag detections on the spawning grounds. Anadromous fish were distinguished from precocious males based on size. Anadromous fish are generally greater than 400 mm and precocials are generally less

than 300 mm. Females were distinguished from males by the mouth shape and the condition of the caudal fin. Males have a kype and females have a white band on the margin of the caudal fin from digging a redd. After a redd was snorkeled, it was flagged and numbered for subsequent redd measurements.

Data Analysis

The mean number of precocious males per redd was calculated by dividing the number of precocious male observed by the number of redds snorkeled in each stream. Stream specific values (i.e., number of precocious males/redd) were multiplied by the total number of redds in each stream to estimate the total number of precocious males.

As stated previously, the origin of all the males could not be determined. The lack of externally marked hatchery fish in 2005, limited the analysis between origins to only female spring Chinook. However, the fork length of the dominant male was estimated using a linear regression model of estimated and actual fork lengths determined from PIT tag recaptures on the spawning grounds. The mean fork length of males paired with hatchery and natural origin female spring Chinook was compared using a Mann-Whitney U-test. Correlation analysis was conducted on female (hatchery and natural) and male fork length. Differences in the size of males for a female of a given length would suggest assortative pairing was occurring.

Results and Discussion

A total of 84 redds (10.3%) were snorkeled in the upper Wenatchee River Basin during spawning ground surveys (Table 1). Water clarity limited snorkeling on the Chiwawa River, which contained the greatest number of redds in the Wenatchee Basin. Of the 49 redds snorkeled on the Chiwawa River, only 2 hatchery, 6 naturally produced, and 2 unknown origin precocious fish were observed. Water clarity was excellent in Nason Creek and the Little Wenatchee River. Of those redds snorkeled, one redd had one naturally produced precocious fish present. Age 0+ precocious males (i.e., FL < 80 mm) were not observed during any of the surveys. The high discharge in the upper Wenatchee River limited our ability to conduct snorkel surveys in this area. Snorkel surveys were not conducted on the White River due to poor water clarity (i.e, glacial till in the river).

Table 1. Precocious males found during spawning ground surveys on the upper Wenatchee River basin in 2005 (H = hatchery; N = natural; U = unknown).

Stream	Redds snorkeled	Number of precocious males			Mean number of precocious males per redd			
		H	N	U	H	N	U	Total
			2004					
Chiwawa	20	2	7	0	0.10	0.35	0.00	0.45
Nason	73	0	2	0	0.00	0.27	0.00	0.03
White (Panther)	2	0	0	0	0.00	0.00	0.00	0.00
Upper Wenatchee	9	0	0	0	0.00	0.00	0.00	0.00
Total Upper Basin	104	2	9	0	0.02	0.09	0.00	0.11
			2005					
Chiwawa	49	2	6	2	0.04	0.12	0.04	0.20
Nason	22	0	1	0	0.00	0.05	0.00	0.05
Upper Wenatchee	7	0	0	0	0.00	0.00	0.00	0.00
Little Wenatchee	6	0	0	0	0.00	0.00	0.00	0.00
Total Upper Basin	84	2	7	2*	0.02	0.09	0.02	0.13

*Origins not determined due to poor visibility.

An estimated 76 precocious males (13 hatchery, 50 natural, and 13 unknown origin) potentially contributed gametes during spawning in 2005. None of the 297 precocious males sampled at Tumwater Dam were detected or recovered on the spawning grounds. The mark rate of the 2003 brood Chiwawa spring Chinook was 97.4%. The mark rate of the precocious fish sampled at Tumwater Dam was 100.0%. Hence, assuming all precocious fish sampled at Tumwater Dam were from the Chiwawa Ponds and all precocious fish migrated below Tumwater Dam, the precocity rate of the 2003 brood Chiwawa spring Chinook was 0.13% (222,131 fish released in 2005). The probability of recovering age-2 fish carcasses was estimated as zero. The mean (standard deviation, SD) size of the age-2 fish sampled at Tumwater Dam was 210 (16) mm. Zhou (2002) reported that no tagged fish less than 350 mm was recovered over 11 years in the Salmon River, Oregon. Thus, carcass surveys likely underestimate the contribution of precocious males and necessitate the need for snorkel surveys.

Observations of pairings on the spawning grounds were severely limited in 2005 because age-4 hatchery fish were not adipose fin-clipped. The origin and sex of a relatively small number of pairings (i.e., both male and female) on the spawning grounds were determined from PIT detections. Of which, 69% were recorded on the White River (Table 2). Female hatchery spring Chinook were paired with similar proportions of hatchery (52%) and natural (48%) male spring Chinook. Conversely, natural origin spring Chinook were paired predominately (88%) with natural origin male spring Chinook. These results are consistent with the differences in the spawning distribution

detected between hatchery and natural origin female spring Chinook (see Chapter 3). Statistical comparisons will be possible as the number of adipose fin-clipped hatchery spring Chinook increases in subsequent years.

No difference was detected in the estimated mean fork length of male spring Chinook paired with hatchery or natural origin female spring Chinook ($Z = -0.74$, $P = 0.46$). Statistical comparisons of the relationship between hatchery and natural origin female fork length and the estimated male fork length could not be performed because requisite assumptions of data normality and equal variances could not be met. However, no significant correlation was found between female and male fork length for either hatchery ($P = 0.67$) or natural ($P = 0.39$) spring Chinook.

Summary

The incidence of precocious males in the Wenatchee River Basin is low and may be due in part to the relatively low prey productivity of the basin. Yearling spring Chinook smolts rarely exceed 100 mm in fork length at time of emigration (WDFW, unpublished data). Precocity in the hatchery population also appears to be very low. Pearsons et al. (2004) reported that 73% of the estimated number of precocious males in the upper Yakima Basin were found in the most downstream reaches of potential spawning habitat. The low abundance of hatchery precocious fish on the spawning grounds in the Wenatchee Basin suggests that most hatchery precocious fish do not successfully migrate to the tributary spawning areas, or they die, as observed in the upper Yakima Basin.

Data collected in 2005 suggests that mate pairing in the Wenatchee Basin is random with respect to the variables that we measured. These data will be used in conjunction with the DNA pedigree analysis (See Chapter 2), which should also provide information about mate selection.

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Table 2. Pairing of hatchery and natural origin spring Chinook on redds in the upper Wenatchee River Basin in 2004 and 2005.

Stream	Female origin	Number of females	Number of males		
			Natural	Hatchery	Unknown (Jacks)
			2004 Single Pairings		
Chiwawa	H	12	7	2	3
	N	16	14	2	0
Nason	H	6	2	0	4
	N	22	18	0	4
Wenatchee	H	1	0	0	1
	N	1	1	0	0
White	H	0	0	0	0
	N	6	5	1	0
Little	H	0	0	0	0
	N	1	1	0	0
			2004 Multiple Male Pairings		
Chiwawa	H	7	8	8	9
	N	19	39	9	12
Nason	H	7	7	3	6
	N	26	50	5	19
Wenatchee	H	0	0	0	0
	N	0	0	0	0
White	H	3	10	0	0
	N	3	7	0	0
Little	H	0	0	0	0
	N	0	0	0	0
			2005 Single Pairings		
Chiwawa	H	3	1	2	0
	N	0	0	0	0
Nason	H	5	2	3	0
	N	1	0	1	0
Wenatchee	H	0	0	0	0
	N	0	0	0	0
White	H	13	6	7	0
	N	3	1	2	0
Little	H	1	1	0	0
	N	0	0	0	0
			2005 Multiple Male Pairings		
Chiwawa	H	2	2	2	0
	N	0	0	0	0
Nason	H	0	0	0	0
	N	0	0	0	0
Wenatchee	H	0	0	0	0
	N	0	0	0	0
White	H	9	11	11	0
	N	2	0	4	0
Little	H	0	0	0	0
	N	0	0	0	0

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Appendix A. Daily number of spring Chinook observed at Tumwater Dam during trapping in 2005 (F = female; M = male; J = jack; P = precocious male).

Date	Natural			Hatchery				Unknown			Daily Total
	F	M	J	F	M	J	P	F	M	J	
05/14/05		1									1
05/15/05		1									1
05/16/05									1		1
05/17/05				1							1
05/18/05	1			1	1						3
05/19/05		1									1
05/20/05	1			2							3
05/21/05	1	2		2	2						7
05/22/05	1			3	1						5
05/23/05											0
05/24/05	3	2		6	3						14
05/25/05	1	1		6	4						12
05/26/05	3			9	5						17
05/27/05	2	2		9	7						20
05/28/05	1	1		10	6						18
05/29/05	1	4		14	9						28
05/30/05	9	8		42	32		1		1		93
05/31/05	4	6		42	17		1	1			71
06/01/05	5	3		33	19		4				64
06/02/05	3	4		19	18		1	1	1		47
06/03/05	2	3		30	12			2			49
06/04/05	1	2		34	17			1			55
06/05/05	1	2		19	10						32
06/06/05	1			8	10	1					20
06/07/05	1	2		19	13						35
06/08/05	1			24	27	3					55
06/09/05	3	3		26	16	2					50
06/10/05	7	8		56	28	1			1		101
06/11/05	7	6		55	14	1		1	1		85
06/12/05	3			33	17				2		55
06/13/05	4	3	1	53	28	1					90
06/14/05		3		40	22	2	1	1			69
06/15/05	5	4		60	37	2		1			109
06/16/05	10	9		60	44	2	2		1		128
06/17/05	11	2	2	72	36	6	1	1			131
06/18/05	10	5	1	50	22		2				90
06/19/05	14	6		54	36	2	4				116

Date	Natural			Hatchery				Unknown			Daily
	F	M	J	F	M	J	P	F	M	J	Total
06/20/05	7	8		65	36	3	2				121
06/21/05	7	6		72	47	1	2				135
06/22/05	5	12	1	66	49	6	3		1		143
06/23/05	4	7		29	36	8	9	3			96
06/24/05	9	6	1	45	37	4	4				106
06/25/05	8	7		54	34	1	5		1		110
06/26/05	11	5		68	51	5	5	1	2		148
06/27/05	8	5		53	35	6	1	1			109
06/28/05	3	6		28	16	3	3				59
06/29/05	3	5		34	34	5	7				88
06/30/05	9	7		57	37	10	8	1			129
07/01/05	6	14		74	40	7			1		142
07/02/05	6			27	36	4	2				75
07/03/05	5	3		18	14	3	7				50
07/04/05	7	15		37	39	4	2				104
07/05/05	8	6		42	28	3					87
07/06/05	7	12		50	24	7	9	1		1	111
07/07/05	4	7		20	19	3	9		2		64
07/08/05	5	3		24	12	3	8	1			56
07/09/05		3		8	2	3	4				20
07/10/05	2	5	1	11	12	1	7	1			40
07/11/05	4	2		17	10	2	9				44
07/12/05	6	4	2	15	8		6				41
07/13/05	5	6		13	10	3	22				59
07/14/05	2	4		4	8	2	11				31
07/15/05	7	1		6	4	1	14				33
07/16/05		1		2	1	1	7				12
07/17/05	2	2		1	2	2	11				20
07/18/05	5	2		8	5	2	15				37
07/19/05	2	2		4	3	1	10				22
07/20/05	4	7		2	4	3	9				29
07/21/05		2		1	3	3	14				23
07/22/05	3			2	5		11				21
07/23/05		1		2	1		9				13
07/24/05	1	2		2		2	2				9
07/25/05				1			3				4
07/26/05					1						1
07/27/05	2	1			2		9				14
07/28/05				3	1		6				10

Date	Natural			Hatchery				Unknown			Daily Total
	F	M	J	F	M	J	P	F	M	J	
07/29/05				0			6				6
07/30/05				0			3				3
07/31/05				0	3	1	3				7
08/01/05				1							1
08/02/05				1							1
08/03/05											0
08/04/05					1		1				2
08/05/05											0
08/06/05				1			1				2
08/07/05		1									1
08/08/05	1						1				2
08/09/05		1	1	1							3
08/10/05											0
08/11/05											0
08/12/05											0
08/13/05											0
08/14/05											0
08/15/05											0
08/16/05											0
08/17/05	1*										1
08/18/05											0
08/19/05											0
08/20/05											0
08/21/05											0
08/22/05	1*										1
08/23/05											0
08/24/05											0
08/25/05											0
08/26/05											0
08/27/05											0
08/28/05											0
08/29/05	1*										1
08/30/05											0
Total	288	275	10	1861	1223	136	297	18	15	1	4124

* Video recorded counts, sex not determined.

Appendix B. Spring Chinook spawn timing in the upper Wenatchee River Basin in 2005.

Date	Stream					Daily Total	Cumulative Total
	Nason	Chiwawa	Wenatchee	Little Wenatchee	White		
08/01/2005	0	0	0	0	0	0	0
08/02/2005	0	0	0	0	0	0	0
08/04/2005	1	1	0	0	0	2	2
08/07/2005	0	0	0	0	0	0	2
08/08/2005	0	0	0	0	0	0	2
08/10/2005	0	0	0	0	0	0	2
08/11/2005	1	4	0	0	0	5	7
08/14/2005	0	0	0	0	0	0	7
08/15/2005	0	5	0	0	1	6	13
08/17/2005	0	3	0	0	0	3	16
08/18/2005	1	15	0	1	0	17	33
08/21/2005	0	0	0	0	0	0	33
08/22/2005	3	10	0	0	2	15	48
08/24/2005	0	31	0	0	1	32	80
08/25/2005	8	15	0	3	12	38	118
08/28/2005	0	23	0	0	3	26	144
08/29/2005	11	33	0	4	14	62	206
08/31/2005	7	46	1	10	10	74	280
09/01/2005	13	27	0	6	9	55	335
09/04/2005	21	21	0	3	4	49	384
09/05/2005	10	18	0	17	5	50	434
09/07/2005	28	15	34	0	6	83	517
09/08/2005	7	16	0	1	1	25	542
09/11/2005	43	6	1	3	3	56	598
09/12/2005	22	7	14	7	7	57	655
09/14/2005	2	8	0	4	4	18	673
09/15/2005	7	6	5	1	3	22	695
09/18/2005	7	5	0	0	1	13	708
09/19/2005	0	5	88	1	0	94	802
09/21/2005	0	12	0	1	0	13	815
09/22/2005	1	0	0	2	0	3	818
09/25/2005	0	0	0	0	0	0	818
09/26/2005	0	0	0	0	0	0	818
09/28/2005	0	0	0	0	0	0	818
09/29/2005	0	0	0	0	0	0	818
Total	193	332	143	64	86	818	818

Appendix C. Spring Chinook spawning ground reaches in the upper Wenatchee River Basin (CG = campground).

River (*Tributary*)	Reach	River kilometer
Chiwawa River		
Mouth to Grouse Creek	C1	0 – 19.5
Big Meadow Creek		0 – 1.5
Grouse Creek to Rock Creek CG	C2	19.5 – 32.2
Chikamin Creek		0 – 1.0
Rock Creek		0 – 1.0
Rock Creek CG to Schaefer Creek CG	C3	32.2 – 37.3
Schaefer Creek CG to Atkinson Flats	C4	37.3 – 42.7
Atkinson Flats to Maple Creek	C5	42.7 – 45.0
Maple Creek to Trinity	C6	45.0 – 50.5
Little Wenatchee River		
Mouth to Old fish weir	L1	0 – 4.5
Old fish weir to Lost Creek	L2	4.5 – 8.7
Lost Creek to Rainy Creek	L3	8.7 – 15.3
Rainy Creek to Waterfall	L4	15.3 – 21.0
Nason Creek		
Mouth to Kahler Cr. Bridge	N1	0 – 6.5
Kahler Cr. Bridge to Hwy.2 Bridge	N2	6.5 – 13.8
Hwy.2 Bridge to Lower Railroad Bridge	N3	13.8 – 22.0
Lower Railroad Bridge to Whitepine Cr.	N4	22.0 – 25.7
Whitepine Cr. to Upper Railroad Bridge	N5	25.7 – 26.3
Upper Railroad Bridge to Falls	N6	26.3 – 27.0
White River		
Mouth to Sears Cr. Bridge	H1	0 – 10.7
Sears Cr. Bridge to Napeaqua River	H2	10.7 – 18.3
Napeaqua River		
Napeaqua R. to Grasshopper Meadows	H3	18.3 – 21.5
Panther Creek		
Grasshopper Meadows to Falls	H4	21.5 – 23.8
Wenatchee River		
Tumwater Dam to Tumwater Bridge	W8	51.5 – 59.3
Tumwater Bridge to Chiwawa River	W9	59.3 – 80.7
Chiwaukum Creek		
Chiwawa River to Lake Wenatchee	W10	80.7 – 90.3

Appendix D. Spring Chinook redd microhabitat variables measured in the Wenatchee river Basin in 2005.

Variable	Hatchery			Natural		
	N	Mean	SD	*N*	Mean	SD
	Chiwawa River (rkm 27.9 – 32.2)					
Bowl Front Depth	16	0.42	1.37	9	0.48	0.12
Bowl Depth	16	0.16	0.04	9	0.17	0.07
Redd Depth	16	0.38	0.07	9	0.43	0.09
Tail Depth	16	0.21	0.08	8	0.20	0.10
Bowl Front Velocity	16	0.42	0.16	9	0.38	0.17
Tail Front Bottom Velocity	16	0.23	0.10	9	0.24	0.10
Distance to Cover	16	2.93	4.04	9	1.17	1.77
Distance to Nearest Redd	16	20.20	24.20	9	18.8	20.19
Tail Substrate Boulder	16	0.00	0.00	9	0.00	0.00
Tail Substrate Cobble	16	11.00	19.00	9	10.78	15.94
Tail Substrate Gravel	16	70.00	25.00	9	73.33	15.00
Tail Substrate Sand	16	13.00	12.00	9	15.89	14.35
Female Fork Length	16	76.00	8.24	9	79.56	4.30
	Chiwawa River (rkm 23.2 – 27.9)					
Bowl Front Depth	3	0.56	0.19	1	0.43	
Bowl Depth	3	0.21	0.02	1	0.22	
Redd Depth	3	0.47	0.20	1	0.37	
Tail Depth	3	0.28	0.11	1	0.13	
Bowl Front Velocity	3	0.34	0.07	1	0.49	
Tail Front Bottom Velocity	3	0.18	0.01	1	0.38	
Distance to Cover	3	1.00	1.73	1	0.00	
Distance to Nearest Redd	3	17.5	28.17	1	8.00	
Tail Substrate Boulder	3	0.00	0.00	1	0.00	
Tail Substrate Cobble	3	15.00	17.32	1	18.00	
Tail Substrate Gravel	3	80.00	18.03	1	80.00	
Tail Substrate Sand	3	5.00	5.00	1	2.00	
Female Fork Length	3	80.67	3.06	1	88.00	
	Chiwawa River (rkm 19.5 – 23.2)					
Bowl Front Depth	19	0.30	0.12	2	0.29	0.04
Bowl Depth	19	0.11	0.05	2	0.08	0.004
Redd Depth	19	0.28	0.08	2	0.24	0.02
Tail Depth	17	0.14	0.05	2	0.12	0.03
Bowl Front Velocity	19	0.23	0.09	2	0.45	0.11
Tail Front Bottom Velocity	19	0.15	0.05	2	0.35	0.16
Distance to Cover	19	4.76	3.89	2	0.75	5.80
Distance to Nearest Redd	19	7.20	8.27	2	5.70	1.06
Tail Substrate Boulder	19	0.26	1.15	2	0.00	0.00
Tail Substrate Cobble	19	39.21	13.05	2	42.50	10.61
Tail Substrate Gravel	19	43.68	12.68	2	50.00	7.07

Variable	Hatchery			Natural		
	N	Mean	SD	*N*	Mean	SD
Tail Substrate Sand	19	16.84	13.15	2	7.50	3.54
Female Fork Length	19	78.68	3.48	2	80.00	7.07
Chiwawa River (rkm 32.2 – 37.3)						
Bowl Front Depth	3	0.33	0.10			
Bowl Depth	3	0.12	0.01			
Redd Depth	3	0.28	0.11			
Tail Depth	2	0.19	0.01			
Bowl Front Velocity	3	0.22	0.10			
Tail Front Bottom Velocity	3	0.17	0.08			
Distance to Cover	3	4.77	2.97			
Distance to Nearest Redd	3	10.3	8.14			
Tail Substrate Boulder	3	0.33	0.58			
Tail Substrate Cobble	3	21.67	16.07			
Tail Substrate Gravel	3	81.67	7.64			
Tail Substrate Sand	3	8.33	2.89			
Female Fork Length	3	80.00	1.00			
Chiwawa River (rkm 37.3 – 42.7)						
Bowl Front Depth	10	0.40	0.10	5	0.35	0.10
Bowl Depth	10	0.14	0.08	5	0.18	0.07
Redd Depth	10	0.33	0.07	5	0.30	0.03
Tail Depth	10	0.15	0.05	4	0.10	0.05
Bowl Front Velocity	10	0.31	0.10	5	0.32	0.19
Tail Front Bottom Velocity	10	0.18	0.07	5	0.23	0.15
Distance to Cover	10	3.64	4.48	5	3.54	3.96
Distance to Nearest Redd	10	22.42	31.52	5	6.20	10.83
Tail Substrate Boulder	10	0.00	0.00	5	0.00	0.00
Tail Substrate Cobble	10	10.00	5.77	5	10.00	3.54
Tail Substrate Gravel	10	79.00	10.49	5	85.00	3.54
Tail Substrate Sand	10	11.00	10.75	5	5.00	3.54
Female Fork Length	10	79.60	3.17	5	82.40	4.34
Nason River (rkm 0 – 6.5)						
Bowl Front Depth	43	0.32	0.08	5	0.28	0.10
Bowl Depth	43	0.09	0.04	5	0.08	0.04
Redd Depth	43	0.30	0.05	5	0.28	0.07
Tail Depth	43	0.17	0.05	5	0.15	0.06
Bowl Front Velocity	43	0.39	0.18	5	0.37	0.17
Tail Front Bottom Velocity	43	0.22	0.12	5	0.21	0.10
Distance to Cover	43	5.79	5.93	5	4.04	4.49
Distance to Nearest Redd	43	33.12	45.93	5	51.16	83.75
Tail Substrate Boulder	43	3.60	5.70	5	4.00	5.48
Tail Substrate Cobble	43	38.02	12.28	5	36.00	15.17
Tail Substrate Gravel	43	38.02	8.46	5	48.00	13.04

Variable	Hatchery			Natural		
	N	Mean	SD	*N*	Mean	SD
Tail Substrate Sand	43	19.88	10.61	5	12.00	4.47
Female Fork Length	43	79.30	5.63	5	73.60	6.19
Nason River (rkm 18.8 – 22.0)						
Bowl Front Depth	13	0.30	0.08	8	0.40	0.19
Bowl Depth	13	0.12	0.03	8	0.12	0.03
Redd Depth	13	0.28	0.06	8	0.34	0.13
Tail Depth	13	0.16	0.04	8	0.17	0.09
Bowl Front Velocity	13	0.34	0.14	8	0.31	0.10
Tail Front Bottom Velocity	13	0.21	0.13	8	0.17	0.08
Distance to Cover	13	10.27	8.53	8	5.29	4.51
Distance to Nearest Redd	13	83.46	107.94	8	67.65	59.97
Tail Substrate Boulder	13	2.31	4.39	8	1.25	3.54
Tail Substrate Cobble	13	56.15	15.02	8	50.00	15.12
Tail Substrate Gravel	13	33.08	15.48	8	38.75	17.27
Tail Substrate Sand	13	8.46	5.55	8	10.00	14.14
Female Fork Length	13	82.46	6.50	8	84.38	3.54