

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

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Executive Summary

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.

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 ($P < 0.05$). Data collected during spawning at a hatchery showed that there was a significant difference in fecundity ($P < 0.05$), but not egg weight ($P > 0.05$), 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 in two of the three years examined ($P > 0.05$). In 2004, hatchery fish had significantly greater higher egg retention rates than naturally produced fish ($P < 0.05$). 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 or if impacts to reproductive success and survival in natural environments has occurred.

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.

In this report, we provide preliminary estimates of relative fitness for hatchery and natural fish for the 2004 parental spawning year. We also estimated relationships between fitness and several traits, including weight, age, and run timing. We are using fractional assignment methods and a sample of subyearling parr trapped in Nason Creek and the Chiwawa and White Rivers in fall of 2005 and a sample of smolts trapped in the tributaries and the lower Wenatchee River near Monitor in 2006 to estimate the relative fitness of hatchery and natural origin fish, and evaluate how weight, run timing, and age contribute to these differences. We also conducted computer simulations to evaluate the effectiveness of the fractional assignment methods. Based on our preliminary results, both male and female hatchery fish produced fewer progeny when spawning naturally than did natural fish, particularly when progeny were counted at the smolt stage. Differences in age structure and to a lesser degree weight and run timing were responsible for a portion of the difference in fitness between hatchery and wild fish. Male size and age had a large influence on fitness, with older and larger males selectively favored. Male run time had a smaller but still significant effect on fitness, with earlier returning fish favored. Female size had a significant effect on fitness, but the effect was much smaller than the effect of size on male fitness. Additional variables that are likely to affect fitness, including spawning location and spawning time, have been measured but not yet analyzed and will be included in subsequent reports.

Reproductive success of hatchery and natural origin fish that spawn in natural environments could differ for a variety of reasons such as differences in spawn time, spawn location, spawn habitat, and redd construction. 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 2006, the composite population of spring Chinook redds were distributed similarly to that of years past. A total of 528 redds were found upstream of Tumwater Dam, of which the female origin was identified on 242 redds. The estimated spawning escapement, based on the number of redds, was 51.5% of the number of spring Chinook counted at Tumwater Dam. After correction for carcass recovery bias, no differences were found in the estimated age composition of the spawning population compared to population sampled at Tumwater Dam. However, the estimated proportion of hatchery fish on the spawning grounds was significantly lower than that of naturally produced fish compared to the population at Tumwater Dam ($P < 0.05$). Hatchery origin female spring Chinook spawned in significantly lower elevations of the Chiwawa River and Nason Creek than natural origin fish ($P < 0.05$). No difference in spawning timing of hatchery and natural origin spring Chinook spawning within the same reaches was detected ($P > 0.05$). However, differences in spawn timing, regardless of origin, was associated with elevation. Microhabitat variables were measured on 93 redds, which included 63 and 30 constructed by hatchery and natural origin females, respectively. However, power analysis of redd microhabitat characteristics suggest additional years of data collection is necessary to obtain the desired statistical power.

Salmon use different mating strategies to increase their chances of producing fit offspring and hatchery production may alter the production of fishes that use different mating strategies. PIT tag detections were used to determine composition of female hatchery and natural origin spring Chinook salmon on individual redds. Snorkel surveys were used to determine the origin and relative abundance of precocious males on redds. The estimated index number of precocious males that were on the spawning grounds and potentially contributed to natural spawning was 260 (13 hatchery and 247 naturally produced). 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. Assortative pairing analysis was limited in 2006 because not all hatchery fish were externally marked. No difference was detected in the mean fork length of males paired with either hatchery or natural origin females ($P > 0.05$).

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 years of work were addressed in Murdoch et al. 2005 and 2006. The project is intended to last until 2012 in order to evaluate two entire spring Chinook salmon generations.

This project is a collaboration between NOAA-Fisheries (Northwest Fisheries Science Center) and the Washington Department of Fish and Wildlife (WDFW). 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 m³/s and a mean monthly spring peak discharge of 257 m³/s.

Of the total river discharge, the Little Wenatchee River (13%) and White River (24%) 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 (9%), Chiwawa River (14%) and Icicle Creek (19%; 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 watersheds (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 into Icicle Creek that were 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 hatchery smolts released since 1991 has been 153,032 (1989-2004 brood). Despite not meeting production goals, the number of hatchery fish on the spawning grounds has been greater than the number of naturally produced fish since 2000 (Table 1).

Table 1. Summary of broodstock, spawner composition, and number of smolt released as part of the Chiwawa River spring Chinook hatchery program (WDFW, unpublished data).

Brood year	Broodstock		Number of spawners		Proportion natural influence (PNI)	Naturally produced		Hatchery	
	Naturally produced	Hatchery	Naturally produced	Hatchery		Number of smolts	Smolt-to-adult	Number of smolts	Smolt-to-adult
1989	28	0	713	0	1.0			43,000	0.48%
1990	18	0	347	0	1.0			53,170	0.04%
1991	32	0	242	0	1.0			62,138	0.06%
1992	78	0	676	0	1.0	56,763	0.09%	85,113	0.04%
1993	94	0	218	4	1.0	17,926	0.59%	223,610	0.13%
1994	7	4	110	73	0.6	22,145	0.25%	27,226	0.08%
1995	0	0	31	2		5,230	0.96%		
1996	8	10	33	25	0.5	17,922	0.99%	15,176	0.52%
1997	32	79	54	128	0.3	39,044	2.33%	266,148	0.99%
1998	13	34	39	47	0.4	24,953	1.37%	75,906	1.54%
1999	0	0	63	31		13,953	0.08%		
2000	9	21	138	174	0.4	50,634	1.19%	47,104	0.76%
2001	112	259	626	1,790	0.3	389,940	0.08%	377,544	0.31%
2002	20	51	263	444	0.3	152,547		149,668	
2003	41	53	148	121	0.5	27,897		222,131	
2004	83	132	477	381	0.5	101,172		494,517	
2005	89	181	102	496	0.2				
2006	93	252	119	410	0.2				

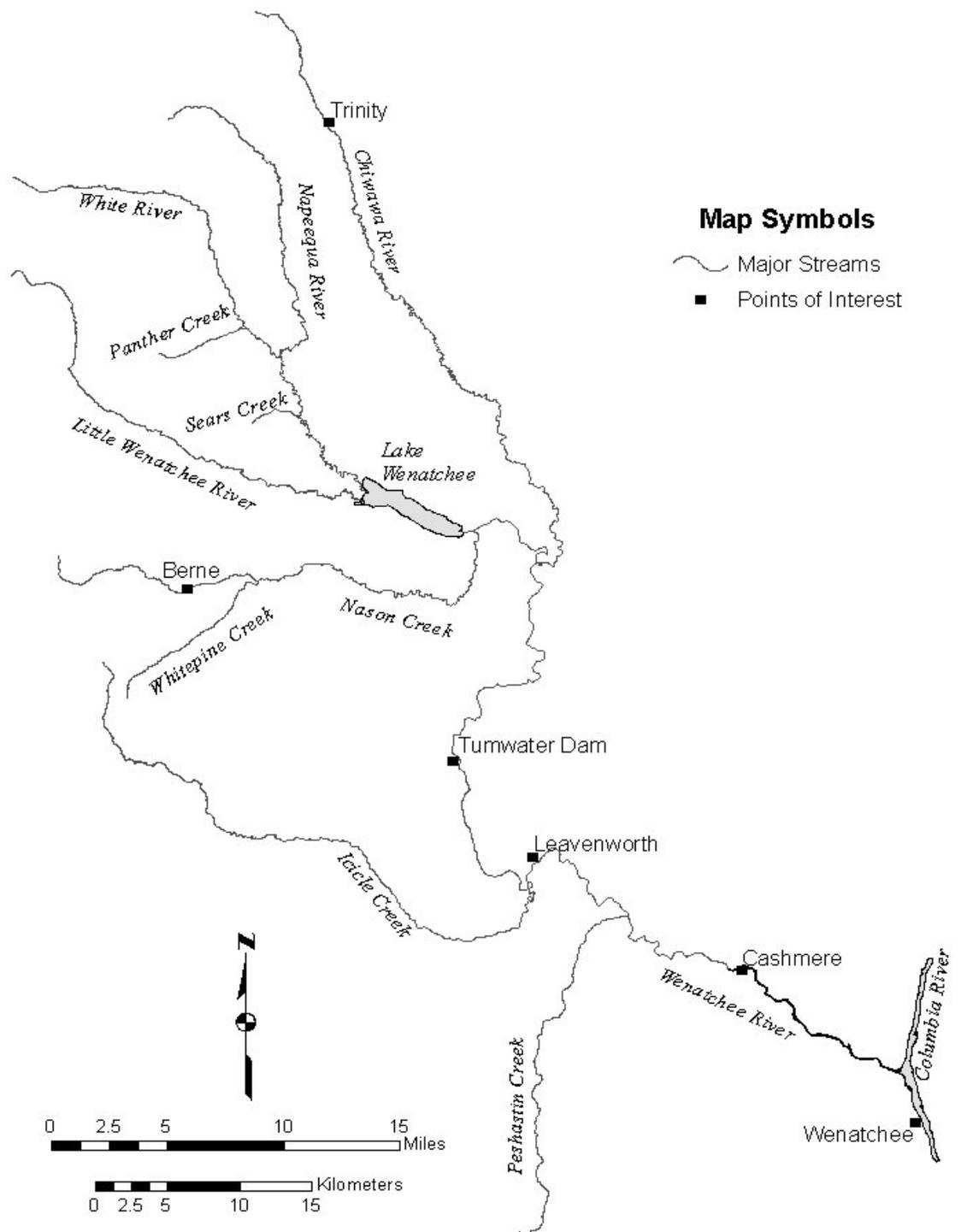


Figure 1. Map of Wenatchee River Basin and spring Chinook spawning tributaries.

References

- Andonaegui, C. 2001. Salmon, steelhead, and bull trout habitat limiting factors for the Wenatchee Subbasin (Water Resource Inventory Area 45) and portions of WRIA 40 within Chelan County (Squilchuck, Stemilt and Clockum drainages). Washington State Conservation Commission, Olympia, WA. 71 – 238 p.
- Chapman, D.W., C. Peven, A. Giorgi, T. Hillman, F. Utter. 1995. Status of spring Chinook salmon in the mid-Columbia region. Report to Chelan, Douglas, and Grant County Public Utility Districts, Washington. Don Chapman Consultants, Inc., Boise, Idaho.
- CCPUD (Chelan County Public Utility District). 2002. Anadromous fish agreement and habitat conservation plan. Chelan County Public Utility District, Wenatchee, WA.
- Craig, J. A. and A. J. Suomeia. 1941. Time of appearance of the runs of salmon and Steelhead trout native to the Wenatchee, Entiat, Methow and Okanogan rivers. United States Fish and Wildlife Service. 35 p. plus 18 affidavits and accompanying letters of corroboration.
- Ford, M., and twelve coauthors. 2001. Upper Columbia River Steelhead and Spring Chinook Salmon Population Structure and Biological Requirements, Final Report, March 2001. Northwest Fisheries Science Center, National Marine Fisheries Service. Seattle, Washington.
- Mosey, T. R., and L. J. Murphy. 2002. Spring and summer Chinook spawning ground surveys on the Wenatchee River basin, 2002. Chelan County Public Utility District, Wenatchee, Washington.
- Mullan, J. W., K. R. Williams, G. Rhodus, T.W. Hillman and J.D. McIntyre. 1992. Production and habitat of salmonids in Mid-Columbia River tributaries. U.S. Fish and Wildlife Service, Monograph 1, Leavenworth, WA. 8 p.
- Murdoch, A.R., T.N. Pearsons, T.W. Maitland, M.F. Ford, and K. Williamson. 2005. Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. BPA Project No. 2003-039-00. Bonneville Power Administration, Portland, Oregon.
- Murdoch, A.R., T.N. Pearsons, T.W. Maitland, M.F. Ford, and K. Williamson. 2006. Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. BPA Project No. 2003-039-00. Bonneville Power Administration, Portland, Oregon.

RISPA (Rock Island Project Settlement Agreement). 1989. Rock Island Project Settlement Agreement. Federal Energy Regulatory Commission and Chelan County Public Utility District Project No. 943, No. E-9569, Wenatchee, WA.

Utter, F.M., D.W. Chapman, and A.R. Marshall. 1995. Genetic Population Structure and history of Chinook salmon of the Upper Columbia River. *American Fisheries Society Symposium* 17: 149-68.

Chapter 1 -- A comparison of demographic variables of adult hatchery and natural origin spring Chinook salmon 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 ($P < 0.05$). Data collected during spawning at a hatchery showed that there was a significant difference in fecundity ($P < 0.05$), but not egg weight ($P > 0.05$), 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 in two of the three years examined ($P > 0.05$). In 2004, hatchery fish had significantly greater higher egg retention rates than naturally produced fish ($P < 0.05$). 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 or if impacts to reproductive success and survival in natural environments has occurred.

Introduction

Hatcheries can inadvertently change important demographic traits of salmonid populations that have the potential to influence short and long-term reproductive success and survival (Carmichael and Messmer 1995, Olson et al. 2004, Knudsen et al. 2006). 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; Knudsen et al. 2006; Busack et al. in press). Despite strict hatchery guidelines to minimize environmental and genetic differences, supplementation of spring Chinook salmon in the Yakima Basin resulted in changes in sex composition, age at maturation, size-at-age, and spawn timing after only one generation of artificial propagation (Knudsen et al. 2006). Differences were not detected for migration time of hatchery and natural origin adults (Knudsen et al. 2006). Unfortunately, reproductive success estimates of hatchery and natural origin fish spawning in the natural environment of the upper Yakima Basin are not available. However, a pedigree assessment of hatchery and natural origin fish in an experimental spawning channel will soon be available. 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 downstream of current and 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 m³), which the fish enter after being diverted from the adult fish ladder. Two gravity fed chambers provide a constant source of river water. Trapped fish must actively swim through a deniel located at the upstream end of the primary collection chamber. 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 m³), or if fish are to be sampled immediately, into a tank (1.36 m³) fed by a 5 hp pump. The secondary collection chamber is also fed river water through gravity fed chambers. Located at the bottom of the chamber is a large hopper (1.54 m³) that is used to hoist fish from the secondary collection chamber and also serves as an anesthetic tank. The final portion of the trapping facility is the recovery tank (1.72 m³) and return flume, which is supplied with river water from another 5 hp pump. After fish are revived in the recovery tank they are released upstream of the dam.

The fish trap is capable of operating in either passive or active mode. In passive mode, fish are held in the primary collection chamber. In active mode, personnel are present to sort fish as they volitionally swim from the primary collection chamber into the deniel. 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, personnel 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 deniel 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 m³) 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 and adipose fin present) 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 cm² 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 Chapters 4 and 5). 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 (adult fish) or body cavity (jacks and precocious males). 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.

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

Genetic differences between spawning aggregates have been reported in the upper Wenatchee spring Chinook population (Murdoch et al. 2006). Hence, differences in life history traits between hatchery and naturally produced fish sampled at Tumwater Dam may be attributed to genetic differences within the population. Whenever possible, comparisons between Chiwawa hatchery and naturally produced (i.e., collected the Chiwawa weir or on the spawning grounds in the Chiwawa River) were conducted.

Migration timing of hatchery and natural origin fish was compared by run year, origin, and age class using a Kruskal-Wallis analysis of variance (KW test). Age composition and sex ratios of hatchery and naturally produced adult spring Chinook were compared by brood year 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 (BW) of hatchery and wild fish was compared by brood year, age, and gender using a KW test. Any significant differences detected within brood years was reanalyzed with using a t-test. Length and weight comparisons were made for both the population at Tumwater Dam and age-4 Chiwawa River spring Chinook. Fecundity and egg weight of hatchery and naturally produced females of the same brood year and age were also compared using a KW test. A linear regression was performed using fork length (independent variable) and fecundity (dependent variable) for both, hatchery and wild broodstock. The slopes of the regression models were compared using homogeneity of slopes test. Subsequently, regression models with parallel slopes were analyzed with an analysis of covariance (ANCOVA) to examine differences in length-fecundity relationships between hatchery and naturally produced fish. Using the linear regression models, the estimated fecundity for all females examined for egg retention on the spawning grounds 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 by run year and spawning location using a KW test. All statistical tests were run with a significance level of 0.05.

Results and Discussion

Trap Operation

The trap was operated from 7 May through 6 August 2006. We operated the trap operated passively from 7 May to 20 May due to low fish passage. During this time period, personnel checked the trap and sampled fish multiple times daily. Between 21 June and 6 August, the trap was operated actively during the hours of daylight and passively during night when fish passage was low. No trapping occurred from 17 May through 25 May because the fish ladders were closed due to very high river flows and subsequent debris load. The only other break in trapping occurred on 30 May, when Chelan PUD replaced the water pump that supplies the deniel. At which time, trapping was interrupted for approximately 8 hours. No mortality occurred during the trapping period.

A total of 2,175 spring Chinook adults and jacks and 201 precocious males (age-2) were counted at Tumwater Dam (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 1,590 hatchery adults, 559 natural adults, 26 unknown origin, 200 hatchery and 1 naturally produced precocious male (100% of all spring Chinook accounted for at Tumwater

Dam). After trapping was completed, no additional spring chinook were observed on videotapes migrating upstream of Tumwater Dam.

Run timing

Naturally produced spring Chinook migrated upstream of Tumwater Dam between 17 June and 05 August (50 days). Hatchery spring Chinook were captured at Tumwater Dam between 19 June and 31 July (43 days). Precocious hatchery Chinook were observed between 3 July and 5 August (Figure 1, Appendix A). Differences in run timing were detected at Tumwater Dam for between groups ($P < 0.001$), but not within groups of the same age in 2006 (Table 1). Differences in run timing at Tumwater Dam were also detected between years (2004 - 2006) and age classes ($P < 0.001$). Older aged spring Chinook migrated earlier than younger spring Chinook within all years. Age-3 hatchery and naturally produced spring Chinook did not differ in run timing within years, but hatchery fish in 2006 were significantly different when compared to 2004 and 2005 ($P < 0.001$; Figure 2). Conversely, no difference was detected in age-4 hatchery and naturally produced spring chinook in 2006, but differences were found among groups in 2004 and 2005 ($P < 0.001$). Age-4 fish also exhibited a later run timing in 2006 than that observed in 2004 or 2005 ($P < 0.001$). Age-5 hatchery and naturally produced fish had similar run timing within each year, but both groups were significantly later in 2006 (Table 2; $P < 0.001$).

A more detailed analysis of only age-4 Chiwawa hatchery and naturally produced spring Chinook (i.e., naturally produced fish from other spawning tributaries were excluded) yielded similar results. Differences in run timing between hatchery and natural produced fish were detected within and between years ($P < 0.001$). The only within year difference between hatchery and natural origin fish of the same gender was male spring Chinook in 2005 ($P < 0.03$; Figure 3). In 2006, all groups were significantly later in run timing compared to 2004 and 2005. The later run timing observed in 2006 was likely the result of higher than average discharge in the Wenatchee River during the months of May and June. Higher than normal discharge in 2006 within the Tumwater Canyon section of the Wenatchee River likely formed hydraulic barriers to upstream migration.

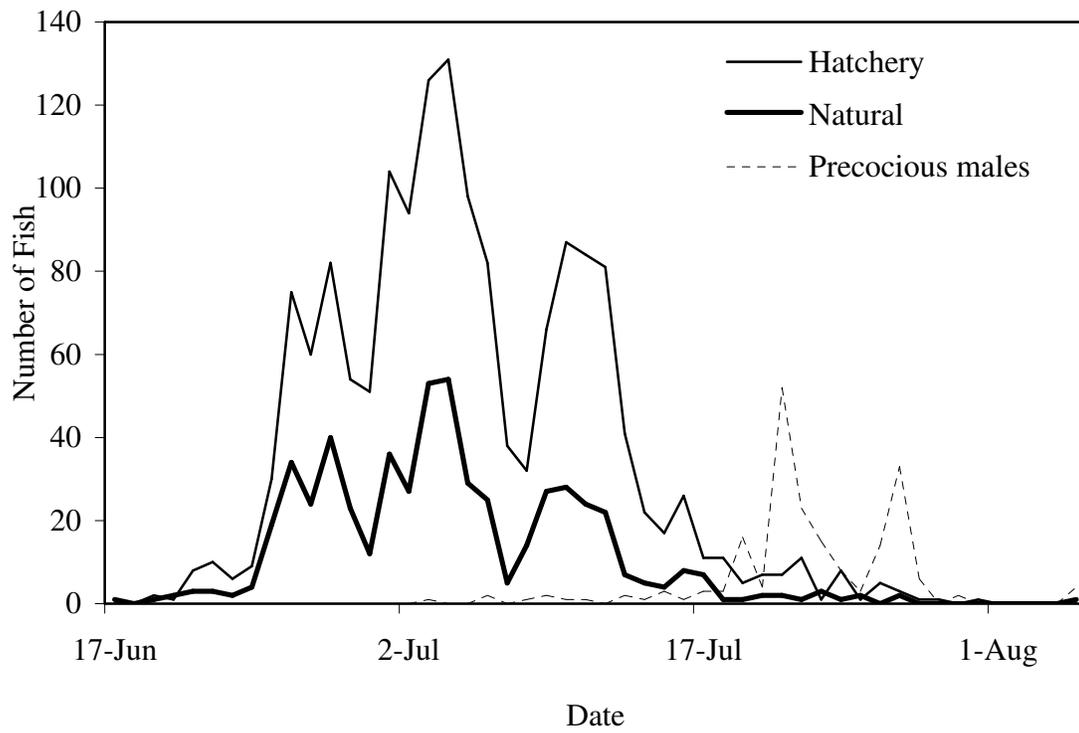


Figure 1. Run Timing of adult hatchery and naturally produced spring Chinook and Chinook sampled at Tumwater Dam in 2006.

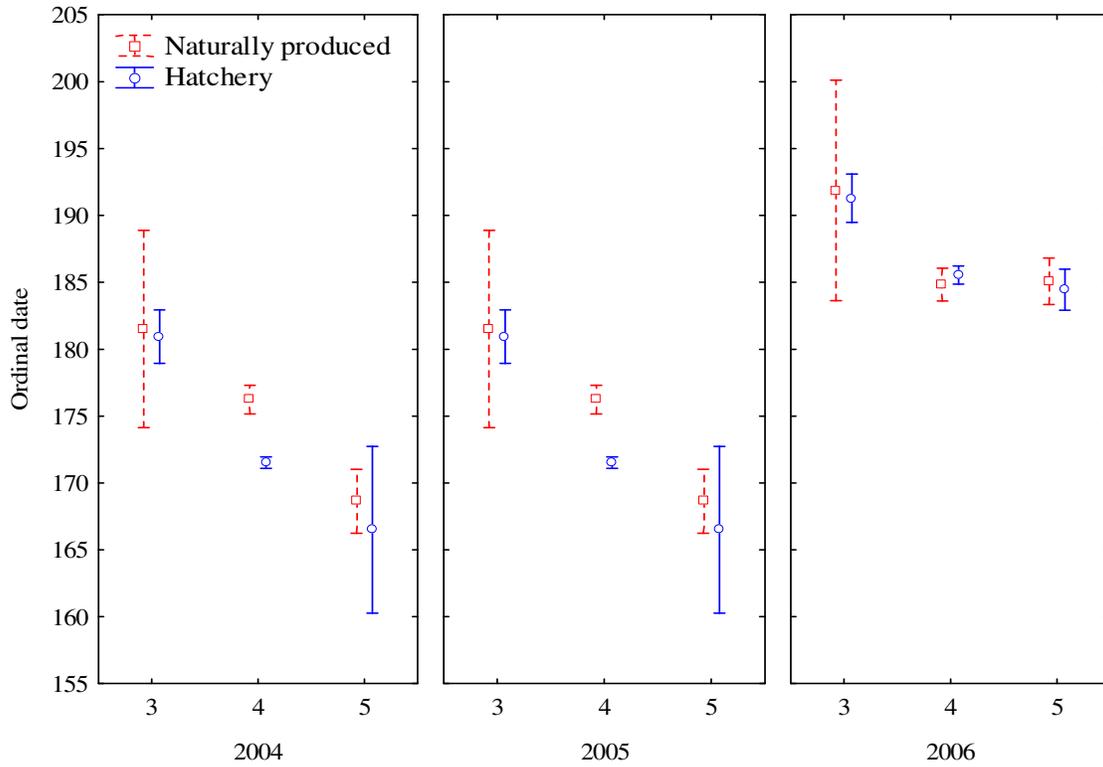


Figure 2. Passage timing by age class of spring Chinook at Tumwater Dam between 2004 and 2006.

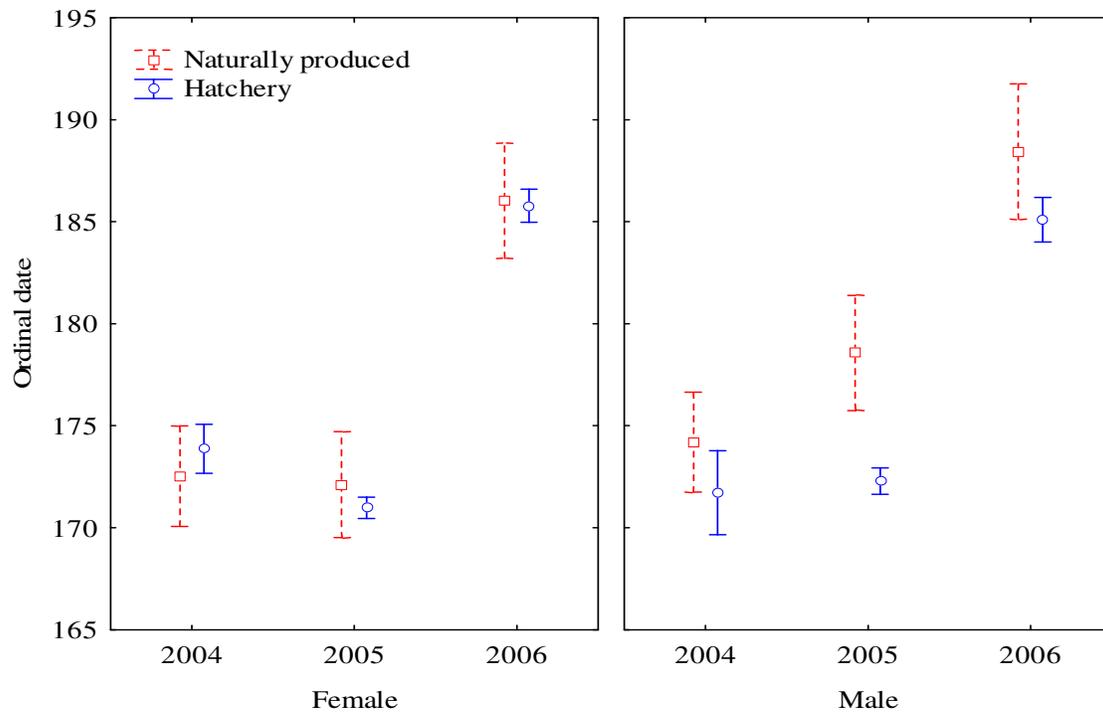


Figure 3. Passage timing of age-4 Chiwawa spring Chinook at Tumwater Dam between 2004 and 2006.

Table 1. Cumulative passage dates of Wenatchee River spring Chinook sampled at Tumwater Dam between 2004 and 2006.

Origin/Age	Cumulative Run Timing		
	10%	50%	90%
<i>2004</i>			
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
<i>2005</i>			
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
<i>2006</i>			
Hatchery (All ¹)	27-Jun	04-Jul	13-Jul
Age-2	18-Jul	22-Jul	27-Jul
Age-3	02-Jul	11-Jul	17-Jul
Age-4	27-Jun	04-Jul	12-Jul
Age-5	26-Jun	03-Jul	12-Jul
Natural (All ¹)	26-Jun	03-Jul	12-Jul
Age-2	26-Jul	26-Jul	26-Jul
Age-3	30-Jun	12-Jul	27-Jul
Age-4	26-Jun	03-Jul	12-Jul
Age-5	26-Jun	03-Jul	12-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, 2005, and 2006 (H = hatchery; N = natural).

Age/Origin	N	Mean	Median	Minimum	Maximum	SD (days)
<i>2004</i>						
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
<i>2005</i>						
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
<i>2006</i>						
2W	1	Jul 26	Jul 26	Jul 26	Jul 26	-
2 H	200	Jul 22	Jul 22	Jul 03	Aug 05	5
3 H	166	Jul 10	Jul 11	Jun 27	Jul 28	6
3 N	7	Jul 12	Jul 12	Jun 30	Jul 27	9
4 H	1,164	Jul 05	Jul 04	Jun 19	Jul 31	6
4 N	365	Jul 04	Jul 03	Jun 17	Jul 27	6
5 H	229	Jul 03	Jul 03	Jun 21	Jul 26	7
5 N	181	Jul 04	Jul 03	Jun 21	Aug 05	7

Age Composition

In 2006, ages were determined through scale samples from 1,559 and 553 hatchery and natural spring Chinook, respectively (Table 3). All 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 upstream of Tumwater Dam could not be determined. Murdoch et al. (2006) found that differences in age composition within a given year were the result of variation in the number of hatchery fish released (range 47,104 – 377,544). Because of these differences, comparisons of age composition by brood year were performed.

Significant differences were detected in the 2001 brood between hatchery and natural origin fish ($\chi^2 = 623.7$, $df = 2$, $P < 0.001$). A greater proportion of hatchery fish returned at age-3 and fewer hatchery fish returned as age-5 than natural origin fish (Table 4). Murdoch et al. (2006) also reported that naturally produced age-5 fish in the 2000 brood returned in a greater proportion than hatchery fish, but no difference in age-3 fish was found. 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. 2006).

Table 3. Age composition of Wenatchee River spring Chinook sampled at Tumwater Dam in 2004, 2005, and 2006 (Age-2 fish not included).

Origin	Total Age			N
	3	4	5	
<i>2004</i>				
Hatchery	64.1%	35.4%	0.5%	1,273
Natural	3.50%	95.2%	1.30%	888
All	39.2%	60.0%	0.80%	2,161
<i>2005</i>				
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
<i>2006</i>				
Hatchery	10.65%	74.66%	14.69%	1,559
Natural	1.27%	66.00%	32.73%	553
All	8.19%	72.40%	19.41%	2,112

Table 4. Age composition of the 2000 and 2001 brood Wenatchee River spring Chinook sampled at Tumwater Dam in between 2003 and 2006.

Origin	Total Age			N
	3	4	5	
<i>2000 brood</i>				
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
<i>2001 brood</i>				
Hatchery	20.4%	73.9%	5.7%	4,047
Natural	4.6%	68.5%	26.9%	677
All	18.1%	73.2%	8.7%	4,724

Differences between brood years of the same origin were also examined. Both natural and hatchery origin fish differed in age composition between 2000 and 2001 brood years. The 2001 brood natural origin fish had a lesser proportion of age-4 ($\chi^2 = 39.8$, $df = 1$, $P < 0.001$) and greater proportion of age-5 fish than the 2000 brood ($\chi^2 = 67.1$, $df = 1$, $P < 0.001$). The 2000 and 2001 brood hatchery fish differed in all age classes. Of which, the 2001 brood had a greater proportion of age-3 ($\chi^2 = 301.5$, $df = 1$, $P < 0.001$) and age-5 ($\chi^2 = 361.5$, $df = 1$, $P < 0.001$) fish and a lesser proportion of age-4 ($\chi^2 = 42.4$, $df = 1$, $P < 0.001$) fish than the 2000 brood. Differences in age composition between and within origins may be influenced by many factors outside the scope of the study (i.e., smolt size, ocean conditions, and differential survival or harvest rates). Understanding the differences may provide additional information that could explain potential differences in reproductive success. Because hatchery fish mature at an earlier age than naturally produced fish, they may be less competitively dominant on the spawning grounds, which could result in lower reproductive success (i.e., access to fewer females or less than ideal spawning locations).

Sex Ratio

The gender of each fish was determined at Tumwater Dam based on external morphological characteristics. Due to the early run timing of spring Chinook, secondary sexual characteristics may not be prominent and correct gender identification may not be accurate. A comparison of the gender of individual fish determined at Tumwater Dam to those fish subsequently recovered on the spawning grounds and during hatchery spawning found that gender determination was correct 94.3 % for female and 93.1% for males. After correction, the male to female ratio of the natural and hatchery fish for 2006 was 0.86 to 1.0 and 0.74 to 1.0, respectively (Table 5). The overall male to female ratio for the spawning population upstream of Tumwater Dam (broodstock not included) was 0.78 to 1.0.

Comparisons between gender and origin of the 2001 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 2004 and 2006 (Table 6; age-2 fish excluded). No difference was detected in the proportion hatchery and natural origin males or females ($\chi^2 = 0.43$, $df = 1$, $P=0.51$). The overall male to female ratio of hatchery and natural origin fish was 1.14:1 and 1.05:1, respectively.

Although no difference was detected for the entire brood, analysis by age class found that age-4 hatchery fish had a significantly lower proportion of males than age-4 natural origin fish ($\chi^2 = 7.89$, $df = 1$, $P < 0.01$). No difference was detected in the sex ratio of age-5 hatchery and natural origin fish ($\chi^2 = 0.29$, $df = 1$, $P = 0.59$). 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.

Table 5. The estimated number of male and female spring Chinook counted at Tumwater Dam and the corrected number based on carcass recoveries in 2006.

Age	Origin	Sex	Tumwater Dam	Corrected Number
3	Hatchery	Male	166	166
		Female	0	0
4	Hatchery	Male	7	7
		Female	0	0
	Natural	Male	165	165
		Female	200	200
5	Hatchery	Male	86	88
		Female	143	141
	Natural	Male	81	81
		Female	100	100
Unknown	Hatchery	Male	9	9
		Female	22	22
Unknown	Natural	Male	5	5
		Female	1	1
Unknown	Unknown	Male	10	10
		Female	16	16

Table 6. Summary of the 2000 and 2001 brood hatchery and natural origin Spring Chinook by sex and age observed at Tumwater Dam between 2003 and 2006.

Age	Male		Female	
	Hatchery	Natural	Hatchery	Natural
2000 brood				
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%
2001 brood				
3	20.3%	4.6%	0.1%	0.0%
4	30.6%	34.6%	43.3%	34.0%
5	2.2%	12.0%	3.5%	14.8%

Size-at-Age

Of those fish sampled at Tumwater Dam, no difference in POH or body weight was detected for naturally produced and hatchery age-3 fish at Tumwater Dam within all brood years examined (2001-2003). The POH of 2000 brood age-4 females was significantly different ($P < 0.001$), but not body weight. The mean POH of hatchery females was 1.3 cm greater than that of naturally produced females (t-test, $P < 0.001$; Table 7). In the 2001 brood, age-4 male fish differed in both POH ($P < 0.001$) and body

weight ($P < 0.001$). Hatchery males were 3.0 cm and 0.86 kg greater in POH and body weight, respectively (t-test, $P < 0.001$). Differences were only detected between the mean body weights of age-4 females in the 2002 brood ($P < 0.001$). Age-4 hatchery females were 0.39 kg lower in body weight than naturally produced age-4 females (t-test, $P < 0.001$). No differences in POH or body weight were found within brood years between hatchery and naturally produced age-5 fish of the same gender.

Table 7. Mean fork length (FL, cm) and body weight (BW, kg) by brood year for Wenatchee River spring Chinook sampled at Tumwater Dam between 2004 and 2006.

Brood	Age	Origin	Sex	FL (SD)	BW (SD)	N
1999	5	Natural	Male	91.6 (4.8)	7.86 (1.01)	5
			Female	91.3 (5.7)	8.22 (1.77)	7
		Hatchery	Male	98.0 (1.4)	9.10 (0.42)	2
			Female	82.8 (8.4)	6.15 (1.84)	4
2000	5	Natural	Male	96.2 (6.5)	9.46 (2.21)	44
			Female	91.7 (4.1)	8.13 (1.31)	51
		Hatchery	Male	90.2 (6.8)	8.10 (2.20)	6
			Female	88.1 (6.4)	7.23 (1.41)	8
2000	4	Natural	Male	78.5 (6.5)	5.33 (1.27)	438
			Female	77.9 (4.0)	5.29 (0.81)	407
		Hatchery	Male	80.2 (6.6)	5.49 (1.40)	115
			Female	79.6 (4.5)	5.51 (0.98)	343
2001	5	Natural	Male	95.5 (7.4)	9.12 (1.85)	78
			Female	90.2 (4.4)	7.72 (1.24)	10
		Hatchery	Male	97.1 (5.8)	9.32 (1.83)	85
			Female	90.6 (4.2)	7.67 (1.21)	14
2001	4	Natural	Male	78.4 (6.6)	5.21 (1.28)	231
			Female	79.3 (4.8)	5.38 (0.96)	234
		Hatchery	Male	82.5 (5.9)	6.08 (1.31)	1,188
			Female	79.3 (4.0)	5.42 (0.87)	1,804
2001	3	Natural	Male	50.7 (5.4)	1.52 (0.56)	31
		Hatchery	Male	52.9 (5.9)	1.76 (0.66)	821
			Female	62.2 (4.9)	2.85 (0.75)	5
2002	4	Natural	Male	79.0 (7.2)	5.35 (1.44)	162
			Female	77.9 (4.6)	5.15 (1.07)	203
		Hatchery	Male	80.7 (6.2)	5.45 (1.26)	412
			Female	76.9 (4.5)	4.76 (0.91)	752
2002	3	Natural	Male	52.0 (3.7)	1.60 (0.29)	10
		Hatchery	Male	54.8 (4.5)	1.84 (0.46)	136
2003	3	Natural	Male	51.7 (6.1)	1.53 (0.47)	7
		Hatchery	Male	52.7 (4.2)	1.59 (0.44)	166

A more detailed analysis of hatchery and naturally produced fish from the Chiwawa River found no difference in POH ($P = 0.97$) or body weight ($P = 0.85$) between males or females in the 2000 brood (Table 8). For 2001 brood fish sampled at Tumwater Dam, age-4 male hatchery spring Chinook were significantly different in POH and body weight than naturally produced males (Figure 4 and 5). Age-4 hatchery males were 3.6 cm and 1.03 kg greater than naturally produced males in POH (t-test, $P < 0.001$) and body weight (t-test, $P < 0.001$), respectively. For 2002 brood age-4 fish in the Chiwawa River, hatchery female spring Chinook had a significantly lower mean POH ($P < 0.01$) and body weight ($P < 0.01$) than naturally produced female spring Chinook (Figure 5). Age-4 naturally produced females were 2.1 cm and 0.77 kg greater than hatchery females in POH (t-test, $P < 0.001$) and body weight (t-test, $P < 0.001$), respectively.

Table 8. Mean fork length (FL, cm) and body weight (BW, kg) by brood year for age-4 Chiwawa River spring Chinook sampled between 2004 and 2006.

Brood	Origin	Sex	FL (SD)	BW (SD)	<i>N</i>
2000	Natural	Male	63.8 (0.5)	5.63 (0.12)	81
		Female	64.0 (0.5)	5.47 (0.12)	81
	Hatchery	Male	63.8 (0.4)	5.49 (0.10)	116
		Female	64.3 (0.4)	5.52 (0.06)	340
2001	Natural	Male	61.8 (0.5)	5.30 (0.14)	62
		Female	63.6 (0.5)	5.33 (0.13)	73
	Hatchery	Male	65.4 (0.3)	6.33 (0.08)	168
		Female	63.8 (0.2)	5.54 (0.06)	303
2002	Natural	Male	63.2 (0.6)	5.52 (0.16)	45
		Female	64.4 (0.5)	5.53 (0.14)	62
	Hatchery	Male	64.1 (0.2)	5.45 (0.05)	412
		Female	62.3 (0.2)	4.76 (0.04)	752

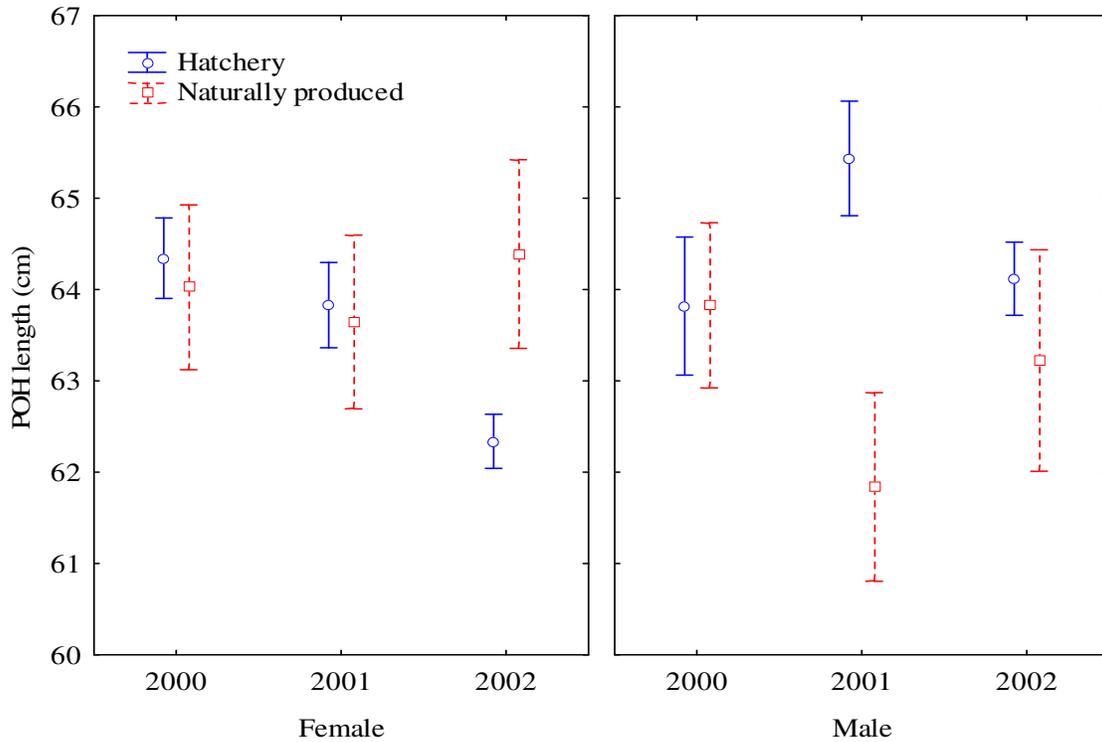


Figure 4. Mean post-orbital to hypural plate length by brood year of age-4 Chiwawa spring Chinook sampled on the spawning grounds and as broodstock. Vertical bars denote 95% confidence intervals.

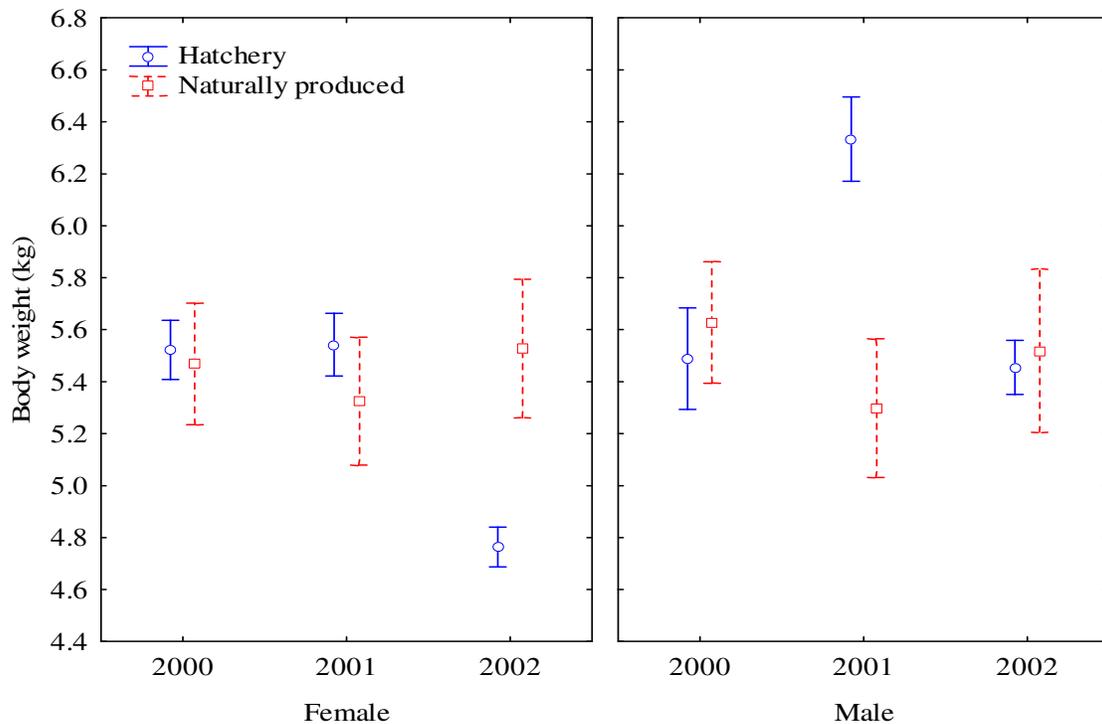


Figure 5. Mean weight by brood year of age-4 Chiwawa spring Chinook sampled on the spawning grounds or as broodstock. Vertical bars denote 95% confidence intervals.

Fecundity and Egg Weight

A total of 398 spring Chinook were initially collected and held at Eastbank Fish Hatchery for broodstock in 2006. Surplus hatchery males ($N = 6$) and females ($N = 44$) were returned to the Chiwawa River to spawn naturally. Age and origin was determined through scale analysis and CWT decoding for 249 and 91 hatchery and wild fish that were retained as broodstock, respectively (Table 8). The origin for an additional eight fish (4 hatchery and 4 naturally produced) was based on the presence or absence of an adipose fin clip and/or CWT. Mean fecundity and egg weight was determined for hatchery and naturally produced age-4 and age-5 female spring Chinook (Table 9).

Analysis of fecundity and egg weight data was limited to age-4 fish because age-5 female hatchery fish were unintentionally not collected for broodstock in 2004 or 2005. Differences in fecundity were detected within and between brood years ($P < 0.001$). The only within year difference between hatchery and naturally produced fish was observed in 2002 brood (Figure 6). Age-4 hatchery fish differed in mean fecundity between all years ($P < 0.001$), while naturally produced fish only differed between 2000 and 2001 brood years ($P < 0.001$).

Differences were detected in the slope of the fecundity regression lines of 2002 brood age-4 hatchery and natural produced fish ($P < 0.05$). Hence, separate regression models were used to estimate the fecundity of hatchery and naturally produced fish. When data from all years was incorporated into the analysis, differences were detected between years ($P < 0.002$) and the interaction term year \times origin ($P < 0.04$). Subsequently, results of the separate slopes model using the same data also detected no difference in origin ($P = 0.19$), but differences were detected between years ($P < 0.01$) and the interaction term year \times origin ($P < 0.04$). These results suggest that age-4 hatchery and naturally produced Chiwawa spring Chinook have varying fecundity to length relationships within and between years.

Mean egg weight of age-4 fish was different between years ($P < 0.001$; Figure 7). The 2000 brood hatchery and naturally produced fish had lower mean egg weight than 2001 and 2002 broods. The lower egg weight corresponds with an observed increase in fecundity and POH. However, no differences within years between hatchery and naturally produced fish were detected (Figure 8).

Table 8. Age composition of Chiwawa spring Chinook hatchery broodstock at Eastbank Fish Hatchery for brood years 2000 to 2002.

Origin	Total Age			N
	3	4	5	
<i>2000</i>				
Hatchery	37.3%	62.7%	0.0%	193
Natural	4.3%	92.5%	3.2%	93
All	26.6%	72.4%	1.0%	286
<i>2001</i>				
Hatchery	4.4%	94.5%	1.1%	183
Natural	1.0%	84.9%	14.1%	99
All	3.2%	91.1%	5.7%	282
<i>2002</i>				
Hatchery ¹	0.4%	82.3%	17.3%	249
Natural	2.2%	70.3%	27.5%	91
All	0.9%	79.1%	20.0%	340

¹ Fish released were not included.

Table 9. Summary statistics for Chiwawa spring Chinook broodstock fecundity and egg weights for brood years 2000 to 2002.

Origin	Age	Fecundity			Egg Weight		
		Mean	SD	N	Mean	SD	N
<i>2000</i>							
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
<i>2001</i>							
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
<i>2002</i>							
Hatchery	4	3,761	727	106	0.220	0.032	106
Hatchery	5	4,930	711	25	0.262	0.054	25
Natural	4	4,308	727	27	0.223	0.033	27
Natural	5	5,390	823	17	0.257	0.054	17

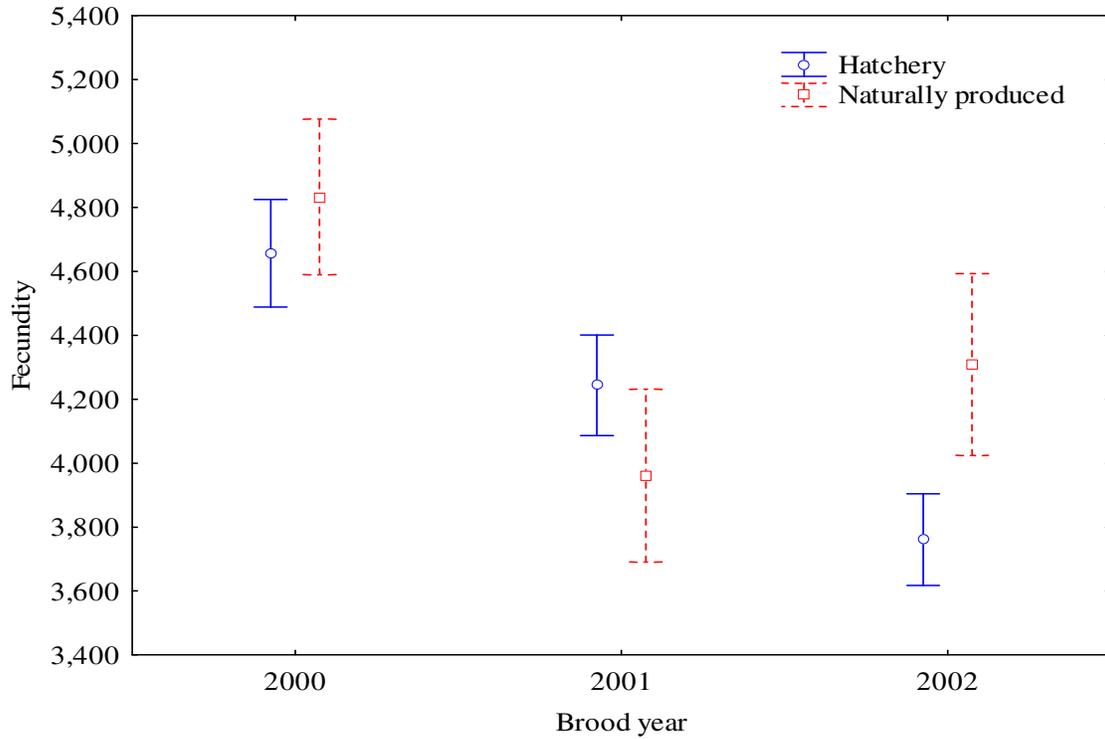


Figure 6. Mean fecundity of age-4 hatchery and natural origin Chiwawa spring Chinook. Vertical bars denote 95% confidence intervals.

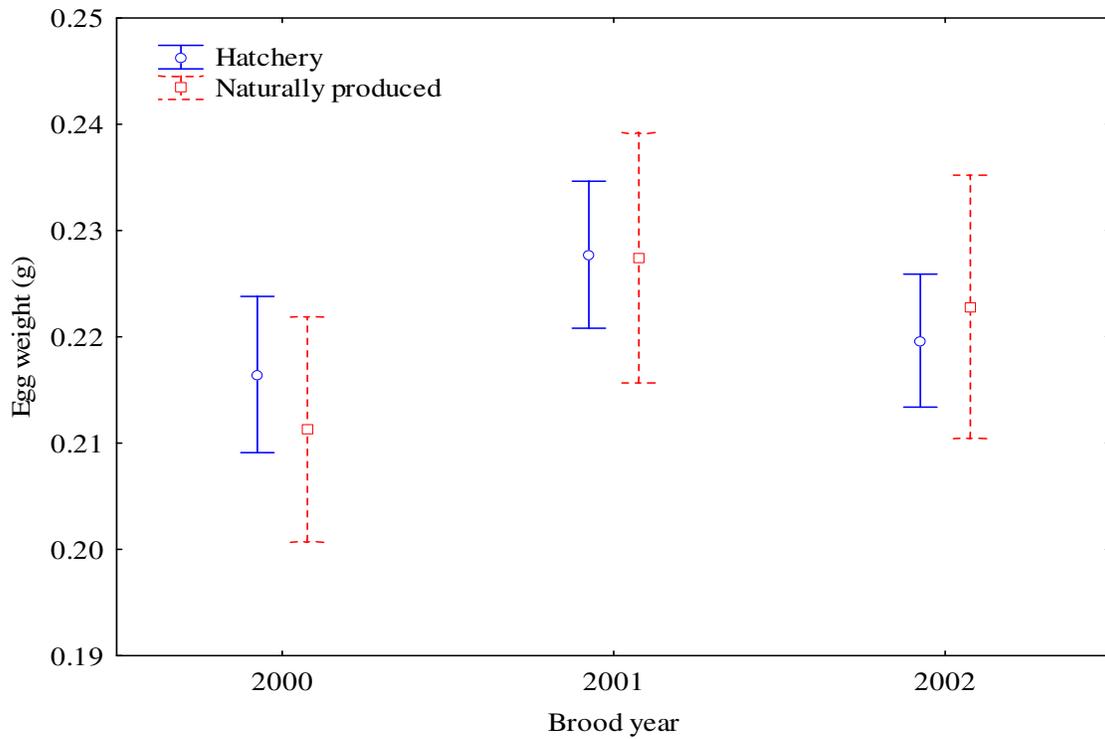


Figure 7. Mean egg weight of age-4 Chiwawa spring Chinook. Vertical bars denote 95% confidence intervals.

Egg Retention

In 2006, a total of 167 hatchery and 72 naturally produced fish were examined to determine the number of eggs retained in the body cavity after spawning (Table 10). The estimated mean (SD) percentage of eggs retained in 2006 for hatchery and naturally produced fish was 1.24 (5.41) and 0.22 (0.82), respectively. Significant differences were detected between years ($P < 0.02$), streams ($P < 0.001$), and origins ($P < 0.04$; Figure 8). A more detailed analysis of only those fish recovered in the Chiwawa River found similar differences between years and origin ($P < 0.02$; Figure 9). The cause of the relatively higher egg retention rates of hatchery fish in 2004 is unknown.

Table 10. Number of female spring Chinook examined and the mean (SD) number and proportion (SD) of eggs retained in the body cavity after spawning between 2004 and 2006.

Stream	Hatchery			Natural		
	<i>N</i>	Mean number of eggs	Mean proportion of eggs	<i>N</i>	Mean number of eggs	Mean proportion of eggs
<i>2004</i>						
Chiwawa	22	63 (255)	1.35 (5.40)	32	13 (53)	0.27 (1.13)
Nason	14	37 (75)	0.86 (1.79)	56	12 (42)	0.26 (0.93)
Wenatchee	6	10 (6)	0.19 (0.17)	3	2 (4)	0.04 (0.07)
White	2	10 (13)	0.28 (0.40)	5	5 (11)	0.13 (0.22)
Little Wenatchee	0			1	8 (--)	0.20 (--)
<i>2005</i>						
Chiwawa	179	11 (47)	0.26 (1.06)	35	10 (51)	0.26 (1.35)
Nason	99	31 (106)	0.81 (2.92)	25	21 (52)	0.50 (1.30)
Wenatchee	46	46 (107)	1.12 (2.51)	1	0 (--)	0.00 (--)
White	32	3 (6)	0.07 (0.15)	7	1 (2)	0.01 (0.04)
Little Wenatchee	23	5 (8)	0.13 (0.22)	11	21 (59)	0.46 (1.24)
<i>2006</i>						
Chiwawa	101	17 (42)	0.50 (1.30)	25	14 (60)	0.30 (1.25)
Nason	50	74 (355)	2.14 (9.32)	36	9 (20)	0.23 (0.52)
Wenatchee	6	179 (156)	5.80 (5.36)	0		
White	6	60 (90)	1.45 (2.15)	8	1 (2)	0.03 (0.04)
Little Wenatchee	3	67 (115)	1.62 (2.77)	3	2 (2)	0.04 (0.06)

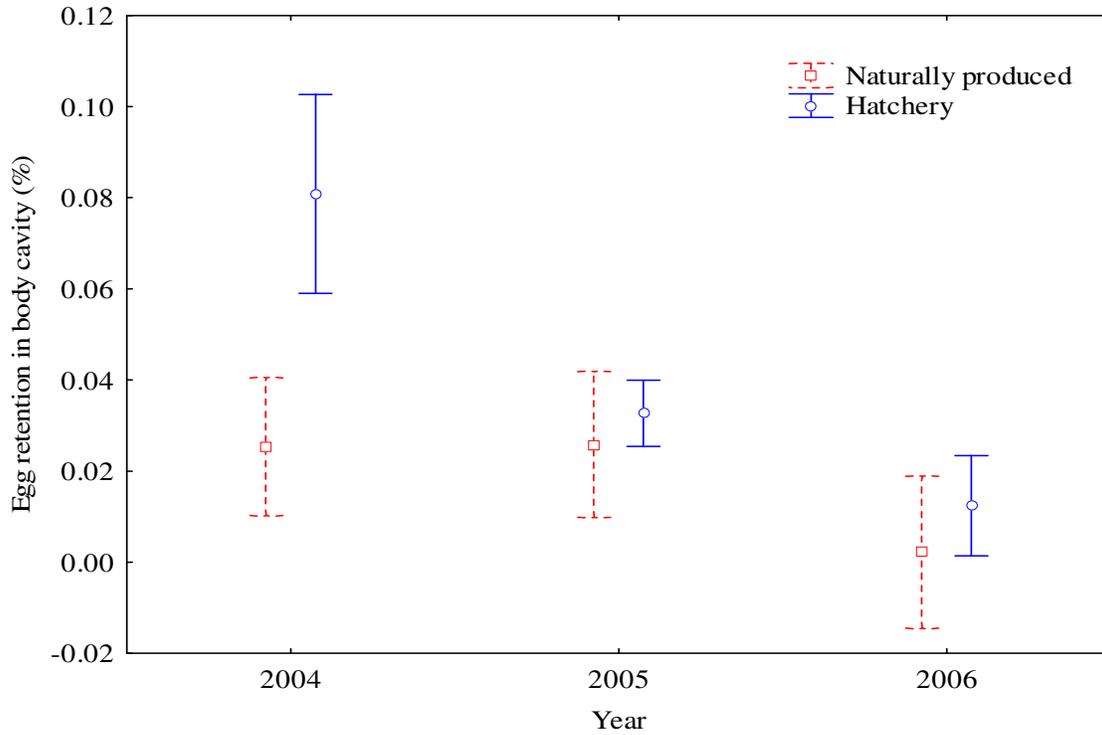


Figure 8. Mean (%) egg retention of hatchery and naturally produced Wenatchee River spring Chinook. Vertical bars denote 95% confidence intervals.

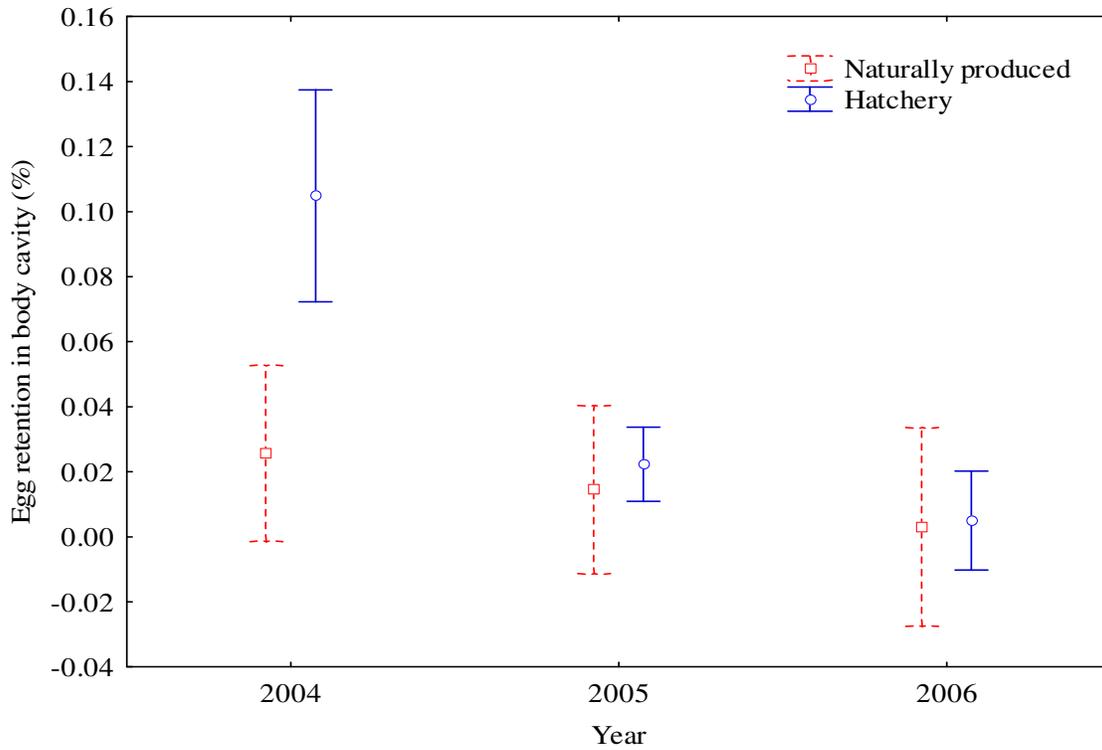


Figure 9. Mean (%) egg retention of hatchery and naturally produced Chiwawa River spring Chinook. Vertical bars denote 95% confidence intervals.

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 1,813 adults and jacks and 201 precocious males (Table 12).

Table 12. Distribution of spring Chinook detected at Tumwater Dam in 2004, 2005, and 2006. Data includes 8 natural and 1 hatchery origin spring Chinook detected from video counts in 2004 and 3 natural origin spring Chinook detected from video counts in 2005 (HPM = hatchery precocious males, NPM = natural precocious males).

Origin	Below Tumwater Dam		Above Tumwater Dam			Total
	Fallback	Eastbank Hatchery	Prespawn Mortality	Chiwawa Weir	Spawning Grounds	
<i>2004</i>						
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
<i>2005</i>						
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
<i>2006</i>						
Hatchery	3	143	11	109	1,324	1,590
HPM	0	0	0	0	200	200
Natural	0	0	0	93	466	559
NPM	0	0	0	0	1	1
Unknown	0	1	0	2	23	26
Total	3	144	11	204	2,014	2,376

Summary

Run timing of Wenatchee spring Chinook was influenced by age at return. Older aged fish, both hatchery and naturally produced, returned earlier than younger aged fish. In 2006, all age classes had a later run timing due to higher than average river discharge that likely delayed passage through Tumwater Canyon. Hatchery spring Chinook return at a younger age than naturally produced fish. Of those hatchery fish, male spring Chinook comprised a greater proportion of age-3 and a lesser proportion of age-5 fish compared to naturally produced fish. However, no significant difference was detected in the overall sex ratio of all age classes. Differences in POH or body weight of naturally produced fish were not detected. However, hatchery fish exhibited more variation in size across years.

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. 2006). Differences in size observed in the Wenatchee Basin may be because of the larger size disparity of hatchery and natural origin smolts. For brood years 2000 through 2003, Chiwawa hatchery yearling smolts were 5.1 cm (SD = 0.5 cm) greater in fork length than naturally produced Chiwawa yearling smolts ($P < 0.001$; WDFW unpublished data). Although no correlation was detected between the size difference of smolts and adults of the same brood year, the difference in size between hatchery and naturally produced fish decreased with age (Figure 10). The size advantage of hatchery fish was generally eliminated or reversed by the time naturally produced fish reached age-5.

No significant differences were detected in the fecundity of hatchery and naturally produced, except the 2002 brood hatchery fish, which were smaller in size at return. Differences in egg weight were not detected within years, but both the 2000 brood hatchery and naturally produced fish had smaller eggs due to a greater size at return than 2001 or 2002 brood years.

Egg retention rates were similar between hatchery and naturally produced fish, except in 2004 when hatchery fish were significantly higher than naturally produced fish. Egg retention was shown to differ significantly between streams and environmental factors (i.e., high water temperature) are likely the cause. The greater the proportion of hatchery fish that spawn in the Wenatchee River would likely result in a decreased the number of eggs deposited and subsequently reduce reproductive success.

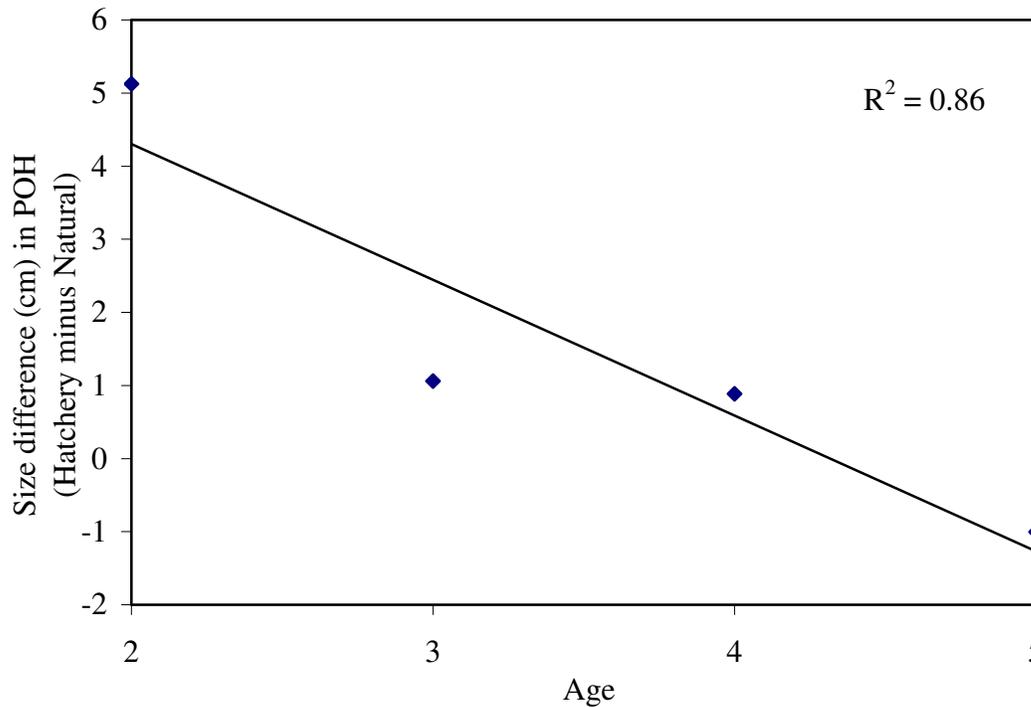


Figure 10. Size differences between Chiwawa hatchery and naturally produced spring Chinook (brood years (2000-2003) for age-2 (yearling smolts) through age-5.

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References

- Busack, C.A., and K.P. Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium 15: 71-80.
- Busack, C., C.M. Knudsen, G. Hart, and P. Huffman. In Press. Differences in body shape between first-generation hatchery and wild upper Yakima spring Chinook salmon. Transactions of the American Fisheries Society.
- Carmichael, R. W., and R.T. Messmer. 1995. Status of supplementing Chinook salmon natural production in the Imnaha River Basin. American Fisheries Society Symposium 15: 284-291.
- Gallinat, M.P. 2004. Tucannon River Spring Chinook Salmon Hatchery Evaluation Program 2003 Annual Report to U.S. Fish and Wildlife Service, Cooperative Agreement 1411-03-J051. Washington Department of Fish and Wildlife, Olympia, Washington. Report # FPA04-12. (54 pp.)
- Knudsen C.M., S.L. Schroder, C.A., Busack, M.V. Johnston, T.N. Pearsons, and W.J. Bosch, and D.E. Fast. 2006. Comparison of life-history traits between first-generation hatchery and wild Upper Yakima River spring Chinook salmon. Transactions of the American Fisheries Society 135: 1130-1144.
- Kostow, K.E., A.R. Marshall, and S.R. Phelps. 2003. Naturally spawning hatchery steelhead contribute to smolt production but experience low reproductive success. Transactions of the American Fisheries Society 132: 780-790.
- McLean, J.E., P. Bentzen, and T.P. Quinn. 2003. Differential reproductive success of sympatric, naturally spawning hatchery and wild steelhead trout (*Oncorhynchus mykiss*) through adult stage. Canadian Journal of Fisheries and Aquatic Science 60:433-440.
- Murdoch, A.R., T.N. Pearsons, T.W. Maitland, M.F. Ford, and K. Williamson. 2006. Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. BPA Project No. 2003-039-00. Bonneville Power Administration, Portland, Oregon.
- Olson, D.E., B. Spateholts, M. Paiya, and D.E. Campton. 2004. Salmon hatcheries for the 21st Century: A model at Warm Springs National Fish Hatchery. American Fisheries Society Symposium 44: 585-602.
- Yates, F. 1934. Contingency tables involving small numbers and the χ^2 test. Journal of the Royal Statistical Society Supplement 1:217-235.
- Zar, J.H. 1999. Biostatistical Analysis, 4th edition. Prentice Hall, Upper Saddle River, New Jersey.

Chapter 2 -- Spawning distribution and redd characterization of hatchery and natural origin spring Chinook salmon in the Wenatchee River Basin

Abstract

Reproductive success of hatchery and natural origin fish that spawn in natural environments could differ for a variety of reasons such as differences in spawn time, spawn location, spawn habitat, and redd construction. 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 2006, the composite population of spring Chinook redds were distributed similarly to that of years past. A total of 528 redds were found upstream of Tumwater Dam, of which the female origin was identified on 242 redds. The estimated spawning escapement, based on the number of redds, was 51.5% of the number of spring Chinook counted at Tumwater Dam. After correction for carcass recovery bias, no differences were found in the estimated age composition of the spawning population compared to population sampled at Tumwater Dam. However, the estimated proportion of hatchery fish on the spawning grounds was significantly lower than that of naturally produced fish compared to the population at Tumwater Dam ($P < 0.05$). Hatchery origin female spring Chinook spawned in significantly lower elevations of the Chiwawa River and Nason Creek than natural origin fish ($P < 0.05$). No difference in spawning timing of hatchery and natural origin spring Chinook spawning within the same reaches was detected ($P > 0.05$). However, differences in spawn timing, regardless of origin, was associated with elevation. Microhabitat variables were measured on 93 redds, which included 63 and 30 constructed by hatchery and natural origin females, respectively.

Introduction

Reproductive success of hatchery and natural origin fish that spawn in natural environments could differ for a variety of reasons such as differences in spawn time, spawn location, spawn habitat, and redd construction. Spawn time of fish in natural environments is important to survival of offspring because it affects what conditions embryos will experience (e.g., floods) as well as the conditions that newly emergent fry will encounter. Fry emergence during periods of low food abundance or harsh conditions could result in poor survival of fry. Non-representative broodstock collection or spawn timing can skew run and spawn timing (Leider et al. 1984; Nickelson et al. 1986; Chandler and Bjornn 1988). Even when attempts to collect representative broodstock occur, spawn timing of hatchery fish can still differ (Knudsen et al. 2006). Collecting, holding, and spawning salmon broodstock can remove selection pressures (e.g., competing for mates, digging deep redds, maintaining energy stores and other factors) that are used for spawning in the natural environment (Schroder et al. submitted). Any deviation from naturally produced fish can be assumed to be maladaptive in natural environments (Waples 1999).

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, reaches 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 impacted even if they are genetically, demographically, and behaviorally identical to natural origin fish. Furthermore this impact could occur across multiple generations if homing fidelity of progeny of hatchery fish return to spawn in the same locations as their birthplace. Even if hatchery fish spawn in the same river reaches as natural origin fish, hatchery fish that select locations with suboptimal flow, depth, or substrate has the potential to impact egg-to-fry survival. Suboptimal flows and substrates could result in decreased oxygenation and waste removal, factors that are considered to be important in survival (Groot and Margolis 1991). Redd depths that are too deep could be subject to scouring flows as discharges increase. Conversely, redd depths that are too shallow could be subject to desiccation as discharges decrease.

Hatchery and natural origin fish may construct redds that differ in their quality to promote good egg-to-fry survival. Fish with low energy expenditures or differences in size and morphology may not be able to dig redds as large, deep, or cover eggs with sufficient substrate (Crisp and Carling 1989). Sizes and shapes of hatchery and natural origin fish can differ even in hatchery supplementation programs where the goal is to minimize differences between hatchery and natural origin fish (Knudsen et al. 2006; Busack et al. in press). The impact of poor nest construction could cause increased susceptibility of eggs to scouring or desiccation (Crisp and Carling 1989).

The objective of this Chapter is to determine if differences in spawn timing, spawning distribution between and within tributaries, redd 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 was 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 live female was determined by PIT tag detection when possible. Redds were identified as locations that had areas of clean gravel which also exhibited the typical redd morphological characteristics (e.g., well developed bowl and tail spill). Test redds were identified as locations that had areas of clean gravel, but lacked typical redd morphological characteristics (e.g., no tail spill). Post spawned females guarding redds were scanned for PIT tags using an underwater antenna mounted on an extension pole. Using this technique, we were able to identify an individual fish and correspond 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 that 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 and age-3 hatchery fish (i.e., not adipose fin clipped) all snouts were collected and the presence of a CWT were 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 marked by removing the caudal fin to prevent double sampling. 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 derived from sex ratios calculated at Tumwater Dam (See Chapter 1). The proportion of hatchery and natural origin fish was calculated by multiplying the proportion of carcasses recovered within each reach by the estimated spawning population for that reach.

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 high probability that hatchery and natural origin redds would be created within 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 end of the tail, the shallowest point of the tail, the downstream end of the tail, and left and right side of the redd (Figure 1).

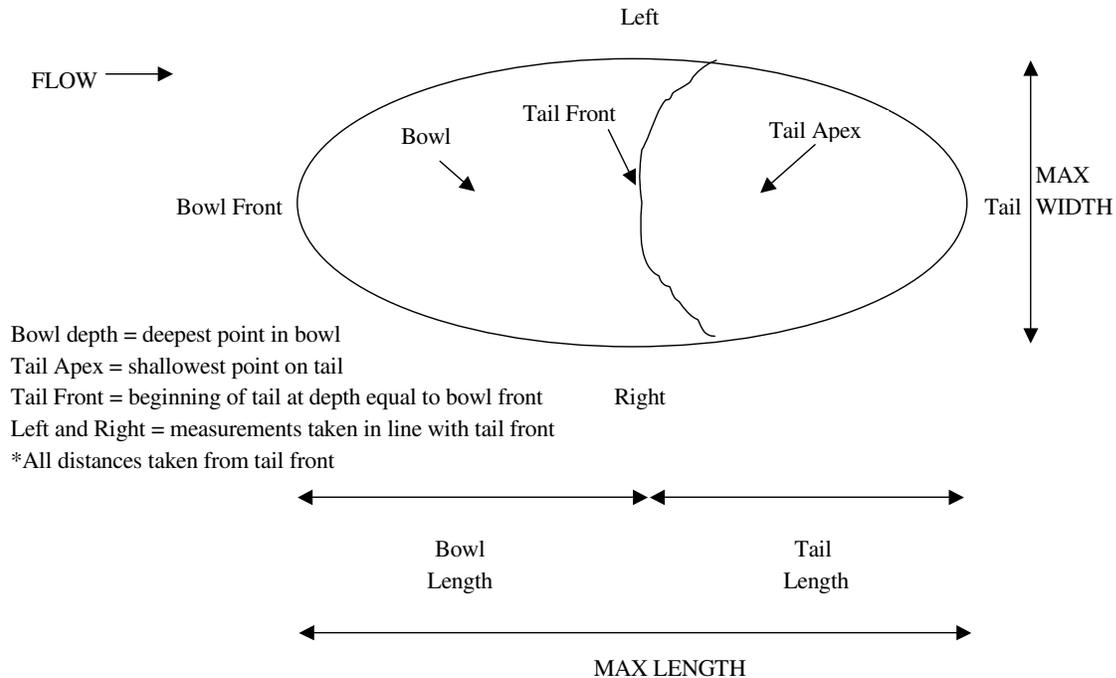


Figure 1. Locations of redd microhabitat characteristic measurements.

Water velocity (m/s) was measured using a Marsh McBirney Model 2000 or Swiffer Model 2100 flow meters. Water velocity was recorded at the upstream end of the bowl (60% depth), maximum depth of the bowl (60% depth), upstream end of the tail (60% depth, surface, bottom), downstream end of the tail (60% depth), and the left and right side of the redd (60% depth). Average redd water depth was calculated from water depth measurements recorded at the left and right side of the redd and the upstream end of the bowl. The depth of bowl was calculated by subtracting the average depth from the maximum depth of the bowl. Average tail depth and velocity was calculated from measurements recorded at the left, right, and center (i.e., tail front) of the tail. Tail height was derived by subtracting the depth at the tail apex from the average depth measured at the front and back of the tail. 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 to age compositions estimated from carcass recoveries from the spawning grounds.

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 by return year, sex, and origin of carcasses recovered on the spawning grounds were tested using a Kruskal-Wallis analysis of variance (KW test). Significant differences in spawning distribution were analyzed using a multiple comparison of ranks test to determine the source of differences.

Spawning time was assessed at the hatchery during routine spawning operations and on the spawning grounds. At the hatchery, spawning time (ordinal date) of female spring Chinook was compared by return year and origin using a KW test. Pearson Product moment correlation statistic was to examine the relationship between passage timing and spawn timing. On the spawning grounds, spawn timing was assessed within specific river reaches, based on historical data, in 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 redd microhabitat. We controlled for elevation and reach specific differences in spawning habitat by comparing only hatchery and naturally produced fish in the same reach. A KW test was used to compare the spawn timing by return year and reach of hatchery and natural origin female spring Chinook based on both the spawn and carcass recovery date because statistical assumptions of data normality and equal variances could not be met.

Power analysis for two sample t-tests were conducted for microhabitat characteristics of redds constructed by hatchery and natural origin fish. Statistical comparisons will be conducted after required sample sizes have been obtained for each respective variable. Correlation analysis was performed to examine the relationship between fish size, discharge, and redd microhabitat characteristics. Substrate composition data was transformed (arcsine square root) to meet normality assumptions. All statistical tests were performed at a significance level (α) of 0.05.

Results and Discussion

Spawning ground surveys

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.

Chiwawa River

A total of 297 redds were found in the Chiwawa River basin in 2006. Of those redds, 287 redds (96.6%) were found in the Chiwawa River, while 10 redds (3.4%) were found in tributaries (i.e., Chikamin and Rock creeks). In past years, redds were constructed first in the higher elevation reaches and progressively downstream as the spawning season ended (Table 1). In 2006, spawning was observed more uniformly across all reaches. Spawning began the first week of August and continued until second week of September, with peak spawning occurring during the third week of August (Appendix B). The origin of the female constructing a redd was determined for 136 redds (45.8%). Of which, 94 redds were constructed by hatchery fish and 42 redds by naturally produced fish.

Table 1. Number of spring Chinook redds located within historical reaches during spawning ground surveys on the Chiwawa River in 2006.

Survey Week	Historical Reach (rkm)						Totals redds
	0-20	20-32	32-37	37-43	43-45	45-51	
07/30	0	0	0	0	0	0	0
08/06	3	0	1	2	4	3	13
08/13	9	19	3	3	7	8	49
08/20	14	54	3	10	7	12	100
08/27	15	56	2	6	5	7	91
09/03	5	20	1	4	1	1	32
09/10	5	7	0	0	0	0	12
09/17	0	0	0	0	0	0	0
09/24	0	0	0	0	0	0	0
Total	51	156	10	25	24	31	297

Nason Creek

During surveys on Nason Creek a total 152 redds were found in 2006. 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 fourth week of September, with peak spawning occurring in the first week of September (Appendix B). The origin of the female constructing a redd was determined for 72 redds (47.4%). Of those redds, 41 were hatchery and 31 were naturally produced.

Table 2. Number of spring Chinook redds located within historical reaches during spawning ground surveys on Nason Creek in 2006.

Week	Historical reach (rkm)				Total redds
	0-7	7-14	14-22	22-26	
07/30	0	0	1	0	1
08/06	0	0	0	2	2
08/13	1	0	0	4	5
08/20	3	6	13	6	28
08/27	11	9	20	9	49
09/03	21	15	12	2	50
09/10	4	2	2	3	11
09/17	3	2	0	0	5
09/24	1	0	0	0	1
Total	44	34	48	26	152

Upper Wenatchee River

A total of 27 redds were located by raft or on foot on the upper Wenatchee River in 2006. Only the two highest elevation reaches were surveyed because historical spring Chinook spawning ground surveys indicated that redds were not found in other locations. Of those redds, 24 redds (89%) were found in the Wenatchee River, while only 3 redds (11%) were found in a small tributary (i.e., Chiwaukum creek). The temporal distribution of redds was primarily confined to the upper most reach (Table 3). Spawning began the fourth week of August and continued until the fourth week of September, with peak spawning occurring during the first week of September (Appendix B). Female origin was determined for two redds (7%), both constructed by a hatchery female.

Table 3. Number of spring Chinook redds located within historical reaches during spawning ground surveys on the Wenatchee River in 2006.

Survey Week	Historical Reach (rkm)		Totals redds
	59-81	81-90	
08/13	0	0	0
08/20	0	0	0
08/27	3*	0	3
09/03	0	15	15
09/10	0	6	6
09/17	0	2	2
09/24	0	1	1
Total	3	24	27

* Redds located on Chiwaukum Creek

White River

Survey crews found a total of 31 redds in the White River basin in 2006. Of those, 30 redds (96.8%) were found in the White River, while 1 redd (3.2%) was found in the Napeequa River. Redds were distributed primarily in the mid elevation reach (Table 4). Spawning activity started during the first week of August and continued until the second week of September, with peak spawning occurring in the third week of August (Appendix B). The origin of the female was determined for 26 redds (84%). Of the redds in which origin was determined, 5 were hatchery and 21 were naturally produced.

Table 4. Number of spring Chinook redds found within historical reaches during spawning ground surveys on the White River in 2006.

Survey Week	Historical Reach (rkm)			Totals redds
	11-18	18-22	22-24	
07/30	0	0	0	0
08/06	0	1	0	1
08/13	0	5	0	5
08/20	0	17	0	17
08/27	0	4	0	4
09/03	0	2	0	2
09/10	1*	1	0	2
09/17	0	0	0	0
09/24	0	0	0	0
Total	1	30	0	31

* Redd located in the on Napeequa River

Little Wenatchee River

A total of 21 redds were found during spawning ground surveys on the Little Wenatchee River in 2006. 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 6 redds (28.6%). Of those redds, it was determined that 3 were hatchery and 3 were naturally produced.

Table 5. Number of spring Chinook redds located within historical reaches during spawning ground surveys on the Little Wenatchee River in 2006.

Survey Week	Historical Reach (rkm)			Totals redds
	5-9	9-15	15-21	
07/30	0	0	0	0
08/06	0	0	0	0
08/13	0	1	0	1
08/20	0	5	0	5
08/27	3	4	0	7
09/03	1	4	0	5
09/10	0	2	0	2
09/15	0	1	0	1
09/24	0	0	0	0
Total	4	17	0	21

Carcass Surveys

Chiwawa River

Of the 241 carcasses sampled throughout the Chiwawa River basin in 2006, scale analysis determined the proportion of hatchery and naturally produced fish was 81% ($N = 195$) and 19% ($N = 46$), respectively. Based on a male to female ratio derived from the broodstock of 0.78 to 1 (i.e., 1.78 fish per redd), spawning escapement was estimated to be 428 hatchery and 101 naturally produced fish. All snouts were collected and sent to the WDFW CWT lab in Olympia to determine if CWTs were present and then the tag was 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 distribution of redds (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 between 2004 and 2006.

River (km)	Redds	Carcasses		
		Hatchery	Natural	Total
<i>2004</i>				
0-19.5	0.15	0.62	0.24	0.41
19.5-32.2	0.46	0.31	0.52	0.41
32.2-37.3	0.02	0.01	0.02	0.02
37.3-42.7	0.20	0.01	0.12	0.07
42.7-45.0	0.09	0.01	0.07	0.05
45.0-50.5	0.08	0.04	0.03	0.04
<i>2005</i>				
0-19.5	0.23	0.53	0.25	0.48
19.5-32.2	0.53	0.41	0.56	0.44
32.2-37.3	0.02	0.02	0.05	0.03
37.3-42.7	0.11	0.02	0.06	0.03
42.7-45.0	0.05	0.01	0.05	0.02
45.0-50.5	0.06	0.01	0.03	0.01
<i>2006</i>				
0-19.5	0.17	0.43	0.22	0.39
19.5-32.2	0.53	0.41	0.41	0.41
32.2-37.3	0.03	0.02	0.04	0.03
37.3-42.7	0.09	0.04	0.17	0.06
42.7-45.0	0.08	0.03	0.09	0.04
45.0-50.5	0.10	0.07	0.07	0.07

Nason Creek

A total of 190 carcasses were recovered in Nason Creek during 2006. Scale analysis determined the proportion of hatchery and naturally produced fish was 59% ($N=111$) and 41% ($N=77$), 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 160 hatchery and 111 naturally produced fish spawned in Nason Creek during 2006. The largest proportion of hatchery carcasses was 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 between 2004 and 2006.

River (km)	Redds	Carcasses		
		Hatchery	Natural	Total
<i>2004</i>				
0-6.5	0.31	0.52	0.28	0.38
6.5-13.8	0.20	0.31	0.33	0.32
13.8-22.0	0.32	0.13	0.17	0.16
22.0-25.7	0.17	0.04	0.22	0.14
<i>2005</i>				
0-6.5	0.56	0.75	0.41	0.69
6.5-13.8	0.16	0.10	0.13	0.10
13.8-22.0	0.19	0.13	0.28	0.16
22.0-25.7	0.09	0.02	0.18	0.05
<i>2006</i>				
0-6.5	0.29	0.44	0.31	0.39
6.5-13.8	0.22	0.28	0.22	0.26
13.8-22.0	0.32	0.15	0.35	0.23
22.0-25.7	0.17	0.13	0.12	0.12

Upper Wenatchee River

In the upper Wenatchee River a total of 15 carcasses were recovered during spawning ground surveys in 2006. Scale analysis determined the proportion of hatchery and natural origin fish recovered was 87% ($N = 13$) and 13% ($N = 2$), 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 42 hatchery and 6 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 between 2004 and 2006.

River (km)	Redds	Carcasses		
		Hatchery	Natural	Total
<i>2004</i>				
51.5-59.3	0.02	0.00	0.17	0.06
59.3-80.7	0.98	1.00	0.83	0.94
<i>2005</i>				
51.5-59.3	0.00	0.03	0.00	0.03
59.3-80.7	1.00	0.97	1.00	0.97
<i>2006</i>				
51.5-59.3	0.11	0.00	0.00	0.00
59.3-80.7	0.89	1.00	1.00	1.00

White River

Of the 25 carcasses recovered in the White River during 2006, scale analysis determined the proportion of hatchery and natural origin fish was 24% ($N=6$) and 76% ($N=19$) 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 ($N = 45$; 64%) was greater than the number of carcasses recovered (36%). Based on the proportion of hatchery (13%) and natural fish (87%) detected on the spawning grounds, the number of fish on the spawning grounds was 7 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 between 2004 and 2006.

River (km)	Redds	Carcasses		
		Hatchery	Natural	Total
<i>2004</i>				
11.0-18.0	0.00	0.00	0.11	0.10
18.0-22.0	0.91	1.00	0.89	0.90
22.0-24.0	0.09	0.00	0.00	0.00
<i>2005</i>				
11.0-18.0	0.09	0.08	0.09	0.08
18.0-22.0	0.90	0.92	0.91	0.92
22.0-24.0	0.01	0.00	0.00	0.00
<i>2006</i>				
11.0-18.0	0.03	1.00	0.16	0.12
18.0-22.0	0.97	0.00	0.84	0.88
22.0-24.0	0.00	0.00	0.00	0.00

Little Wenatchee River

Of the 13 carcasses recovered in the Little Wenatchee River during 2006, scale analysis indicated that the proportion of hatchery and natural origin fish was 31% ($N=4$) and 69% ($N=9$), respectively (Table 10). The estimated spawning population was 11 and 26 hatchery and naturally produced fish, respectively.

Table 10. Proportion of redds and carcasses by reach in the Little Wenatchee River between 2004 and 2006.

River (km)	Redds	Carcasses		
		Hatchery	Natural	Total
<i>2004</i>				
4.5-8.7	0.15	0.00	0.00	0.00
8.7-15.3	0.85	0.00	1.00	1.00
15.3-21.0	0.00	0.00	0.00	0.00
<i>2005</i>				
4.5-8.7	0.39	0.31	0.27	0.30
8.7-15.3	0.61	0.69	0.73	0.70
15.3-21.0	0.00	0.00	0.00	0.00
<i>2006</i>				
4.5-8.7	0.19	0.25	0.22	0.23
8.7-15.3	0.81	0.75	0.78	0.77
15.3-21.0	0.00	0.00	0.00	0.00

PIT Tag Retention and Detection Efficiency

PIT tag retention by spring Chinook was slightly higher than in 2006 (94%) than observed in 2005 (93%) or 2004 (85%). Additional training on proper technique and placement conducted early in the field season likely contributed to the higher retention rates. In 2006, only 20 mm PIT tags were only inserted in adults, while only 12 mm PIT tag were inserted into jacks and precocious males. 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 in 2007.

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 2007. Therefore, it is important to include potential production from these streams. Chelan County Public Utility District (CCPUD) personnel conducted the spawning ground surveys and sampled carcasses during surveys using similar methodologies previously described for this study.

Icicle Creek

A total of 50 redds were found during spawning ground surveys in 2006. Historically, fish recovered on the Icicle River originated from the Leavenworth National Fish Hatchery (LNFH), which is also located on the Icicle River. Of the 7 carcasses sampled, scale analysis determined that 6 were hatchery origin. The spawning population was

estimated at 90 hatchery fish. All hatchery fish sampled were sent to the WDFW CWT lab in Olympia to have CWTs extracted and decoded.

Peshastin Creek

CCPUD personnel found 10 redds in Peshastin Creek and Ingalls Creek. However, no carcasses were recovered in 2006. No hatchery adults were expected to return to Peshastin Creek in 2006 (i.e., no hatchery releases). Therefore, the spawning population ($N = 18$) was assumed to be natural origin fish.

Spawning Ground Summary

The composition of the spawning population upstream of Tumwater Dam was 66% hatchery and 34% 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 74% and 26%, respectively. In 2006, 90% of the spring Chinook redds were found upstream of Tumwater Dam. Based on the number of potential spawners at Tumwater Dam ($N = 1,824$) and the estimated spawning population, the survival to spawning was 51.5% (Table 11).

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. Surveys were conducted to ensure that carcasses were recovered in similar proportions to the spawning populations (See Carcass Recovery Section in this Chapter). However, the age composition of the hatchery and natural spring Chinook was different (See Chapter 1). If age composition of hatchery and natural fish are different and carcass recovery probability of different age classes are 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 = 0.038; age-3 = 0.237; age-4 = 0.515; age-5 = 0.734) and the expected and observed age composition of carcasses recovered on the spawning grounds was significantly different than that observed at Tumwater Dam ($\chi^2 = 152.5$, $df = 3$, $P < 0.001$). Excluding age-2 fish from the analysis (i.e., recovery probability of age-2 fish near zero) did not influence the results ($\chi^2 = 49.4$, $df = 2$, $P < 0.001$). 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

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.

River	Number of redds	Sample Rate	Number of fish		
			Hatchery	Natural	Total
<i>2004</i>					
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	0.2500	692	916	1,608
Icicle	30	0.2963	50	4	54
Peshastin	55	0.4590	99	0	99
Subtotal	85	0.3660	149	4	153
Wenatchee Basin Total	585	0.2641	841	920	1,761
<i>2005</i>					
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	0.5614	1,178	295	1,473
Icicle	8	0.1429	14	0	14
Peshastin	3	0.0000	0	5	5
Subtotal	11	0.1053	14	5	19
Wenatchee Basin Total	829	0.5560	1,221	270	1,491
<i>2006</i>					
Chiwawa	297	0.4559	410	119	529
Nason	152	0.7022	153	118	271
Little Wenatchee	21	0.3478	11	26	37
White	31	0.4531	7	48	55
Wenatchee	27	0.3121	42	6	48
Subtotal	528	0.5150	623	317	940
Icicle	50	0.0777	90	0	90
Peshastin	10	0.0000	0	18	18
Subtotal	60	0.0648	90	18	108
Wenatchee Basin Total	588	0.4685	713	335	1,048

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 ($\chi^2 = 4.7$, $df = 2$, $P = 0.10$), natural origin spawners ($\chi^2 = 2.7$, $df = 2$, $P = 0.26$), or when combined ($\chi^2 = 4.4$, $df = 2$, $P = 0.11$). These results suggest that there is no differential survival based on size or age of fish from Tumwater Dam to the spawning grounds. Similar results were reported for 2004 and 2005 (Murdoch et al. 2005, 2006). Alternatively, the statistical power of our test may be very low. Although survival by age class was similar, overall 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
	<i>N</i>	%	<i>N</i>	%		
<i>2004</i>						
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
<i>2005</i>						
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
<i>2006</i>						
Age-3	170	0.096	22	0.046	0.057	0.117
Age-4	1,260	0.711	337	0.700	0.156	0.687
Age-5	342	0.193	122	0.254	0.212	0.196

Spawning Distribution

In the Chiwawa River, differences were detected in the distribution of hatchery and natural origin female spring Chinook in all years examined (Figure 2; $P < 0.04$). No difference was detected in the distribution of hatchery and naturally produced male spring Chinook except in 2004 ($P < 0.01$). The spawning distribution of female hatchery spring Chinook in Nason Creek was also significantly different than naturally produced fish in all years (Figure 3; $P < 0.04$). No difference was detected in the spawning distribution of hatchery and naturally produced male spring Chinook in Nason Creek. The spawning

distribution in other major spawning area within the upper Wenatchee Basin (i.e., Little Wenatchee, White, and upper Wenatchee rivers) was not analyzed because limited available spawning habitat or low sample size.

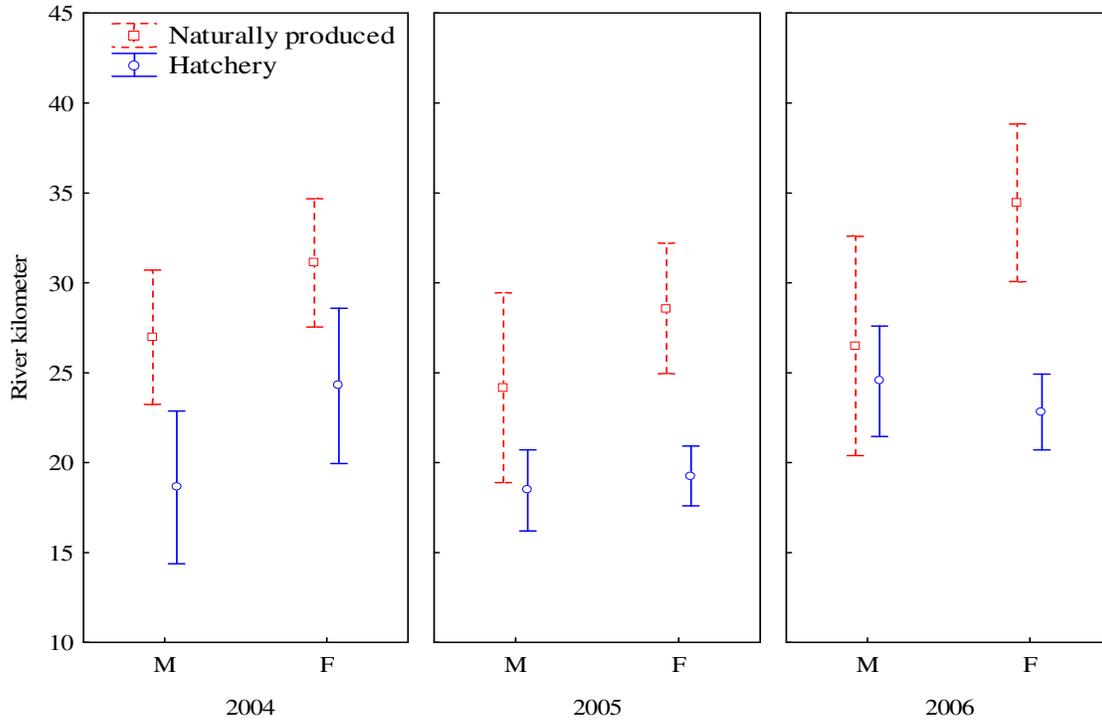


Figure 2. Mean carcass recovery locations of hatchery and natural origin spring Chinook in the Chiwawa River between 2004 and 2006. Vertical bars denote 95% confidence intervals.

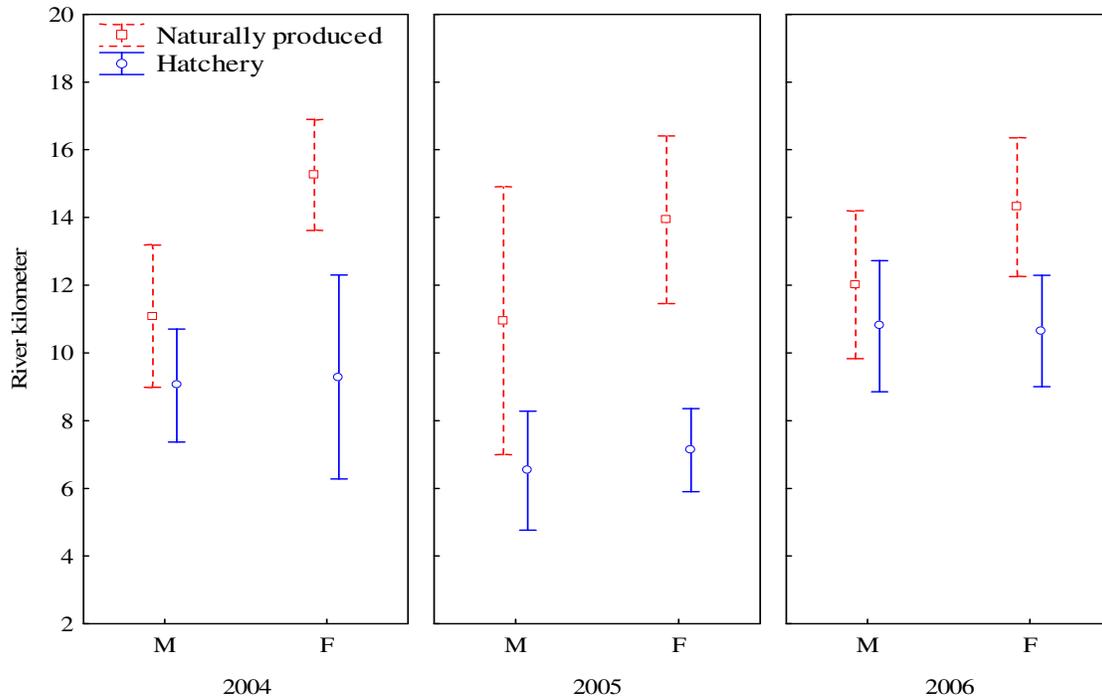


Figure 3. Mean carcass recovery locations of hatchery and natural origin spring Chinook in Nason Creek in between 2004 and 2006. Vertical bars denote 95% confidence intervals.

Spawn Timing

Passage and spawn timing for hatchery and natural origin fish collected as broodstock was significantly but weakly correlated ($r = 0.17$, $P < 0.03$) in 2006. When analyzed separately, hatchery female broodstock were not significantly correlated ($r = 0.13$, $P = 0.14$), while a significant correlation between run and spawn timing of natural origin fish was found ($r = 0.37$, $P < 0.02$). A similar poor correlation was found between run and spawn timing when all years (2004-2006) were combined ($r = 0.16$, $P < 0.002$). Conversely, when data from all years was analyzed separately, hatchery females were significantly correlated ($r = 0.15$, $P < 0.02$), while naturally produced fish were not ($r = 0.18$, $P = 0.06$).

Passage date at Tumwater Dam and the spawn date of the female spring Chinook in the Chiwawa River were also significantly correlated ($r = 0.28$, $P < 0.01$) in 2006. Analysis of 2006 spawn timing by origin found that spawn timing of naturally produced females was positively correlated with run timing at Tumwater Dam ($r = 0.55$, $P < 0.001$), while no correlation was present for hatchery females ($r = 0.06$, $P = 0.58$) consistent with the results for broodstock. When data from all years (2004-2006) was included in the analysis, no correlation was detected between run timing and spawn timing for female spring Chinook in the Chiwawa River ($r = 0.02$, $P = 0.78$). Similar results were found when hatchery ($r = -0.04$, $P = 0.62$) and naturally produced ($r = 0.10$, $P = 0.41$) fish were analyzed separately. Lack of correlation between passage and spawn timing was also reported in the Yakima Basin (Knudsen et al. 2006). Correlations between run and

spawn timing within the broodstock may be influenced by the frequency of spawning (i.e., once per week). In contrast, spawning observations in the natural environment were made four times per week.

During spawning at the hatchery, no difference in spawn timing (2004 – 2006) was detected within or between years among hatchery and natural origin fish (KW test, $H = 8.9$, $P = 0.11$). 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. Differences in the spatial distribution (i.e., elevation of the redd) required that the influence of elevation be controlled in the analysis. The same reaches used in the redd microhabitat analysis (Chiwawa $N = 2$; Nason $N = 2$) were also used to test for differences in spawn timing.

Differences in spawn timing based on redd construction were found between years in both the lower Chiwawa River (KW test, $H = 25.01$, $P < 0.001$) and Nason Creek (KW test, $H = 14.87$, $P < 0.02$) reaches. Although, no within year differences in spawn timing were found between hatchery and naturally produced fish within the same reach (Figure 4). Similar results were found when the female carcass recovery date was used, except in lower reach of Nason Creek. In 2006, differences were detected in the female carcass recovery date of hatchery and naturally produced fish (KW test, $H = 15.35$, $P < 0.01$; Figure 5). 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.

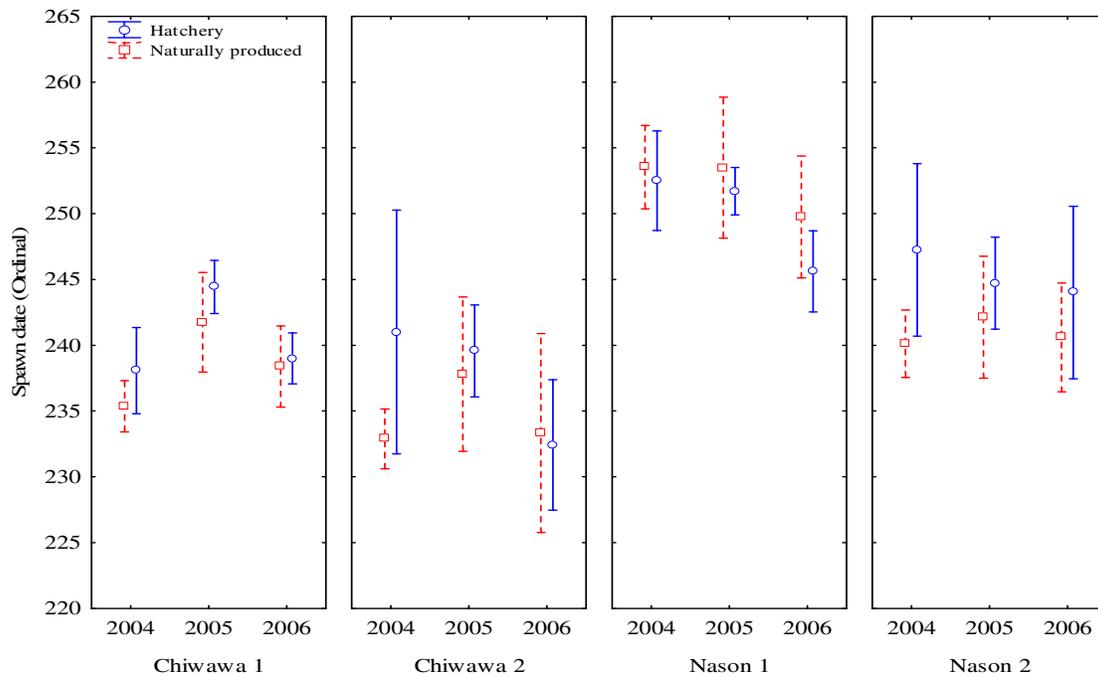


Figure 4. Mean date redds were constructed by female hatchery and natural origin spring Chinook fish spawning in selected reaches of the Chiwawa River and Nason Creek between 2004 and 2006. Vertical bars denote 95% confidence interval.

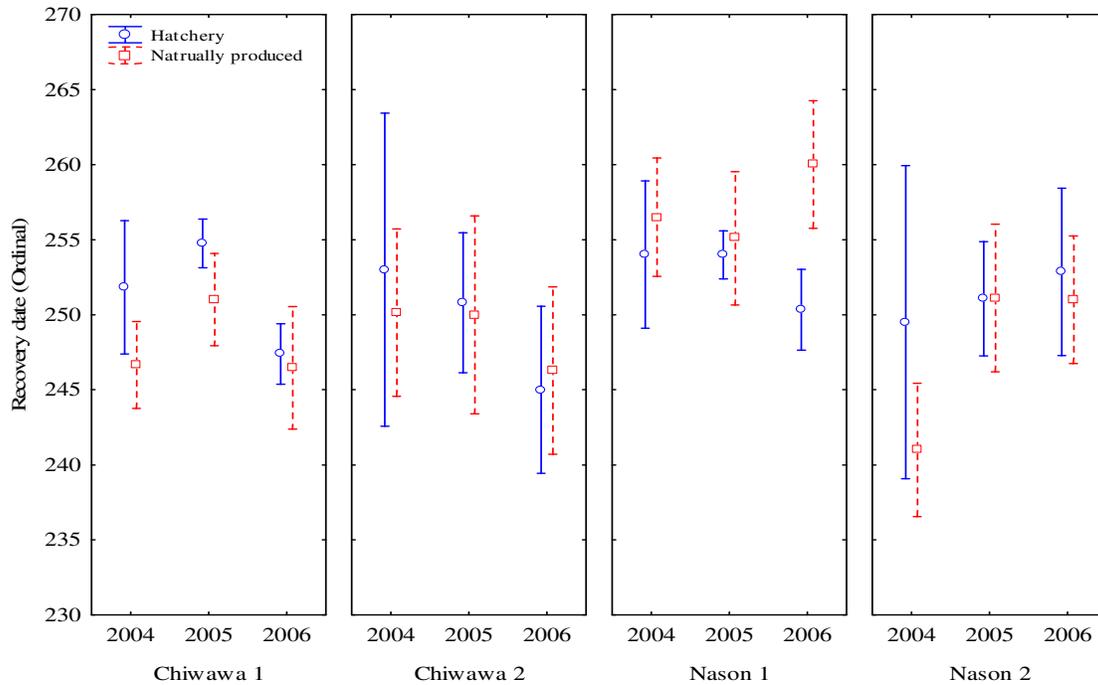


Figure 5. Mean date female spring Chinook carcasses were recovered in selected reaches of the Chiwawa River and Nason Creek between 2004 and 2006. Vertical bars denote 95% confidence interval.

Survival to Spawning

In 2006, the Wenatchee River Basin experienced a second consecutive year of low water conditions during the spawning period (August–September). The proportion of spring Chinook that migrated upstream of Tumwater Dam and subsequently accounted for on the spawning grounds was 51.5%. Differences between run and spawning escapement estimates may be the result of fall back, undetected spawning, or inaccurate redd expansion values. Fall back at Tumwater Dam has not been a significant factor. In 2006, only three PIT tag recaptures of fish tagged at Tumwater Dam were reported at a hydroelectric dam (Rocky Reach), and no PIT tagged fish were recovered at LNFH. Furthermore, the number of redds and lack of PIT tagged carcasses found downstream of Tumwater Dam does not account for the differences observed upstream of Tumwater Dam.

Due to low discharge observed in 2006, it is unlikely that any significant spawning was undetected. Although, high undetected redd superimposition rates would explain the observed difference between run and spawning escapement estimates. Redd superimposition rates in spawning tributaries were inversely related to the amount of available spawning habitat. In 2006, redd superimposition was detected in Chiwawa River (4%), Nason Creek, (8%) and the White River (10%). While the overall redd superimposition rate upstream of Tumwater Dam was only 5% ($N = 25$). Given the large observed difference in run and spawning escapement estimates, the undetected redd superimposition rates required would need to be near 100%. Given the frequency of

surveys (2-4 times per week) and the detection of individual female fish on redds or as carcasses, it is highly unlikely that undetected redd superimposition is responsible for the observed difference between run and spawning escapement estimates.

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 than that reported.

Poor survival was attributed to extreme environmental conditions prior to and during spawning as a result of low discharge 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). Although no difference in survival was detected between age classes (See Spawning Ground Summary), differences were detected between the proportion of hatchery and natural origin fish at upstream of Tumwater Dam and on the spawning grounds based on carcass recoveries ($\chi^2 = 57.4$, $df = 1$, $P < 0.0001$) and the estimated spawning population ($\chi^2 = 22.2$, $df = 1$, $P < 0.001$). 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. In 2004 and 2005, after the bias was corrected using carcass recovery probabilities, no difference was detected in the estimated proportion of hatchery and natural spring Chinook on the spawning grounds and that observed at Tumwater Dam. After correcting for the size related bias, an estimated 49.8% of the hatchery fish and 62.3% of the naturally produced fish that could have spawned naturally were accounted for on the spawning grounds in 2006.

In 2006, hatchery spring Chinook were transported to the Chiwawa River from Tumwater Dam ($N = 104$) in an effort to reduce the stray rate and from Eastbank FH ($N = 46$) as surplus to broodstock needs. Differential survival of transported and non-transported hatchery fish may account for difference observed on the spawning grounds. However, no difference was detected in the proportion of carcass recovered by age class of transported and non-transported hatchery fish ($\chi^2 = 0.18$, $df = 2$, $P = 0.92$). Furthermore, no transported fish were recovered on the spawning grounds other than the Chiwawa River. A possible explanation for the difference in the proportions of hatchery and naturally produced fish was that female hatchery fish were significantly smaller in size (length and weight) than naturally produced fish. In previous years, hatchery fish were similar or greater in size than naturally produced fish. Thus, survival of hatchery fish to the spawning grounds may be related to size (i.e., inadequate energy reserves).

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
2006										
Tumwater Dam	165	5	959	301	186	156	1,310	462	0.74	0.26
Spawning grounds	38	3	464	186	121	129	623	318	0.66	0.34
Estimated number	104	8	461	185	89	94	653	288	0.69	0.31

Redd Microhabitat Characteristics

In 2006, spring Chinook redd microhabitat variables were measured on 67 redds in the Chiwawa River and 26 redds in Nason Creek (Appendix D). Results from the power analysis suggest that many variables have inadequate power (i.e., < 0.8) to make statistical inferences based on data collected between 2004 and 2006 (Table 15). However, adequate power should be obtained for many of the more biologically important variables within the next three years as sample sizes increase (Figure 6). Based on the power analysis, data from approximately 300 redds from both hatchery and naturally produced spring Chinook should be collected.

Differences in size of hatchery and naturally produced fish have been detected in the Wenatchee Basin (See Chapter 1). Hence, differences in redd microhabitat characteristics may simply be a function of fish size (i.e., small fish build small redds). No relationships between female length and all microhabitat variables were found except for bowl depth ($r = 0.17$, $P < 0.02$) and redd width ($r = 0.13$, $P < 0.05$). Differences in redd microhabitat characteristics may also be influenced by discharge when the redd was constructed or when microhabitat characteristics were measured. Of the 326 redds measured between 2004 and 2006, eleven redds (7 wild and 4 hatchery) were excluded from this analysis because the difference in discharge between the date the redd was constructed and the date microhabitat variables were measured was excessive (i.e., > 150%). All eleven redds were measured on the same day in Nason Creek during a freshet in 2004. Of the 15 flow variant variables examined (i.e., water depth and velocity), no relationship ($P > 0.05$) was detected between discharge on the day the redd constructed, discharge the day microhabitat characteristics were measured, or the change in discharge. The only exception was water depth at the tail apex ($r = -0.13$, $P < 0.03$) and only for discharge on the day microhabitat characteristics were measured.

Table 15. Summary of spring Chinook redd microhabitat variables measured in the Wenatchee River Basin in between 2004 and 2006.

Variable	Hatchery			Natural			Power
	Mean	SD	N	Mean	SD	N	
Redd morphology							
Redd length (m)	6.42	1.60	187	6.39	1.59	139	0.05
Redd width (m)	3.98	1.08	187	4.00	1.17	139	0.05
Redd area (m ²)	26.30	11.48	187	26.12	11.67	139	0.05
Bowl length (m)	2.50	1.15	187	2.75	0.95	139	0.55
Tail length (m)	3.93	1.21	187	3.65	1.25	139	0.53
Depth of bowl (m)	0.12	0.07	187	0.14	0.06	139	0.58
Tail height (m)	0.17	0.11	187	0.15	0.09	139	0.62
Water depth (m)							
Redd left side	0.32	0.13	187	0.34	0.14	139	0.28
Redd right side	0.32	0.14	187	0.34	0.14	139	0.25
Mean redd	0.34	0.12	187	0.36	0.11	139	0.59
Bowl front	0.38	0.16	187	0.43	0.14	139	0.87
Maximum bowl	0.46	0.15	187	0.51	0.13	139	0.77
Tail apex	0.16	0.10	187	0.19	0.10	139	0.77
Tail front	0.37	0.13	187	0.39	0.11	139	0.35
Tail back	0.29	0.20	187	0.28	0.11	139	0.08
Mean tail	0.33	0.11	187	0.35	0.11	139	0.41
Water velocity (m/s)							
Bowl front	0.43	0.23	187	0.47	0.25	139	0.22
Redd left side	0.40	0.26	187	0.46	0.24	139	0.57
Redd right side	0.36	0.23	187	0.48	0.33	139	0.96
Tail front	0.43	0.24	187	0.51	0.32	139	0.73
Tail back	0.54	0.34	187	0.59	0.32	139	0.27
Mean tail	0.34	0.19	187	0.42	0.23	139	0.92
Redd substrate (%)							
Bowl – sand	28.4	17.7	187	24.7	14.8	139	0.37
Bowl – gravel	47.3	22.7	187	53.9	20.9	139	0.72
Bowl – cobble	19.9	16.0	187	18.8	16.8	139	0.10
Bowl – boulder	3.1	6.5	187	1.8	5.6	139	0.70
Tail – sand	13.4	11.1	187	8.9	8.7	139	0.46
Tail – gravel	56.7	24.1	187	63.9	26.5	139	0.66
Tail – cobble	27.0	21.4	187	25.1	25.1	139	0.16
Tail – boulder	1.7	4.5	187	0.7	2.9	139	0.72
Redd location (m)							
Distance to nearest redd	22.2	47.0	185	33.3	53.2	129	0.49
Distance to left bank	8.5	7.1	187	7.7	6.2	139	0.18
Distance to right bank	6.6	6.4	187	8.1	7.3	139	0.50
Distance to cover	5.0	5.7	187	5.3	5.6	139	0.08
Female size (cm)							
Female FL	79.33	5.29	172	80.04	5.15	69	0.31

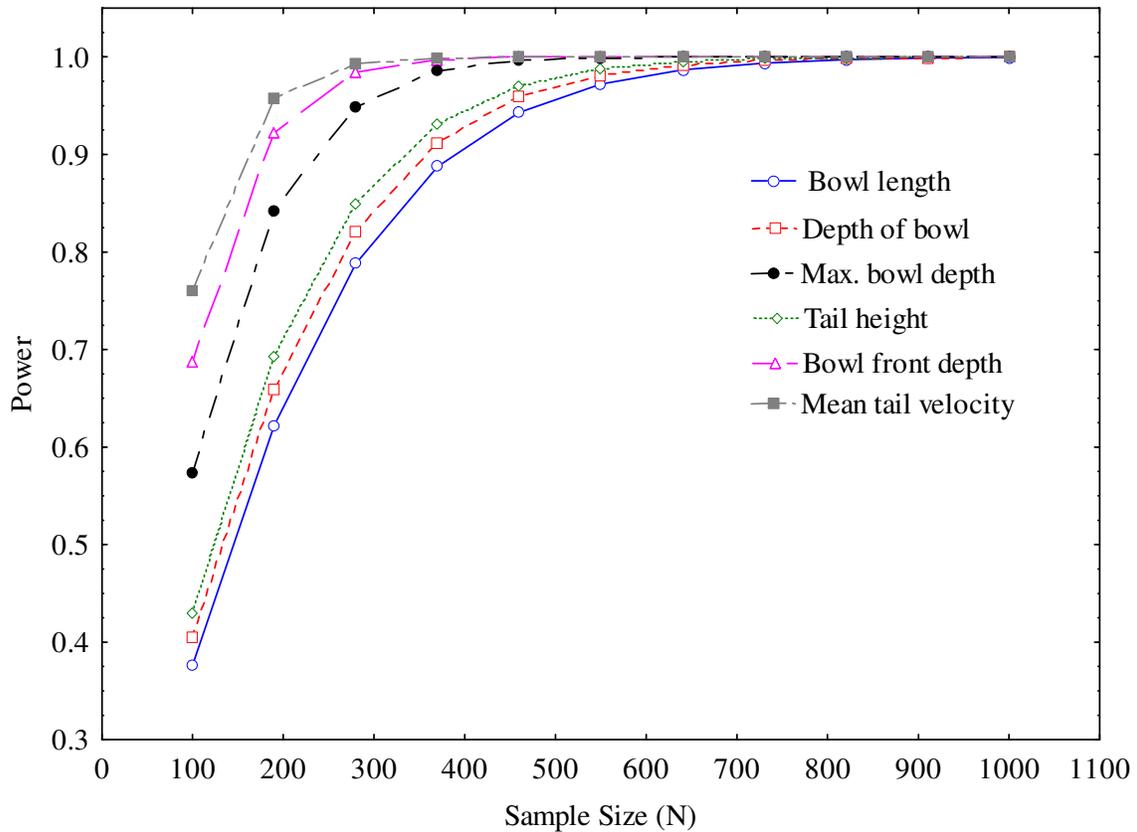


Figure 6. Sample size required to obtain statistical power for redd microhabitat characteristics in the Wenatchee River Basin.

Summary

In 2006, 528 spring Chinook redds were found upstream of Tumwater Dam and 60 redds were found in tributaries downstream of the dam. Based on carcass recoveries, the spawner composition upstream of Tumwater Dam was 66% hatchery and 34% naturally produced spring Chinook. The overall spawner composition was estimated at 68% hatchery and 32% naturally origin spawners. While no difference was detected in survival within age classes regardless of origin, the survival of hatchery fish (50%) was estimated to be significantly lower than that of naturally produced fish (62%). Differential survival of hatchery and naturally produced fish was not detected in 2004 or 2005. Differences detected in 2006 may be due to the smaller size of hatchery females or statistical power in 2004 and 2005 was inadequate to detect differences.

The spawning distribution of hatchery female spring Chinook was different than naturally produced females. Hatchery female spring Chinook spawned in the lower reaches of both Nason Creek and the Chiwawa River whereas natural origin fish spawned over a greater geographic area. No difference was detected in the spawning distribution of male spring Chinook. 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. No difference in spawn timing was detected in the natural or hatchery environment.

Power analysis of the redd microhabitat variables suggested that the required sample sizes for most variables were not obtained. However, adequate statistical power should be reached after several additional years of data collections. Contrary to expectations, no strong relationships between female size or discharge and redd microhabitat characteristics were found. These results suggest that redd microhabitat data can be pooled across years and streams providing the necessary statistical power once sample size targets have been reached.

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The Bonneville Power Administration provided funding for this project. We thank Jonathan McCloud, the Project Manager, for supporting our unique contracting requirements. We would like to thank Chelan County Public Utility District for assistance and funding in conducting spawning ground and carcass surveys. We would also like to thank the numerous members of the WDFW Supplementation Research Team technicians for their assistance in data collection. John Sneva of the WDFW Scale Lab read all the scale samples.

References

- Blouin, M. 2003. Relative reproductive success of hatchery and wild steelhead in the Hood River. Final report to Bonneville Power Administration, Contract No. 9245. Bonneville Power Administration, Portland, Oregon.
- Busack, C., C.M. Knudsen, G. Hart, and P. Huffman. In Press. Differences in body shape between first-generation hatchery and wild upper Yakima spring Chinook salmon. Transactions of the American Fisheries Society.
- Chandler, G. L., and T. C. Bjornn. 1988. Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. Transactions of the American Fisheries Society 117:432-443.
- Crisp, D. T., and P. A. Carling. 1989. Observations on siting, dimensions and structure of salmonid redds. Journal of Fish Biology 34:119-134.
- Groot, C. and L. Margolis. 1991. Pacific Salmon Life Histories. UBC Press, Vancouver.
- Knudsen C.M., S.L. Schroder, M.V. Johnston, C. Busack, T.N. Pearsons, and D. Fast. 2005. A comparison of life-history traits in first generation hatchery- and wild origin Upper Yakima River spring Chinook. Annual Report 2004. Bonneville Power Administration, Portland, Oregon.
- Knudsen C.M., S.L. Schroder, C.A., Busack, M.V. Johnston, T.N. Pearsons, and W.J. Bosch, and D.E. Fast. 2006. Comparison of life-history traits between first-generation hatchery and wild Upper Yakima River spring Chinook salmon. Transactions of the American Fisheries Society 135: 1130-1144.
- Leider, S. A., M. W. Chilcote, and J. J. Lock. 1984. Spawning characteristics of sympatric populations of steelhead trout (*Salmo gairdneri*): evidence for partial reproductive isolation. Canadian Journal of Fisheries and Aquatic Sciences 41:1454-1462.
- Mosey, T. R., and L. J. Murphy. 2002. Spring and summer Chinook spawning ground Surveys on the Wenatchee River Basin, 2002. Chelan County Public Utility District, Wenatchee, Washington.
- Murdoch, A.R., T.N. Pearsons, T.W. Maitland, M.F. Ford, and K. Williamson. 2005. Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. BPA Project No. 2003-039-00. Bonneville Power Administration, Portland, Oregon.
- Murdoch, A.R., T.N. Pearsons, T.W. Maitland, M.F. Ford, and K. Williamson. 2006. Monitoring the reproductive success of naturally spawning hatchery and natural

spring Chinook salmon in the Wenatchee River. BPA Project No. 2003-039-00. Bonneville Power Administration, Portland, Oregon.

Nickelson, T. E., M. F. Solazzi, and S. L. Johnson. 1986. Use of hatchery coho salmon (*Oncorhynchus kisutch*) presmolts to rebuild wild populations in Oregon coastal streams. *Canadian Journal of Fisheries and Aquatic Sciences* 43:2443-2449.

Waples, R. S. 1999. Dispelling some myths about hatcheries. *Fisheries* 24(2)12-21.

Zhou, S. 2002. Size-Dependent Recovery of Chinook Salmon in Carcass Surveys. Oregon Department of Fish and Wildlife.

Chapter 3 -- 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

Salmon use different mating strategies to increase their chances of producing fit offspring and hatchery production may alter the production of fishes that use different mating strategies. 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 relative abundance of precocious males on redds. The estimated index number of precocious males that were on the spawning grounds and potentially contributed to natural spawning was 260 (13 hatchery and 247 naturally produced). 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. Assortative pairing analysis was limited in 2006 because not all hatchery fish were externally marked. No difference was detected in the mean fork length of males paired with either hatchery or natural origin females ($P > 0.05$).

Introduction

Salmon use different mating strategies to increase their chances of producing fit offspring and hatchery production may alter the production of fishes that use different mating strategies. Two major mating strategies of salmon are mate selection and sneaking (Myers and Hutchings 1987; Foote 1988). Mate selection occurs when spawners intentionally spawn with a desired partner. In contrast, some undesirable males will dart in (sneak) and spawn with females as she is spawning with a selected male. Both strategies have been shown to be effective at producing offspring. Artificial propagation has the potential to skew the success of different mating strategies by altering abundance of different types of fish that are more likely to engage in particular strategies.

Salmon are known to select mates based on factors such as competitive dominance and fish size (Myers and Hutchings 1987; Foote 1988). 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 and differences between hatchery and natural origin spawners has been detected (Knudsen et al. 2006; Busack et al. in press). Natural origin fish may pair with other natural origin fish because they are larger (Knudsen et al. 2006), migrate and spawn at different times (Knudsen et al. 2006), or have a different shape (Busack et al. in press). 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.

Hatcheries may also increase the proportion of fish that engage in sneaking behavior. Sneaking has generally been associated with small male size such as age 0+, 1+ (precocious males), and 2+ (jacks). It has been demonstrated that some hatcheries increase the proportion of jacks and potentially age 1+ males (Larsen et al. 2004; 2006; Knudsen et al. 2006; Pearsons et al. 2006). The number of age 1+ precociously mature salmon on the spawning grounds may be significantly increased by hatchery programs (Reviewed by Mullan et al. 1992a, b) 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; Pearsons et al 2006). 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 poses ecological interaction risk with native conspecifics and other non-target species of concern (Pearsons and Hopley 1999; Pearsons 2002; Pearsons and Temple 2007). Large numbers of precocious males on the spawning grounds would alter the age structure, sex ratio and, potentially, other phenotypic characters of the spawning population. 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 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

Surveys were conducted weekly and lasted throughout the spawning season. Active redds (i.e., the presence of an anadromous fish) were found by walking upstream. We also attempted to avoid disturbing fish on redds. 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 precocious fish are generally less than 300 mm. Females were distinguished from males by the body color, secondary sexual characteristics, and the condition of the caudal fin. Male spring Chinook were typically dark in color and possess a distinct kype. While female spring Chinook are green in color, did not possess a kype and have a severely eroded caudal fin with a white band on the margin of the caudal fin as a result from digging a redd.

During spawning ground surveys, surveyors visually estimated the fork length of all fish observed on a redd. Unequal surveyor bias associated with fork length estimates could increase error in making comparisons between hatchery and naturally produced fish. Data from spring Chinook that were encountered on the spawning grounds and subsequently scanned for PIT tags (i.e., fork length measured at Tumwater Dam) were included in a linear regression analysis (dependent variable = estimated fork length; independent variable = fork length measured at Tumwater Dam) to correct fork length estimates of fish that were not scanned for PIT tags.

Precocious males may spawn with multiple females throughout the spawning period. Due to their small size (i.e., poor PIT detection and low carcass recovery probability) the distance precocious males may travel to seek females is unknown. These factors may lead to overestimating the total abundance of precocious males (i.e., double counting). Data collected from 2004 and 2005 indicate that distribution of hatchery precocious males is limited to the lower reaches of Chiwawa River. We assumed the distribution of naturally produced precocious males is likely closely associated with the distribution of redds. We used specific reaches in the Chiwawa River (3.6 km) and Nason Creek (1.7 km) where little to no spawning is present as natural reach breaks and assumed that naturally produced precocious males would not travel between reaches in seeking females. Given the limited amount of spawning habitat in the Little Wenatchee, White, and upper Wenatchee rivers, we assumed naturally precocious males could spawn with any female.

Data Analysis

The mean number of precocious males per redd was calculated by dividing the number of precocious males observed by the number of redds snorkeled in each stream. These data will be used to assess the relative contribution of precocious males to the next generation (i.e., progeny attributed to unknown male parentage). In addition, an index of relative abundance was calculated for both hatchery and naturally produced precocious males by stream and reach. Weekly abundance estimates of precocious males were calculated by multiplying the mean values of each origin (i.e., number of precocious males/redd) by the total number of active redds in each stream or reach. The greatest weekly abundance estimate for each reach and origin was used as the index of relative abundance.

The mean corrected 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 corrected 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

Precocious Males

A total of 78 redds (14.8% of all redds) were snorkeled in the upper Wenatchee River Basin during spawning ground surveys (Table 1). Of the 63 redds snorkeled on the Chiwawa River, there were 9 hatchery, and 60 naturally produced precocious fish observed. Of the 15 redds snorkeled on Nason Creek, there were 9 natural origin precocious males observed. Age 0+ precocious males (i.e., FL < 80 mm) were not observed during any of the surveys. In 2006, the mean number of precocious males per redd was higher in both the Chiwawa River and Nason Creek for both hatchery and naturally produced fish than was observed in 2004 and 2005. 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) or Little Wenatchee River due to low redd abundance.

The index of relative abundance estimates for the upper Wenatchee Basin was 260 precocious males (13 hatchery and 247 natural origin; Table 2). Relative abundance estimates for 2004 and 2005 differ from that previously reported due to changes in methodology that prevent overestimating abundance for reasons previously discussed. In future years, snorkel surveys will be conducted to ensure all streams/reaches are sampled with a similar level of effort to ensure a similar level of precision between streams.

The probability of recovering precocious male carcasses was estimated as 0.5%. Of the three precocious male carcasses recovered in 2006, two were naturally produced and recovered in the Chiwawa and Little Wenatchee River. One hatchery fish was recovered in the Chiwawa River that was PIT Tagged at Tumwater Dam. The mean (standard deviation, SD) size of the hatchery precocious males sampled at Tumwater Dam was 210 (1.68) mm. Naturally produced precocious males had mean fork length of 140 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 illustrate the need for snorkel surveys.

Table 1. Summary of precocious males found during spawning ground surveys in the upper Wenatchee River basin between 2004 and 2006 (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
<i>2004</i>								
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	0.10	0.09	0.00	0.11
<i>2005</i>								
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	0.04	0.09	0.02	0.13
<i>2006</i>								
Chiwawa	63	9	60	0	0.14	0.95	0.00	1.10
Nason	15	0	9	0	0.00	0.60	0.00	0.60
Total Upper Basin	78	9	69	0	0.14	0.89	0.00	1.00

*Origins not determined due to poor visibility.

Table 2. Index of relative abundance for precocious male spring Chinook on the spawning grounds in the upper Wenatchee River Basin between 2004 and 2006. The estimated number of precocial males represent the week with the highest estimate based on the number of active redds and mean number of precocious males per redd for each respective stream/reach (NS = no redds were snorkeled).

River	Reach	Estimated number	
		Hatchery	Naturally produced
<i>2004</i>			
Chiwawa	Lower	31	93
	Upper	0	0
Nason	Lower	0	3
	Upper	0	0
White		0	0
Upper Wenatchee		0	0
Total		31	96
<i>2005</i>			
Chiwawa	Lower	0	0
	Upper	4	23
Nason	Lower	NS	NS
	Upper	0	2
Upper Wenatchee		0	0
Little Wenatchee		0	0
Total		4	25
<i>2006</i>			
Chiwawa	Lower	13	58
	Upper	0	144
Nason	Lower	0	26
	Upper	0	19
Total		13	247

Assortative Mating

Observations of pairings on the spawning grounds were limited in 2006 because only 41.6% of age-4 hatchery fish encountered at Tumwater Dam were 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, 47% were recorded on the White River (Table 3). Female hatchery spring Chinook were paired predominately with hatchery males (62%). Similarly, natural origin female spring Chinook were paired predominately (92%) 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 and do not necessarily reflect a preference by females for males of similar origin (see Chapter 3).

Table 3. Pairing of hatchery and natural origin spring Chinook on redds in the upper Wenatchee River Basin between 2004 and 2006.

Year	Female origin	Number of females	Number of males		
			Natural	Hatchery	Unknown (Jacks)
Single Pairings					
2004	H	19	9	2	8
	N	46	39	3	4
Multiple Male Pairings					
2004	H	17	25	11	15
	N	48	96	14	31
Single Pairings					
2005	H	22	10	12	0
	N	4	1	3	0
Multiple Male Pairings					
2005	H	11	13	13	0
	N	2	0	4	0
Single Pairings					
2006	H	14	2	6	6
	N	9	6	1	2
Multiple Male Pairings					
2006	H	4	4	4	1
	N	9	29	2	1

Of the female spring Chinook included in the assortative pairing analysis, differences in mean fork length of hatchery and naturally produced fish were detected in 2005 and 2006 ($P < 0.03$). In both years, naturally produced female spring Chinook were greater in fork length (~ 3 cm). Despite differences in female size, no within year differences were detected in the mean fork length of the dominant male spring Chinook paired with hatchery or natural origin female spring Chinook (Figure 1; $P > 0.08$). Significant correlations were found between male fork length for both hatchery ($P < 0.05$; $r = 0.36$)

and natural ($P < 0.05$; $r = 0.43$) female spring Chinook fork length for all years combined (Figure 2).

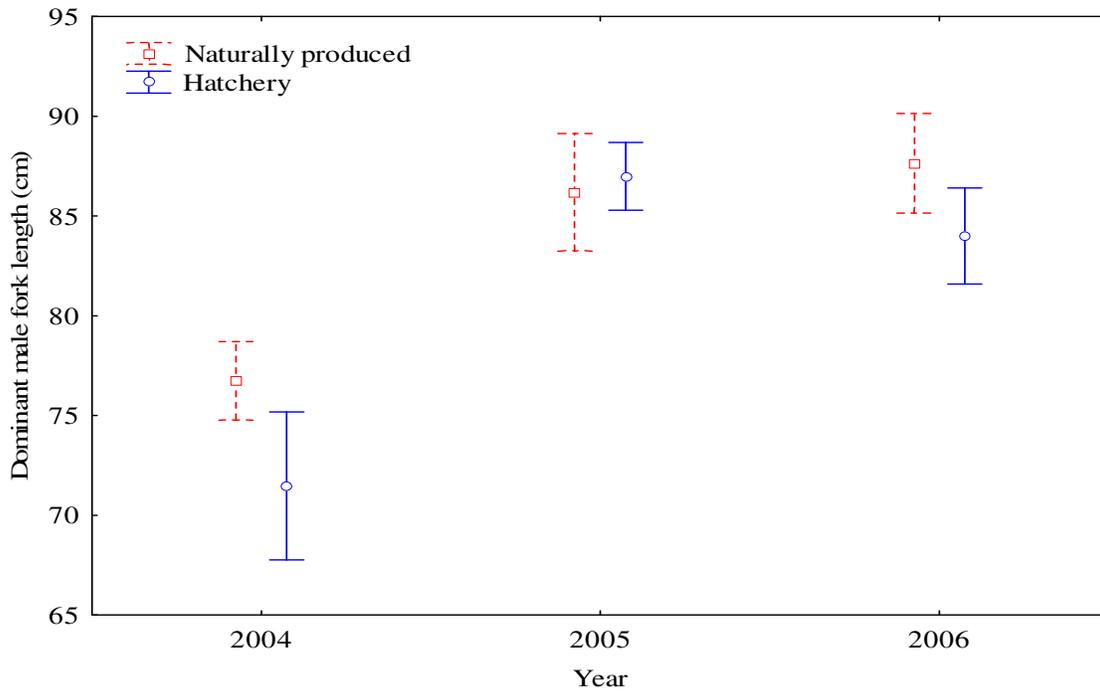


Figure 1. Mean fork length of dominant male spring Chinook paired with hatchery and naturally produced spring Chinook in the Wenatchee Basin between 2004 and 2006. Vertical bars denote 95% confidence intervals.

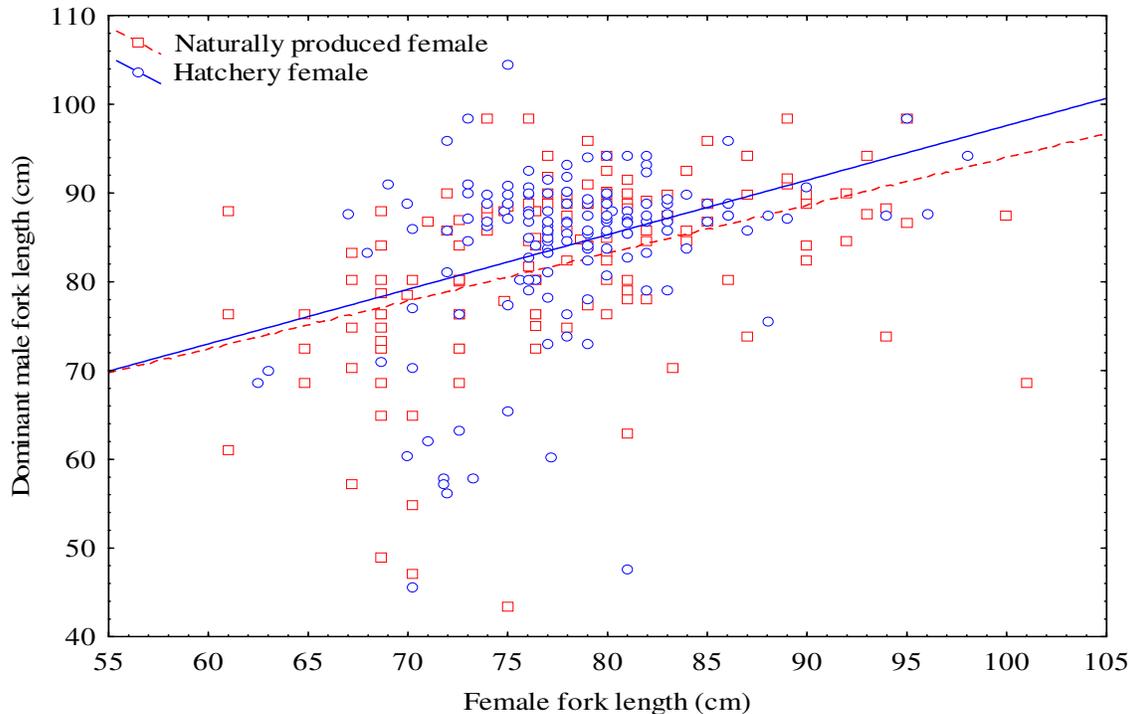


Figure 2. Relationship between female and dominant male fork length on the spawning grounds in the Wenatchee Basin between 2004 and 2006.

Summary

The incidence of hatchery precocious males in the Wenatchee River Basin has remained relatively low and confined to the Chiwawa River. 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. However, the single recapture of a hatchery precocious male in 2006 does indicate that at least some fish have the potential to participate in spawning.

The abundance of naturally produced precocious males increased in 2006, but may be due in part to greater observer efficiency due to excellent water clarity. Furthermore, a majority of the redds snorkeled in 2006 were in the Chiwawa River (81%). The rate of precocious development is likely related to environmental, genetic, or density dependent factors. Hence, the skewed sampling in 2006 may have biased the total abundance estimate. In future years, snorkeling should be conducted in a random systematic approach across the entire basin to ensure that every reach in each spawning tributary was snorkeled.

Data collected between 2004 and 2006 suggests that mate pairing in the Wenatchee Basin is random with respect to the variables that we measured. Although, a relationship albeit

weak does appear to be present between size of the female and dominant male. These data will be used in conjunction with the DNA pedigree analysis (See Chapter 4), which should also provide information about mate selection.

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References

- Busack, C., C.M. Knudsen, G. Hart, and P. Huffman. *In Press*. Differences in body shape between first-generation hatchery and wild upper Yakima spring Chinook salmon. *Transactions of the American Fisheries Society*.
- Foote, C. J. and P. A. Larkin. 1988. The role of male choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). *Behavior* 106:43-62.
- Knudsen C.M., S.L. Schroder, C.A., Busack, M.V. Johnston, T.N. Pearsons, and W.J. Bosch, and D.E. Fast. 2006. Comparison of life-history traits between first-generation hatchery and wild Upper Yakima River spring Chinook salmon. *Transactions of the American Fisheries Society* 135: 1130-1144.
- Larsen, D.A., B.R. Beckman, C. Strom, P. Parkins, K.A. Cooper, M. Johnston, and W. W. Dickhoff. 2004. Growth rate modulation in spring Chinook salmon supplementation, 2003-2004 Annual Report. Project No. 200203100. Bonneville Power Administration, Portland, Oregon.
- Larsen, D. A., B. R. Beckman, C. R. Strom, P. J. Parkins, K. A. Cooper, D. E. Fast, and W. W. Dickhoff. 2006. Growth modulation alters the incidence of early male maturation and physiological development of hatchery-reared spring Chinook salmon: a comparison with wild fish. *Transactions of the American Fisheries Society* 135: 1017-1032.
- Mullan, J. W., K. R. Williams, G. Rhodus, T. W. Hillman, and J. D. McIntyre. 1992. *Productions and Habitat of Salmonids in Mid-Columbia River Tributary Streams*. U.S. Fish and Wildlife Service.

- Mullan, J. W., A. Rockhold, and C. R. Chrisman. 1992b. Life histories and precocity of Chinook salmon in the mid-Columbia River. *The Progressive Fish-Culturist* 54:25-28.
- Myers, R. A. and J. A. Hutchings. 1987. Mating of anadromous Atlantic salmon, *Salmo Salar* L., with mature male parr. *Journal of Fish Biology* 31:143-146
- Pearsons, T. N. 2002. Chronology of ecological interactions associated with the life-span of salmon supplementation programs. *Fisheries* 27(12):10-15.
- Pearsons, T., C. Johnson, B. James, and G. Temple. 2004. Spring Chinook interactions indices and residual/precocial monitoring in the upper Yakima Basin; Yakima/Klickitat Fisheries Project Monitoring and Evaluation Report 5 of 7, 2003-2004 Annual Report. Bonneville Power Administration, Project No. 199506325, Portland, Oregon.
- Pearsons, T. N., and C. W. Hopley. 1999. A practical approach for assessing ecological risks associated with fish stocking programs. *Fisheries* 24(9):16-23.
- Pearsons, T. N., and G. M. Temple. 2007. Impacts of early stages of salmon supplementation and reintroduction programs on three trout species. *North American Journal of Fisheries Management* 27:1-20.
- Schroder, S. L., C. M. Knudsen, T. N. Pearsons, D. E. Fast, and B. D. Watston. 2005. Comparing the reproductive success of Yakima River hatchery and wild-origin spring chinook. Annual Report FY 2004 submitted to Bonneville Power Administration, Portland, Oregon. [DOE/BP-00017478-6](#).
- Schroder, S. L., C. M. Knudsen, T. N. Pearsons, S. F. Young, T. W. Kassler, D. E. Fast and B. D. Watson. 2006. Comparing the reproductive success of Yakima River hatchery and wild-origin spring chinook. Annual Report FY 2005-2006. Report to Bonneville Power Administration, publication [DOE/BP-00022370-3](#), Portland, Oregon.
- Zhou, S. 2002. Size-dependent recovery of Chinook salmon in carcass surveys. *Transactions of the American Fishery Society* 131:1194-1202.

Chapter 4 -- Influence of supportive breeding on genetic diversity of hatchery and natural Wenatchee River spring Chinook salmon

Abstract

Genetic monitoring of weak and endangered natural populations supplemented by captively bred individuals is necessary to predict loss of genetic diversity among local, natural populations as well as the influence of genetic drift and inbreeding on the evolutionary dynamics of captively supplemented populations. We examined the effects of a modern salmon supplementation program on the effective population size (N_e) and genetic diversity of a natural population of Wenatchee River spring-run Chinook salmon. Demographic and genetic estimates were used to estimate N_e . Genetic estimates of N_e were obtained by linkage disequilibrium and temporal methods using data from 11 microsatellites. Despite over a decade of supportive breeding and several years of very low returns, some genetic substructure exists among natural spring-run Chinook populations within the Wenatchee watershed. Demographic estimates of N_e indicated that fluctuating population size had the most substantial impact on reducing N_e in both the hatchery and wild populations, followed by variation in reproductive success. Combining information from the demographic and genetic methods of estimating N_e indicates that small numbers of captive breeders and episodic disproportional contributions to future generations by relatively few captive breeders may have decreased N_e of the entire population below a level that would have been attained if supplementation had not been initiated.

Introduction

Supportive breeding, where a subset of wild individuals are placed in captivity for reproduction and their offspring are released into the natural environment to mix with wild individuals, is intended to increase the size of a natural population without introducing exogenous genes into the population (Ryman and Laikre 1991). This practice, also known as supplementation, is common in the management of some fish species, especially salmonids. In the Pacific Northwest alone, over 30 natural salmon populations are currently undergoing some form of deliberate supplementation (Jim Meyers and Tim Tynan, NOAA Fisheries Service, *personal communication*) although many more populations are subject to inadvertent supplementation as a consequence of stray captively bred individuals. Supplementation of salmon populations typically involves breeding of adults and early rearing of juveniles in a freshwater hatchery, followed by release juveniles into the wild when they are ready to begin migrating to the ocean. Supplementation has been a controversial management practice (e.g., ISAB 2002; Brannon et al. 2004) because it has the potential to produce both benefits and risks to natural populations (reviewed by Waples and Drake 2004). Potential benefits stem largely from the increased abundance that can be achieved by taking advantage of high survival rates in captivity. Potential risks include ecological competition or predation of hatchery-produced fish on wild fish, and impacts to genetic diversity.

One potential risk that has received considerable attention is the possibility that supplementation may decrease a population's effective population size even in cases where population abundance is successfully increased (Ryman and Laikre 1991; Waples and Do 1994; Ryman et al. 1995; Wang and Ryman 2001). Effective population size (N_e) is defined as the size of a hypothetical, ideal population (randomly mating; discrete generations; equal sex ratio; no immigration, mutation, or selection; binomial variance in reproductive success) that undergoes the same rate of genetic change as a real (non-ideal) population (Wright 1969). Populations with small N_e become inbred or lose genetic diversity due to drift at proportionally higher rates than populations with large N_e . The seemingly paradoxical result that supplementation could increase a population's total abundance while decreasing its effective size occurs because supplementation usually involves increasing the reproductive contribution of only a small fraction of the total population. The relatively small number of individuals bred in captivity therefore disproportionately influences N_e .

Previous studies have shown how supportive breeding can alter N_e over a single generation (Ryman and Laikre 1991; Ryman 1994; Ryman et al. 1995) and multiple generations (Waples and Do 1994; Wang and Ryman 2001; Duchesne and Bernatchez 2002). Successful increase in the overall population size requires that the reproductive rate for captive fish be higher than that of the naturally spawning breeders. When a small fraction of individuals are taken into captivity to reproduce and their reproductive output is large relative to that of the naturally spawning population, supportive breeding may also cause more severe decreases in N_e than would have occurred if a supportive breeding program had not been instituted. Decreases in overall N_e can result in the loss of genetic variability through the random extinction of alleles, limit a population's evolutionary potential to adapt to environmental change, and may lead to reduced fitness through inbreeding depression.

Another potential genetic risk of supplementation and other forms of hatchery production more broadly is loss of genetic diversity within and among populations (Busack and Currens 1995). In particular, the geographical scale at which it is practical to implement supplementation may sometimes be larger than the geographic scale at which natural salmon populations are structured (Utter 1998). Salmon naturally have a strong tendency to home to their natal stream, leading to patterns of geographically structured molecular genetic diversity and fine scale local adaptation (reviewed by Taylor 1991). Some hatchery variables, such as the source of rearing water, or location of the fish collection weir or hatchery, may cause large numbers of hatchery fish released in one stream to return to spawn in other streams (straying). Such straying may potentially reduce genetic diversity among the streams receiving the hatchery immigrants (Utter 1998 and 2004). Genetic homogenization of locally adapted native populations due to reproduction with hatchery strays may be disadvantageous by reducing adaptive genetic diversity among local populations (Allendorf and Leary 1988). Habitat affinity (McIssac and Quinn 1988; Quinn 1993), disease resistance (Krueger and May 1991), and age at maturity (Ricker 1972; Hankin et al. 1993) and timing of migration (Sitonen and Gall 1989; Hanson and Jonsson 1991) are unique adaptations under some degree of genetic control that provide populations with a reproductive advantage in particular environments thereby increasing

the probability of persistence in those environments. Populations with low genetic variability may have limited evolutionary potential to adapt to rapid environmental changes (Carson and Templeton 1984). This may, in turn, exacerbate a population's extinction risk from severe, stochastic environmental disturbances and disrupted demographic processes due to reduced numbers (Soule 1987; Lacy 1988; Lande 1988).

While considerable literature is available regarding the propagation, life history, and ecological requirements of salmonid fishes, there are only a few scientific publications (e.g. Docker et al. 2003; Sharma et al 2006; Araki et al. *in press*) that document the influence of supplementation programs on natural salmon populations. In this paper we examine the effects of a modern salmon supplementation program on the effective population size and genetic diversity of a natural salmon population. We focused on the population of spring-run Chinook salmon (*Oncorhynchus tshawytscha*) that spawn in the Wenatchee River, Washington (WA) (Figure 1). This population has been supplemented by hatchery production since 1989, and has been the subject of previous population genetic studies (Utter et al. 1995; Ford et al. 2001; Schwenke et al. 2006). In order to evaluate the effects of supplementation on the genetic diversity of natural Chinook salmon, we examined whether the geographic population structure that currently exists within the Wenatchee R. drainage system remains similar to what existed at the start of the supplementation program and compared patterns of genetic diversity between hatchery- and natural-origin fish (Utter et al. 1995). We also used demographic and genetic data to estimate the effective population size of the hatchery and natural components of the populations, and to evaluate the effects of supplementation on the effective population size of the total combined population.

Methods

Description of study population - The spring-run Chinook salmon that spawn in the Wenatchee, Entiat and Methow Rivers (Figure 1) are considered to be an Evolutionarily Significant Unit (ESU) (Waples 1991; Myers et al. 1998) and are listed as "endangered" under the U.S. Endangered Species Act (NMFS 2005). The Wenatchee R. spring-run Chinook salmon spawn in the upper Wenatchee R. and several of its tributaries upstream of the Tumwater Dam (Figure 1). The population is characterized by a life-cycle pattern typical of "stream-type" Chinook salmon (Healey 1991). Adults return from the ocean to freshwater in the spring and early summer and spawn in late summer and fall. Fry emerge from the gravel the following spring and juveniles then spend a full year in freshwater before migrating to the ocean as smolts. They then spend from one to three years in the ocean, before returning to freshwater. Wenatchee R. Chinook salmon have been subject episodically to artificial propagation for many decades but were primarily naturally self-sustaining from the 1950's to the early 1990's when the population began declining abruptly (Chapman et al. 1995; Myers et al. 1998). In addition to the ESA-listed spring-run population that is the focus of this paper, the Wenatchee R. also contains a spawning population of summer-run Chinook and a non-ESA listed spring-run hatchery stock propagated at Leavenworth National Fish Hatchery. The Wenatchee R. summer-run are "ocean-type" Chinook (Healey 1991), and are readily distinguishable from the

spring-run population both genetically (Waples et al. 2004; Schwenke et al. 2006) and morphologically (Chapman et al. 1995). Carson NFH origin spring Chinook are produced in the Leavenworth, Entiat, and Winthrop NFHs. The primary breeders used by Carson NFH (Wind River) originated with collection of spring Chinook passing the Bonneville Dam in 1958. The majority of these fish were likely returning to spawn in the Snake River Basin, although other stock, from middle and upper Columbia River tributaries, also contributed significantly to the Carson gene pool (Hymer et al. 1992).

The Chiwawa River hatchery supplementation program was established in 1989 (RISPA 1989) using a small number of natural origin spring Chinook salmon captured in the Chiwawa R. (Figure 1). During the first five years of hatchery operation only wild fish collected from the Chiwawa R. were available as breeders. Since 1994 the proportion of hatchery-origin fish used as breeders has ranged from 36-72%. Very low escapements to the Wenatchee R. basin in 1995 and 1999 (Mosey and Murphy 2002) resulted in no fish being taken for captive breeding during those years (A. Murdoch, WA Dept. of Fish and Wildlife, personal communication). The intent behind using locally collected natural-origin fish in the supplementation program is to prevent genetic divergence of the hatchery stock due to a different selection regime within the captive environment (Lynch and O'Hely 2001; Ford 2002). Juveniles are reared on well water for 6-7 months at a central hatchery facility at Eastbank Hatchery on the Columbia R. (Figure 1). In order to promote homing back to the Chiwawa R., juveniles are moved to rearing ponds supplied with Chiwawa R. water where they are acclimatized for 6-7 months before being volitionally released into the Chiwawa R. During the winter rearing period (December-February) ice conditions in the Chiwawa R. drastically limit the water supply. Periodically, Chiwawa R. hatchery fish are supplied with Wenatchee R. water in order to prevent a catastrophic loss due to ice conditions in the Chiwawa R. Despite the on-site release of juveniles, considerable straying of Chiwawa R. hatchery adults occurs throughout the upper Wenatchee R. tributaries. In 1997, for instance, 33% of the adults sampled on Nason Crk. had Chiwawa R. hatchery coded wire tags (Ford et al. 2001).

Genetic sampling – Spring Chinook (adults, jacks, and precocial males) were sampled at the Tumwater Dam (Figure 1) as they migrated upstream to spawn. Fin clips and scale samples were collected non-lethally from essentially all spring Chinook salmon migrating past Tumwater Dam in 2004 and 2005. In 2004, 2969 samples were taken between May 18th and August 28th, and in 2005, 4203 samples were taken between May 14th and August 9th. Other data collected during sampling included secondary sexual characteristics, and absence/presence of an adipose fin. A passive integrated transponder (PIT) tag placed in each fish sampled permitted the tracking of individuals to different tributaries. Carcasses recovered on the spawning grounds were evaluated for the presence of PIT and coded wire tags (CWT). US Fish and Wildlife Service personnel provided fin clip samples from 350 Leavenworth National Fish Hatchery (LNFH) adult spring-run Chinook salmon released into Peshastin Creek in 2004. The latter served as a reference population since Utter et al. (1995) showed that the LNFH stock was genetically indistinguishable from the Carson stock, but distinct from naturally spawning populations in Wenatchee R. tributaries. Genetic data collected by WDFW from 48 Chiwawa R. wild juvenile spring Chinook (born in 1992) sampled in 1994 (Ford et al.

2004) and 60 White R. wild spring Chinook (born in 2004) sampled in 2005 at a rotary screw trap within each respective tributary were also incorporated into this study.

Microsatellite genotyping - Genomic DNA was extracted from fin clips using a QIAgen DNA tissue extraction kit, and quantified using a FL_x 800 Microplate Fluorescence reader (Bio-Tek Instruments, Winooski, Vermont). Individuals were genotyped at 11 microsatellite loci (Table 1). Microsatellite alleles were amplified by Polymerase Chain Reaction (PCR) assays using 15 ng of genomic DNA, 1.75 or 2.0 mM MgCl₂, 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. PCR products and size standards (GeneScan 500) were resolved using an ABI3100 capillary electrophoresis system (Applied Biosystems, Inc. (ABI), Foster City, California). Individual genotypes were scored using Genotyper software version 3.7 (ABI). 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.

Identifying Summer-run and stray spring-run hatchery fish- Putative spring Chinook were assigned to either a spring- or summer-run baseline population using the maximum-likelihood method implemented in the software program Genetic Mixture Analysis (GMA) (Kalinowski 2003). The first 574 (out of 2969) spring-run adults collected at Tumwater Dam and 192 summer-run adults (based on non-overlapping migration time and physical appearance) were used as baseline populations. Leavenworth NFH spring Chinook were identified during the spawning ground surveys based on CWTs implanted by LNFH prior to release. Individuals that were assigned to the summer-run baseline, that had an ambiguous assignment, or that carried a LNFH CWT were excluded from the dataset.

Identifying Chiwawa River hatchery fish - Hatchery fish were identified by an absent adipose fin and/or CWT. Wild fish were identified by the presence of an adipose fin. In addition, scale growth pattern analysis (John Sneva, WDFW, personal communication) was used to positively discriminate hatchery and wild origin since not all hatchery fish are marked. Individuals whose origin could not be ascertained were removed prior to population genetic analyses. The remaining 2004 and 2005 (N=2823 and 4095, respectively) fish were grouped according to hatchery (N=1947 and 3485, respectively) or wild (N=876 and 570, respectively) origin. Hatchery and wild fish were subsequently sub-grouped, where possible, based on carcass recovery location (Chiwawa and White River, Nason Creek) and age.

Identifying closely related juveniles- To avoid over representation by only a few families in the 1994 Chiwawa R. and 2005 White R. wild juvenile spring Chinook samples kinship within each sample was evaluated using the software program Pedigree v.2.0

(provided by Christophe Herbinger, Dalhousie University, <http://herbinger.biology.dal.ca:5080/Pedigree>). 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. Since no one family dominated in the juvenile fish samples, all individuals were included in subsequent analyses.

Genetic analysis– Patterns of variation at microsatellite loci were characterized separately in hatchery and wild Chinook populations, as well as in hatchery and wild fish grouped by age and carcass recovery location, respectively. Allele frequencies, observed number of alleles, expected heterozygosity (H_e) under Hardy-Weinberg equilibrium (HWE), and observed heterozygosity (H_o) for 11 microsatellites loci were calculated using the program GENETIX v. 4.05 (Belkhir et al. 2003, available at <http://www.University-montp2.fr/~genetix/genetix.htm>). Global F_{IS} tests for Hardy-Weinberg expectations and pair-wise comparisons of loci for linkage disequilibrium using all 11 loci were made by estimation of exact P-values by the Markov Chain method (Guo and Thompson 1992) as implemented by the program GENEPOP (dememorization steps 1000; 50 batches; 1000 iterations per batch) (Raymond and Rousset 1995) for hatchery and wild fish grouped by age and carcass recovery location, respectively. Sequential Bonferroni adjustments to α were applied, where appropriate, for simultaneous tests to decrease the chance of erroneously rejecting null hypotheses (Rice 1989). Similar analyses were performed on 8 of the 11 loci (all in Table 1, except Ots201b, 211, and 213) used to genotype the 1994 Chiwawa R. wild juvenile spring Chinook (Ford et al. 2004).

Characterization of spring Chinook population structure – A pair-wise F_{ST} matrix was calculated using 11 loci (Table 1) using GENETIX v. 4.05 (Belkir et al. 2003). The 1994 Chiwawa R. as well as the LNFH spring-run out-plant and Wenatchee R. summer-run samples were included in the analysis. Significance ($\alpha = 0.05$) of pair-wise F_{ST} values was assessed using 1000 bootstrap replicates of the entire data set. A Neighbor-Joining phenogram, based on Cavalli-Sforza (1967) cord distance units, was created using PHYLIP v. 3.6b (Felsenstein 2002). The phenogram was constructed using data from 11 loci (Table 1), 1000 boot-strap replicates of the data set, and was rooted using the 2004 Wenatchee R. summer-run Chinook as an out group.

Estimation of N, sex ratio, recruits, and yearly contribution to the next generation - The total number of naturally spawning hatchery and wild adults was estimated for each year based on expansions from weekly redd counts and sampling a portion of carcasses from each population. Numbers of female and male fish used for breeding each year were obtained from Chiwawa R. hatchery records. Estimates of spawner age composition for years 1989 -1992 were based on all recovered carcasses recovered between 1986 and 1993 in the Wenatchee R. Basin (Chapman et al. 1995). Spawner age composition for years 1993-2005 was based on annual carcass surveys of hatchery and wild fish. The abundance of spawners by gender was estimated from redd counts (i.e., 1 redd = 1 female). The origin and age of each gender was subsequently derived from carcass surveys. This permitted the partitioning of spawners into individual cohorts, thereby the yearly proportional contribution to the subsequent generation could be reconstructed. These data were used to estimate the number of recruits (R_i), adults that returned to

spawn in the next generation, produced by spawners in year i , and the total number of spawners in the next generation produced by spawners in the current generation ($R_T = \sum R_i$ over a generation) between 1989 and 2000. The relative contribution of naturally spawning hatchery and wild adults in year i to the next generation (X_i) was calculated as R_i / R_T .

Demographic estimates of effective population size in the natural and hatchery components of the population - Demographic variables such as unequal sex ratio, greater than binomial variance in reproductive success, and fluctuating population size all have the effect of reducing a population's N_e below the observed number of breeders. The unusual life history of Pacific salmon (semelparity combined with variable age at maturity) introduces added complexity in estimating N_e (Waples 2002a and 2006b). The separate hatchery and natural demographic estimates of number and age of spawning adults, recruitment, proportional contribution to the next generation and number of breeders (assumed to be equal to census size) were combined into a total population estimate of effective number of breeders ($N_{b(i)}$) for each year i using the method of Ryman and Laikre (1991). Finally, the method of Waples (2002a and 2006b) was used to combine these annual estimates of N_b into an estimate of inbreeding N_e per generation (below).

Relationship between effective number of breeders in a year and unequal sex ratio (SR) – The effect of unequal sex ratio on the breeding segment of a population reduces effective number of breeders in a year, N_b , according to the following relationship:

$$N_{b(\text{sex ratio})} = \frac{4(N_f \times N_m)}{(N_f + N_m)}$$

where N_f and N_m are the actual number of female and male breeders, respectively.

Relationship between effective number of breeders (N_b) and variation in reproductive success (VRS) – The effective number of female and male breeders (N_{bf} and N_{bm} , respectively) may be expressed as a function of the mean and variance (k and σ_k^2 , respectively) of the number of progeny produced by an individual over its lifetime (Crow and Kimura 1970; Crow and Denniston 1988). The effective numbers of female and male breeders are estimated, respectively, as:

$$N_{bf} = \frac{(N_f \bar{k}_f - 1)}{[\bar{k}_f + (\sigma_{kf}^2 / \bar{k}_f) - 1]} \quad \text{and} \quad N_{bm} = \frac{(N_m \bar{k}_m - 1)}{[\bar{k}_m + (\sigma_{km}^2 / \bar{k}_m) - 1]}$$

where N_f and N_m are defined as above. The formulas for effective number of males and females were applied to single years so they reflect the effective number of breeders of each sex whereas the original formulas by Crow and Denniston (1988) reflect the effective number of each sex. Estimates of k and σ_k^2 are not available for Wenatchee R. spring Chinook. We used mean number of offspring produced by males (k_m) and females (k_f) and the variance of progeny numbers produced by males (σ_{km}^2) and females (σ_{kf}^2) measured at the juvenile stage as reported by Schroder et al. (2005) for hatchery- ($k_m=8.1$, $\sigma_{km}^2=8391$; $k_f=12.1$, $\sigma_{kf}^2=2104$) and wild-origin ($k_m=104.4$, $\sigma_{km}^2=8949$; $k_f=87.3$,

$\sigma_{kf}^2 = 3628$) spring Chinook in the nearby Yakima River. Using these data to compute inbreeding N_e effectively assumes that survival to adulthood is random after reaching the juvenile stage. Violation of this assumption, such as non-random differences in survival among families, will lead to an overestimate of N_b and N_b/N .

Estimate of the effective number of breeders (N_b) in year i – The effective number of breeders in year i was estimated from the effective number of male and female breeders per year caused by unequal SR and VRS as:

$$N_{b(\text{Demo})i} = \frac{4(N_{bf} \times N_{bm})}{(N_{bf} + N_{bm})},$$

where N_{bf} and N_{bm} are defined as above.

Estimate of combined hatchery and natural effective number of breeders, and effective population size – Captive breeding may reduce variance (N_{eV}) and inbreeding effective size (N_{eI}), which quantify the amount of genetic drift and increase of inbreeding, respectively, of the combined captive and wild populations (Ryman and Laikre 1991; Ryman et al. 1995). For the purpose of this paper N_e will refer to inbreeding N_e unless stated otherwise.

The combined hatchery and natural effective number of breeders ($N_{b(i)}$) for each year i was calculated in a manner analogous to the method of Ryman and Laikre (1991). Numbers of returning hatchery and wild adults were estimated as discussed above. It was assumed that $N_b = N$ for both hatchery and wild fish for the purpose of estimating N_e for the overall (hatchery and naturally spawning) population. The proportional contribution of hatchery fish to the next generation (X_{hi}) in year i was calculated as the ratio of the total number of hatchery recruits in subsequent years produced by hatchery adults spawning in year i to the total number of hatchery- and natural-origin recruits produced by adults spawning in year i . The proportional contribution of wild fish to the next generation (X_{wi}) was calculated as $1 - X_{hi}$. The method of Waples (2002a and 2006b) was then used to estimate N_e per generation based on the yearly estimates of $N_{b(i)}$, where the ratio of the proportional contribution of recruits to the next generation (X_i) produced by hatchery and naturally spawning fish combined in year i are summed over all years in a generation. The estimate of N_e was made using a generation interval of 4 years (Chapman et al. 1995; A. Murdoch, WDFW, unpublished data).

Relationship between effective size and fluctuating population size (FPS) – The influence of fluctuating population size (FPS) on separate estimates of hatchery and wild N_e was evaluated using the method of Waples (2002a and 2006). Accordingly, the ratio of the proportional contribution of hatchery or naturally spawning adults to the next generation (X_i) to effective number of hatchery or natural breeders (N_b) in year i were summed over all years in a generation. The proportional contribution of hatchery fish to the next generation (X_i) in year i was calculated as the ratio of the total number of hatchery recruits in subsequent years produced by hatchery adults spawning in year i to the total number of hatchery-origin recruits produced by adults spawning in that generation. The

proportional contribution of naturally spawned fish to the next generation was similarly calculated. As before, $N_b/N=1$ was assumed over all years in a generation to isolate the influence of fluctuating population size (FPS) on N_e and each generation interval consisted of 4 years (Chapman et al. 1995; A. Murdoch, WDFW, unpublished data).

Once included in the supportive breeding program, wild individuals do not contribute to the next generation as part of the naturally spawning population segment. It is possible that N_e of the wild population segment was reduced by having included a large number of wild fish in supportive breeding. To test this idea the numbers of wild female and male breeders taken for supportive breeding were added back to the naturally spawning population starting after 1993 (hatchery fish first returned in 1994), and the recruitment estimates recalculated using the new numbers of hatchery and wild spawners. Overall effective size was then re-estimated (Waples 2002a and 2006b) and compared to that obtained when wild fish were included in captive breeding once the program had produced returning adults. Finally, N_e of the wild population segment without a supportive breeding program being present, specifically excluding naturally spawning hatchery fish, was estimated using the method of Waples (2002a and 2006b).

Genetic estimates of contemporary N_b and N_e - As an alternative to using demographic data such as sex ratio or variance in reproductive success, N_b and N_e can also be estimated directly from population genetic data from either the degree of non-random association among alleles at different loci (linkage disequilibrium) or the temporal variance in allele frequencies sampled across generations. Linkage disequilibrium (LD) can be related to the correlation among alleles at different loci (r), and in a finite size population, LD (and r) will depart from zero due to the effect of genetic drift at a magnitude inversely proportional to N_e . Reduced N_e increases rate of genetic drift and inbreeding which can result in temporal changes in allelic frequency that are larger than would have occurred in a population with a larger N_e .

Estimate of N_b via linkage disequilibrium method- The effective number of breeders (N_b) that produced hatchery and wild fish samples was estimated via the linkage disequilibrium approach of Hill (1981) using the bias (due to small sample size and/or N_e) correction developed by Waples (2006a). We used a software package provided by Waples and Do (*unpublished*) to calculate the ‘corrected’ mean squared correlation of allele frequencies at pairs of loci (r^2) and the expected contribution to r^2 from sampling a finite number of individuals ($E(r^2_{sample})$) via the composite Burrows method (Weir 1996). For each sample of hatchery and wild fish, r^2 was calculated, excluding alleles with frequencies < 0.02 , as the weighted average r^2 over 55 pair-wise locus comparisons ($J=L(L-1)/2$, where $L = \#$ loci). Confidence intervals (CI; $\alpha=0.05$) for uncorrected r^2 were calculated using the following equation from Waples (2006a):

$$1-\alpha \text{ CI for } r^2 = \left[\frac{J(r^2)}{X^2_{\alpha/2[J]}}, \frac{J(r^2)}{X^2_{1-\alpha/2[J]}} \right],$$

where $X^2_{\alpha/2[J]}$ and $X^2_{1-\alpha/2[J]}$ are the $\alpha/2$ and $1-\alpha/2$ points of the χ^2 distribution (77.380 and 36.398, respectively) with $J (J=L(L-1)/2)$ degrees of freedom. Corresponding 95% CIs

$$r^{2'} = r^2 - E(r^2_{\text{sample}})$$

for $r^{2'}$ were calculated using the r^2 CIs and the sample specific $E(r^2_{\text{sample}})$ value calculated via software package provided by Waples and Do (*unpublished*) according to the expression:

In turn, the originally calculated $r^{2'}$ and its 95% CIs were used to obtain the point estimate and 95% CIs, respectively for the estimated N_b that produced each sample of fish according to the expression developed by Waples (2006a; Table 2, random mating model):

$$\text{Estimated } N_b = \frac{1/3 + (1/9 - 2.76r^{2'})^{1/2}}{2r^{2'}}$$

Estimate of N_b via temporal method - The computer program *SalmonNb* (Waples et al. 2006) was used to estimate N_b in individual years for hatchery and naturally spawning fish. The program uses three or more temporal samples (in this case, cohorts broken down by age) to generate pair-wise comparisons of allele frequencies to estimate N_e in any given year, which in turn, provides a basis for estimating N_b in individual years (Waples 2006a). Effective population size over each generation was calculated as the harmonic mean of the pair-wise N_{bi} estimates multiplied by the estimated generation interval (g). Alleles with frequency < 0.02 were excluded to reduce potential bias in estimating N_b . Assumptions of the program include: alleles are selectively neutral and assort independently, populations are closed to immigration, and fecundity is equal regardless of age.

Results

Hatchery fish were grouped according to age only since too few could be grouped based on carcass recovery location to perform statistically relevant analyses. Greater percentages of heterozygous genotypes compared to HWE proportions were observed in successively younger hatchery cohorts that returned in 2004. Over all 11 loci, age four hatchery fish had about 1.1% fewer heterozygotes than expected under HW equilibrium conditions ($F_{IS} = 0.011$, $p < 0.001$). Age two and three hatchery fish had about 2.5% and 1.8% more heterozygotes than expected under HW equilibrium conditions ($F_{IS} = -0.025$ and -0.018 , $p < 0.001$, respectively). Out of 55 pair-wise comparisons of loci, most (43-53) exhibited significant ($\alpha = 0.0045$ after Bonferroni correction) LD in age 2, 3, and 4 hatchery fish.

Population genetic statistics were calculated for subsets of wild fish recovered on the Chiwawa R. ($N=106$) and Nason Crk. ($N=85$), and the White R. ($N=11$) in 2004. The multilocus F_{IS} values for the Chiwawa R. and Nason Crk. ($F_{IS} = 0.012$ and $F_{IS} = 0.020$, respectively) wild spring Chinook were both significantly different ($p=0.002$) from HW

expectations. Only 11 adults returned to spawn in the White R. in 2004, so the number of fish sampled from this tributary was augmented with 60 juveniles collected in a rotary screw trap during 2005. The White R. spring Chinook population, which included adults and juveniles collected during 2004 and 2005, respectively, did not deviate from HW expectations ($F_{IS} = 0.020$, $p=0.36$). In contrast to hatchery fish, the wild samples had far fewer (1-2) pair-wise comparisons of loci in LD. Most (96%) of the 876 wild spring Chinook that returned in 2004 were 4 year-old fish. Accordingly, statistically relevant analyses based on the age of wild fish could not be performed. Similar to the 2004 wild fish, the 1994 Chiwawa R. sample showed no significant ($\alpha=0.0045$ after Bonferroni correction) deviations from HW equilibrium at eight microsatellite loci and no pair-wise LD (data not shown).

Analyses of population subdivision using F-statistics indicated almost all pair-wise F_{ST} comparisons were significant ($\alpha=0.05$) except the comparison between the 1994 wild juvenile and 2004 wild adult Chiwawa R. spring-run populations (Table 2). Both the LNFH spring and the Wenatchee R. summer-run Chinook were well differentiated from the Wenatchee R. hatchery and wild spring-run fish (Table 2).

Cluster analysis was performed using all 2004 and 2005 Wenatchee R. spring fish (Figure 2). Strong bootstrap support (99%) was obtained for the node separating LNFH and Wenatchee R. spring Chinook. Similarly, hatchery fish produced in 2001 and 2002 clustered together with high bootstrap support (98%) (Figure 2). Cluster analysis indicated geographic structure among wild spring Chinook populations. Temporal samples of fish sampled on Nason Crk. and the White R. clustered with moderate bootstrap support (84% and 64%, respectively) (Figure 2). In addition, the White R. population clustered separately from all other spring populations.

Demographic estimates of N_b and N_e – The estimated number of naturally spawning hatchery and wild-origin fish varied greatly from 1989 to 2005 (Figure 3). Over the periods 1989-92, 1993-96, 1997-2000, and 2001-04, number of hatchery breeders ranged from 11-371 and the estimated number of naturally spawning fish ranged from 58-4130 (Table 3).

Influence of SR on decreasing N_b/N – Unequal sex ratio accounted for N_b/N ratios of 0.93 and 0.96 in Chiwawa R. hatchery and naturally spawning spring Chinook salmon between 1993 and 2005 (Table 3). In general, N_b/N ratios were comparable in magnitude between hatchery and wild fish.

Influence of VRS on decreasing N_b/N – Using values for the mean and variance of progeny numbers produced by Yakima R. hatchery and wild spring Chinook (Schroder et al. 2005), we estimated the effective number of female and male breeders in Chiwawa R. hatchery and naturally spawning spring Chinook for each year from 1993-2005 (Table 3). Both hatchery and naturally spawning fish have higher average N_{bf} (14.8 and 114.1, respectively) than N_{bm} (10.3 and 124.2, respectively), and the wild population had a higher average effective number of breeders ($N_{b(Demo)} = 237.9$) compared to the hatchery population ($N_{b(Demo)} = 24.3$) in each year. Given the assumptions of the analyses (values

of k and σ_k^2 reported by Schroder et al. (2005)) variation in reproductive success in the hatchery and naturally spawning populations produced similar average N_b/N ratios (0.56 and 0.60, respectively).

Influence of FPS on N_e – By assuming that $N_b/N = 1$ (i.e. equal sex ratio and binomial variance in reproductive success among individuals) in each year the effect of fluctuating population size (FPS) on N_e could be isolated. The estimated generation intervals (g) for Wenatchee R. hatchery and naturally spawning spring Chinook were 3.5 and 4.0 yr., respectively. A four year generation interval was assumed for both hatchery and wild fish so that comparisons between the two could be more easily interpreted. Similar results were obtained whether a 3 or 4 yr. interval was used to calculate N_e based on FPS. From 1989 to 2000, FPS had the greatest influence on reducing N_e/N_T ratios for hatchery and naturally spawning fish (Table 4; 0.39 and 0.52, respectively). Hatchery fish (in 1889 and 1991 combined) and naturally spawning fish in 1996 comprised ~15% and 10% of the spawners, respectively, but produced 87% and 40% of subsequent recruits, respectively (Table 3). These results indicate that neither hatchery, nor natural spawners contributed to subsequent generations in direct proportion to their abundance within each generation.

Impact of supportive breeding on decreasing inbreeding effective population size - Compared to estimates of N_e for the naturally spawning population segment, supplementation decreased overall (hatchery and natural) N_e from 1989 to 2000 (Tables 3 and 4). The decrease occurred whether or not wild-origin fish were included in the supportive breeding program (Table 4). If wild-origin fish had not been included in the program, N_e would have been decreased further, and N_e for the naturally spawning population segment would have increased only slightly (data not shown). Had supplementation not been implemented, however, N_e of the natural population segment would have been larger during the first and third generations (Table 4).

Genetic estimates of contemporary N_b and N_e – Direct comparisons of demographic and genetic estimates of N_e were not possible since the latter were made using contemporary (2004-5) samples while demographic estimates spanned 1989-2000. Estimates of $N_{b(i)}$ based on LD for hatchery fish were, in general, lower than the corresponding $N_{b(i)}$ estimates using the temporal method (Table 5). Estimates of $N_{b(i)}$ could only be made for wild fish produced in 2000 and 2001 since very few age 3 and no age 2 fish were present in the wild samples. For both years $N_{b(i)}$ estimates using LD were approximately double the magnitude of those obtained via the temporal method for wild fish.

Discussion

Initial results indicate that despite over a decade of supportive breeding and a demographic bottleneck, some genetic substructure among local wild spring-run Chinook salmon populations exists within upper Wenatchee R. tributaries (Table 2 and Figure 2). In particular, the White R. population is genetically distinct from other wild populations within the Wenatchee R. Basin. An earlier population genetic study of Wenatchee R.

spring-run Chinook salmon provided similar results, suggesting that patterns of variation among the spring Chinook spawning in the different Wenatchee R. tributaries have not changed notably since the start of supplementation. In particular, Utter et al. (1995) examined allele frequency variation at 32 allozyme loci and found that White R. spring Chinook were genetically differentiated from Nason Crk. and Chiwawa R. populations and a low, but statistically significant level of genetic differentiation exists between Nason Crk. and Chiwawa R. populations. Although there is ample opportunity for gene flow from the hatchery to local wild populations to occur, it appears that it has not been enough to genetically homogenize the latter.

Temporal genetic variation was greater in the hatchery than in the wild population segment. Low, but statistically significant allele frequency differences exist between hatchery fish grouped by age and, in general, F_{ST} 's between different age hatchery fish were higher than those for wild populations sampled in different years (Table 2 and Figure 2). The greater temporal allele frequency differences in the hatchery population directly lead to lower estimates of N_b in the hatchery than in the wild (Table 5). The LD based estimates of N_b support this as well (Table 5). It is plausible that the fluctuating number of breeders used for supplementation has promoted an 'artificial signal' of temporal genetic diversity leading to lower estimates of N_b . Using sex ratio to calculate N_b , considered as a maximum estimate of N_b , a value > 50 was observed in only three separate years during the first three generations of supportive breeding (Table 3). The patterns of departure from HW equilibrium and high linkage disequilibrium are consistent with what would be expected in a hatchery population with a low N_e . Waples and Smouse (1990) and Waples and Teel (1990) observed similar patterns of substantial LD and allele frequency differences, respectively, in hatchery (but not wild) Chinook salmon stocks. The most likely explanation for both patterns, according to the authors of these previous studies, was low ($N_b < 50$) hatchery N_b . The extensive LD exhibited by the 2004 Chiwawa R. hatchery fish is due, in part, to small numbers of breeders during the 1990s, episodic disproportional contributions to future generations by relatively few breeders (Table 3), and insufficient generations having passed in the absence of any counteracting forces to permit loci to reach linkage equilibrium.

Other explanations for the departures from HW and linkage equilibrium include null alleles, non-random survival among families, and admixture of genetically divergent populations. The presence of null alleles in a population would lead to a higher frequency of homozygotes than expected under Hardy-Weinberg equilibrium. Since only one hatchery population (year 2000) out of four had a significantly elevated global F_{IS} value, the presence of null alleles in the hatchery population leading to such departures is unlikely. However, non-random survival among hatchery families may account for the extensive linkage disequilibrium observed in the hatchery population as a whole. If individuals in a population tend to be more related to one another than by chance alone, non-random associations of alleles between loci are more likely as well.

Population admixture of genetically divergent hatchery and wild fish would become manifest as levels of homozygosity above Hardy-Weinberg expectations. The Chiwawa R. hatchery supplementation program included, on average, 33% wild origin fish. The

basis of including local, wild fish is to prevent genetic divergence of hatchery and wild stocks (Lynch and O'Hely 2001; Ford 2002), however, the results of such practices may be unpredictable (Waples 1991). If hatchery fish began to diverge genetically from the donor wild stock due to selective pressures during the early phase of the supplementation program, the subsequent mixing of wild populations in the upper Wenatchee River system with high numbers of unmarked hatchery origin fish (Murdoch et al. 2006) may account for the observed higher than expected homozygosity in each of the wild populations.

When considering each of the demographic variables independently, FPS (Table 4), followed by VRS then SR (Table 3), had the biggest influence on reducing N_b/N ratios for hatchery and naturally spawning fish. Compared to an earlier study of Steelhead trout (*O. mykiss*) by Ardren and Kapuscinski (2003), SR also caused only a minor decrease in N_b/N , however, VRS rather than FPS appeared to be the dominant influence on decreasing N_e in steelhead.

Supportive breeding of Wenatchee R. spring Chinook salmon may have increased the population census size, but it has decreased the overall effective size (Tables 3 and 4). Supportive breeding over multiple generations results in a change of demographic parameters that have opposing effects on N_e (Wang and Ryman 2001). Increasing population size tends to increase variance effective population size. Variance effective population size (N_{eV}) is defined as the size of a hypothetical, "ideal" population that undergoes the same rate of genetic drift as a real (non-ideal) population. In contrast, effective size tends to be decreased by the differential contribution of offspring to the next generation by hatchery and wild parents (k_h vs. k_w) resulting in a larger overall variance for the population relative to what it would have been without supportive breeding. Waples and Do (1994) modeled change in identity by descent for various durations of supportive breeding and for a crash in population size after supplementation was initiated. Their results indicated that the effect of supplementation on N_e can be even more pronounced if individuals from only part of a generation are taken as breeders and that under the crash scenario individual production years can have an overwhelming effect on the future genetic make up of a population. In 1995 and 1999 no supportive breeding was carried out and the population crashed during the second generation of supplementation. The fluctuations in population sizes of hatchery and naturally spawning fish (Table 3 and Figure 3), and episodic differential contribution of offspring (observed as recruitment) between hatchery and naturally spawning fish (Table 3) and inconsistent sampling across generations have contributed to decreased overall N_e since the supportive breeding program was established. While this study did not examine N_{eV} , it too has likely been decreased since there have been large differences between the reproductive rates of the hatchery and wild populations (Ryman et al. 1995).

Skewed distributions in family size within a population will also influence N_e . For instance, if only a few families in a population produce the majority of offspring, N_e of that population will be small. Hatchery adults that returned in 2004 may represent fewer families overall compared to their wild counterparts. Hence, family structure differences between hatchery and wild fish may also account for their differing patterns of genetic

diversity. A possible driving force behind skewed distributions in family size may be non-random differences in survival to adulthood among families in the hatchery population (Waples 2002b) compared to their wild counterparts. Small, fluctuating breeder numbers and possible non-random variance in survival to adulthood may result in continued future reduction of N_e and an elevated rate of genetic diversity loss in Wenatchee R. hatchery spring Chinook.

It is not known why LD estimates of $N_{b(i)}$ for naturally spawning fish are approximately twice the magnitude of those obtained by the temporal method (Table 5). Sample sizes (S), defined as the harmonic mean of the number of individuals genotyped for each locus used, of hatchery and naturally produced fish (Table 5) are large enough so that sampling error is likely not an important contributor to r^2 , which would have biased LD-based N_b estimates downward. Furthermore, genotyping error rates, a source of potential bias for measures of LD (Akey et al. 2001), were low at 1.23% over all 11 loci (data not shown). One possible explanation for the relatively lower temporal estimates of $N_{b(i)}$ for 2000 and 2001 may be that they are the product of naturally spawning hatchery- and wild-origin parents. The naturally spawning hatchery-origin parents would have been predominately produced by low, variable numbers of breeders in 1997 and 1998 (Table 3; 111 and 47, respectively). A possible ‘echo’ of elevated allele frequency variance between hatchery years may have contributed to higher allele frequency variance between subsequent years of naturally spawning fish, especially since the majority of fish returning to the spawning grounds in 2000 and 2001 were of hatchery-origin, thereby contributing to a lower temporal estimate of $N_{b(i)}$. Araki et al. (in press) observed a similar scenario in a study of N_e of Hood River, WA steelhead trout. Demographic data and microsatellite parentage assignments were used to estimate N_b and N_e of two steelhead populations. The parents were a mix of local and nonnative hatchery fish that had poor reproductive success. Alleles from non-native fish affected the first parental sample and hence the temporal N_b estimate (depressed downwards because of an additional difference in frequency between parents and offspring). However, the non-native breeders produced few progeny so had little effect on the LD estimate.

In contrast to the observed difference between LD- and temporal-based estimates of $N_{b(i)}$ for naturally spawning fish, LD-based estimates of $N_{b(i)}$ for hatchery fish were typically lower than temporal-based estimates. When population size varies, r^2 will be affected by N_e in generations prior to the one sampled (Waples 2006a). In other words, despite a recently increased population size, estimates of N_b can have a downward bias in subsequent generations. Hatchery fish have increased from very low numbers during the 1990s (Figure 3). It is possible that LD-based estimates of contemporary N_b for hatchery fish (Table 5) are biased downward relative to temporal-based estimates due to the demographic bottleneck experienced in the 1990s.

The relative reproductive fitness of hatchery fish and their progeny that spawn naturally plays a role in determining if supplementation influences genetic diversity and N_e of local wild populations. For instance, if hatchery-origin fish were unsuccessful at reproducing in the wild, they would have no effect on homogenizing genetic structure or influence N_e . One of the conclusions from several studies of the fitness of hatchery fish in the natural

environment is that genetic-based fitness differences have been found after only two-to-five generations of hatchery rearing (Berejikian and Ford 2004). A DNA-based pedigree approach to empirically monitor relative reproductive fitness of Wenatchee R. hatchery- and natural-origin spring Chinook in both the hatchery and natural environment would provide vital information regarding the performance of supportive breeding to augment, or its ability to mask possible declines in, local natural populations. Measuring how quickly hatchery stocks can readapt to a full life-cycle in a natural environment will help to define the feasibility and/or utility of supportive breeding in recovery efforts and permit more accurate assessment of the viability of populations receiving large numbers of individuals produced by supplementation. Monitoring reproductive fitness over multiple generations may provide information that permits more refined selection of breeders for a supplementation program. If the progeny of naturally spawning hatchery fish have relatively higher fitness in the natural environment compared to their parents, pedigree analysis could be used to identify putative breeders that are more suitable, on the basis of having a lower potential to contribute to the next generation if left to spawn naturally, for inclusion in a supplementation program. In turn, descendants of naturally spawning hatchery fish that have a greater reproductive advantage in the natural environment would be allowed to spawn naturally. In short, pedigree-based analysis of reproductive success could be used to target supplementation towards the population segment that has the least likelihood of contributing to the next generation and may provide a better understanding of when supportive breeding could be discontinued, thereby limiting any potentially negative genetic influence of supplementation on local wild populations.

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References

- Akey, J.M., K. Zhang, M. Xiong, P. Doris, and L. Jin. 2001. The effect that genotyping errors have on the robustness of common linkage disequilibrium measures. *American Journal of Human Genetics* 68: 1447-1456.
- Allendorf, F.W., and R.F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* 2: 170-184.
- Araki, H., W. R. Ardren, E. Olsen, B. Cooper, and M.S. Blouin. Reproductive Success of Captive-Bred Steelhead Trout in the Wild: Evaluation of Three Hatchery Programs in the Hood River. *Conservation Biology* *in press*. DOI: 10.1111/j.1523-1739.2006.00564.x

- Araki, H., R.S. Waples, W. R. Ardren, B. Cooper, and M. Blouin. Effective population size of steelhead trout: influence of variance in reproductive success, hatchery programmes, and genetic compensation between life-history forms. *Molecular Ecology in press* DOI: 10.1111/j.1365-294.2006.02206.x
- Ardren, W.R., and A. R. Kapuscinski. 2003. Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. *Molecular Ecology* 12: 35-49.
- Banks, MA, MS, Blouin, BA Baldwin, VK Rashbrook, HA Fitzgerald, SM Blankenship, and D Hedgecock. 1999. Isolation and inheritance of Novel Microsatellite Loci in Chinook Salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity* 90 (2): 281-288. Errata in *Journal of Heredity* 90 (3): U1.
- Belkhir, K., Borsa P., Chikhi L., Raufaste N. & Bonhomme F. 1996-2004. GENETIX, program for Windows™ for the genetic analysis of populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, University of Montpellier II, Montpellier (France). Available at <http://www.genetix.univ-montp2.fr/~genetix/genetix.htm>
- Berejikian, B.A. and M.J. Ford. 2004. Review of relative fitness of hatchery and natural salmon. U.S. Department of Commerce, NOAA Technical Memo. NMFS-NWFSC-61, 28 p., distributed by Northwest Fisheries Science Center and available at <http://www.nwfsc.noaa.gov>.
- Brannon, E., D.F. Amend, M.A. Cronin, J.E. Lannan, S. LaPatra, W.J. McNeil, R.E. Noble, C.E. Smith, A.J. Talbot, G.A. Wedemeyer, and H. Wester. 2004. The controversy about salmon hatcheries. *Fisheries* 29: 12-31.
- Buchholz, W.G., S.J. Miller, W.J. Spearman. 1999. Isolation and characterization of chum salmon microsatellites loci and use across species. *Animal Genetics* 32 (3): 162-165.
- Busack, C.A., and K.P. Currens. 1995. Genetic risks and hazards in hatchery operations: Fundamental concepts and issues. *American Fisheries Society Symposia* 15: 71-80.
- Cairney, M., J.B. Taggart, and B. Hoyheim. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. *Molecular Ecology* 9: 2175-2178.
- Carson, H.L. and A.R. Templeton. 1984. Genetic revolutions in relation to speciation phenomenon: the founding of new populations. *Annual Reviews in Ecology and Systematics* 15: 97-151.

- Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 32:550-570.
- Chapman, D.W., C. Peven, A. Giorgi, T. Hillman, F. Utter. 1995. Status of spring Chinook salmon in the mid-Columbia region. Report to Chelan, Douglas, and Grant County Public Utility Districts, Washington. Don Chapman Consultants, Inc., Boise, Idaho. Available from Chelan PUD at [http://www.chelanpud.org/rr_relicense/existing/hcp/Studies/E4\(3\)-95.pdf](http://www.chelanpud.org/rr_relicense/existing/hcp/Studies/E4(3)-95.pdf)
- Crow, J.F., and C. Denniston. 1988. Inbreeding and variance effective population number. *Evolution* 42: 482-495.
- Crow, J.F., and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, New York.
- Docker, M.F., A. Dale, D.D. Heath. 2003. Erosion of interspecific reproductive barriers resulting from hatchery supplementation of rainbow trout sympatric with cutthroat trout. *Molecular Ecology* 12: 3515-3521.
- Duchesne, P. and L. Bernatchez. 2002. An analytical investigation of the dynamics of inbreeding in multi-generation supportive breeding. *Conservation Genetics* 3: 47-60.
- Felsenstein, J. 2002. PHYLIP (Phylogeny Inference Package) version 3.6 α 3. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle. Available at <http://evolution.genetics.washington.edu/phylip.html>
- Fisher, R.A. 1922. On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society* 85: 87-94.
- Ford, Michael J. 2002. Selection in Captivity During Supportive Breeding May Reduce Fitness in the Wild. *Conservation Biology* 16: 515-525.
- Ford, Michael, P. Budy, C. Busack, D. Chapman, T. Cooney, T. Fisher, J. Geiselman, T. Hillman, J. Lukas, C. Peven, C. Toole, E. Weber, P. Wilson. 2001. Upper Columbia River Steelhead and Spring Chinook Salmon Population Structure and Biological Requirements. Final report distributed by Northwest Fisheries Science Center and available at <http://www.nwfsc.noaa.gov>.
- Ford, M.J., T.A. Lundrigan, and P.C. Moran. 2004. Population Genetics of Entiat River spring Chinook salmon. U.S. Dept. of Commerce, NOAA Technical Memo. NMFS-NWFSC-60, 45 p. Distributed by Northwest Fisheries Science Center and available at <http://www.nwfsc.noaa.gov>.

- Ford, M.J., H. Fuss, B. Boelts, E. LaHood, J. Hard, and Jason Miller. 2006. Changes in run timing and natural smolt production in a naturally spawning coho salmon (*Oncorhynchus kisutch*) population after 60 years of intensive hatchery supplementation. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 2343-2355.
- Greig, C., Davis P. Jacobson, Michael A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered chinook salmon (*Oncorhynchus tshawytscha*). *Molecular Ecology Notes* 3: 376-79.
- Greig, C., and M.A. Banks. 1999. Five multiplexed microsatellite loci for rapid response run identification of California's endangered winter Chinook salmon. *Animal Genetics* 30: 319-320.
- Guo, S. W., and E.A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:361-362.
- Hankin, D.G., J.W. Nicholas, and T.J. Downey. 1993. Evidence for inheritance of age of maturity in Chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 50: 347-358.
- Hanson, L.P., and B. Jonsson. 1991. Evidence of a genetic component in the seasonal return pattern of Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology* 38: 251-258.
- Healey, M.C. 1991. Life history of Chinook salmon (*Oncorhynchus tshawytscha*). Pages 311-394 in C. Groot and L. Margolis, editors. *Pacific salmon life histories*. University of British Columbia Press, Vancouver, B.C.
- Hill W.G. 1981. Estimation of effective population size from data on linkage disequilibrium. *Genetical Research* 38, 209-216.
- Hymer, Joe, Pettit, Rich Washington Department of Fisheries, Mike Wastel, Peter Hahn Washington Dept. of Wildlife, and Keith Hatch Columbia River Inter-Tribal Fish Commission. 1992. Stock summary reports for Columbia River anadromous salmonids. Volume IV: Washington sub-basins below McNary Dam. U.S. Dept. of Energy, Bonneville Power Administration, Division of Fish and Wildlife. Project No. 88-108, Contract No. DE-FC79-89BP4402, Pp. 366. (BPA Report DOE/BP-94402-4). Available at <http://www.efw.bpa.gov/publications/I94402-4.pdf>
- ISAB (Independent Science Advisory Board). 2002. Hatchery surpluses in the Pacific Northwest. *Fisheries* 27:16-27.

- Kalinowski, S.T. 2003. Genetic Mixture Analysis 1.0. Department of Ecology, Montana State University, Bozeman MT 59717. Available for download from <http://www.montana.edu/kalinowski>
- Krueger, C.C., and B. May. 1991. Ecological and Genetic Effects of Salmonid Introductions in North America. *Canadian Journal of Fisheries and Aquatic Sciences* 48 (supplement 1): 66-77.
- Lacy, R. C. 1988. A Report on Population Genetics in Conservation. *Conservation Biology* 2 (3): 245-247.
- Lande, R. 1988. Genetics and Demography in Biological Conservation. *Science* (Washington D C) 241 (4872): 1455-1460.
- Lande, R. and G.F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. In: *Viable Populations for Conservation* (Ed. Soule M.E.), pp. 87-123. Cambridge University Press, New York.
- Lynch, M., and M. O'Hely 2001. Supplementation and the genetic fitness of natural populations. *Conservation Genetics* 2: 363-378.
- McIsaac, D.O., T.P. Quinn. 1988. Evidence for a hereditary component in homing behavior of Chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*. 45: 2201-2205.
- Meyers, J. M., R. G. Kope, G. J. Bryant, D. Teel, L. J. Lierheimer, T. C. Wainwright, W. S. Grant, F. W. Waknitz, K. Neely, S. T. Lindley, and R. S. Waples. 1998. Status Review of Chinook salmon from Washington, Idaho, Oregon, and California. NOAA Technical Memorandum NMFS-Northwest Fisheries Science Center-35, 480 p., Distributed by Northwest Fisheries Science Center and available at <http://www.nwfsc.noaa.gov>.
- Mosey, T.R. and L.J. Murphy. 2002. Spring and Summer Chinook spawning ground surveys on the Wenatchee River basin, 2001. 35 pages, with appendices. Chelan County Public Utility District, Fish and Wild life Operations, Wenatchee, WA.
- Murdoch, A.R., T.N. Pearsons, T.W. Maitland, K.S. Williamson, and M.J. Ford. 2006. Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. Report to Bonneville Power Administration, Project No. 2003-039-00, pp. 97.
- Naish, K.A. and L.K., Park. 2002. Linkage relationships for 35 new microsatellite loci in Chinook salmon *Oncorhynchus tshawytscha*. *Animal Genetics* 33 (4): 316-318.

- Nelson, R.J. and T.D. Beacham. 1999. Isolation and cross species amplification of microsatellite loci useful for study of Pacific salmon. *Animal Genetics* 30: 228-229.
- NMFS (National Marine Fisheries Service). 2005. Endangered and threatened species: final listing determinations for 16 ESUs of West Coast salmon and final 4(d) protective regulations for threatened salmonid ESUs. Final Rule. Federal Register, Vol. 70, pg 37160, June 28, 2005.
- Olsen, J.B., P. Bentzen, and J.E. Seeb. 1998. Characterization of seven microsatellite loci derived from Pink salmon. *Molecular Ecology* 7: 1087-1089.
- Quinn, T.P. 1993. A review of homing and straying of hatchery and wild-produced salmon. *Fisheries Research* 18:29-44.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248-249.
- Rice, William, R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- Ricker, W.E. 1972. Heredity and environmental factors affecting certain salmonid populations. Edited by R.C. Simons and P.A. Larkin H.R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C. pp. 19-160.
- RISPA (Rock Island Project Settlement Agreement). 1989. Rock Island Project Settlement Agreement. Federal Energy Regulatory Commission and Chelan County Public Utility District Project No. 943, Docket No. E-9569, Wenatchee, WA. Available at http://www.chelanpud.org/rr_relicense/existing/hcp/RI/RI_HCP.PDF
- Ryman, N. 1994. Supportive Breeding and Effective Population Size: Differences Between Inbreeding and Variance Effective Numbers. *Conservation Biology* 8: 888-890.
- Ryman, N., P.E. Jorde, and L. Laikre. 1995. Supportive Breeding and Variance Effective Population Size. *Conservation Biology* 9: 1619-1628.
- Ryman, N., and L. Laikre. 1991. Effects of Supportive Breeding on the Genetically Effective Population Size. *Conservation Biology* 5: 325-329.
- Schroder, S., C. Knudsen, T. Pearsons, S. Young, T. Kassler, D. Fast, and B. Watson. 2005. "Comparing the Reproductive Success of Yakima River Hatchery- and Wild-Origin Spring Chinook; Yakima/Klickitat Fisheries Project Monitoring and Evaluation", 2004-2005 Annual Report, Project No. 199506325, 40 electronic pages, (Bonneville Power Administration report DOE/BP-00017478-6). Available from <http://www.efw.bpa.gov/Publications/P00017478-6.pdf>

- Schwenke, Piper L., J.G. Rhydderch; M. J. Ford; A R. Marshall and L. K. Park. 2006. Forensic identification of endangered Chinook Salmon (*Oncorhynchus tshawytscha*) using a multilocus SNP assay. *Conservation Genetics* 7: 983-989.
- Sharma, R., G. Morishima, S.Z. Wang, A. Talbot, and L. Gilbertson. 2006. An evaluation of the Clearwater River supplementation program in western Washington. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 423-437.
- Sitonen, L., and G.A.E. Gall. 1989. Response to selection for early spawning date in rainbow trout (*Salmo gairdneri*). *Aquaculture* 35: 132-137.
- Smith, B.R., Herbinger, C.M. and Merry, H.R. 2001. Accurate partition of individuals into full sib families from genetic data without parental information. *Genetics*, 158: 1329-1338.
- Soule, M.E. (Ed.) 1987. *Viable Populations for Conservation*. Cambridge University Press, Cambridge, England. 189 p.
- Taylor, E.B. 1991. A review of local adaptation in Salmonidae with particular reference to Pacific and Atlantic salmon. *Aquaculture* 98: 185-207.
- Utter, F. M. 1998. Genetic problems of hatchery-reared progeny released into the wild, and how to deal with them. *Bulletin of Marine Science* 62:623-640.
- Utter, F. M. 2004. Population genetics, conservation and evolution in salmonids, and other widely cultured fishes: some perspectives over six decades. *Reviews in Fish Biology and Fisheries* 14: 125-144.
- Utter, F.M., D.W. Chapman, and A.R. Marshall. 1995. Genetic Population Structure and history of Chinook salmon of the Upper Columbia River. *American Fisheries Society Symposium* 17: 149-68.
- Wang, J., and N. Ryman. 2001. Genetic Effects of Multiple Generations of Supportive Breeding. *Conservation Biology* 15: 1619-1631.
- Waples R.S. 1990. Conservation genetics of Pacific salmon II. Effective population size and the rate of loss of genetic variability. *Journal of Heredity* 81:267-276.
- Waples, R. S. 1991. Genetic interactions between hatchery and wild salmonids: Lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 48 (Supplement 1): 124-133.
- Waples, R.S. 2002a. Effective Size of Fluctuating Salmon Populations. *Genetics* 161: 783-791.

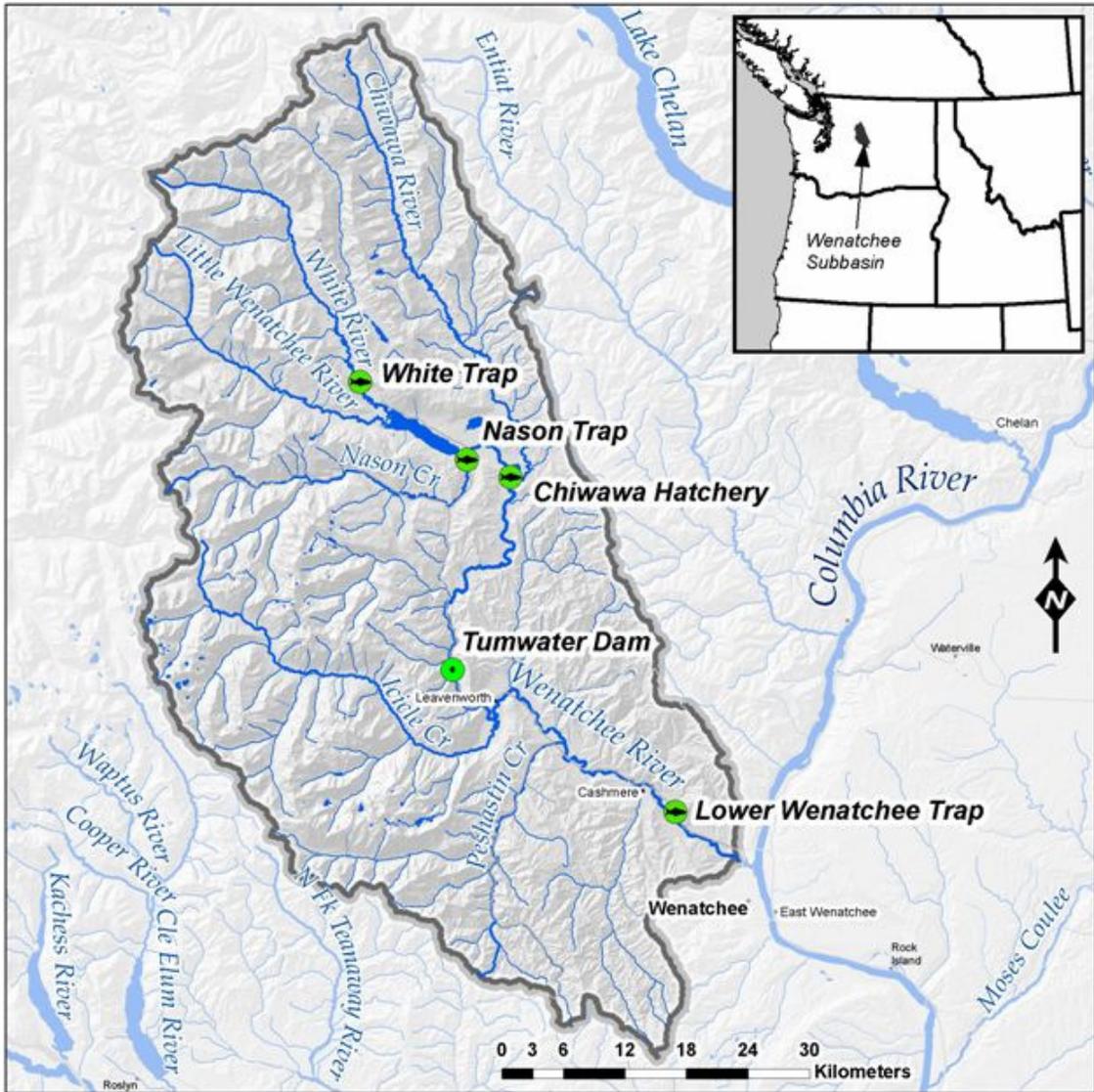
- Waples, R.S. 2002b. Evaluating the effect of stage-specific survivorship on the N_e/N ratio. *Molecular Ecology* 11:1029-1037.
- Waples, R.S. 2006a. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics* 7: 167-184.
- Waples, R.S. 2006b. Seed Banks, Salmon, and Sleeping Genes: Effective Population Size in Semelparous, Age-Structured Species with Fluctuating Abundance. *The American Naturalist* 167: 118-135.
- Waples, R.S., and C. Do. 1994. Genetic Risks Associated with Supplementation of Pacific Salmonids: Captive Brood stock Programs. *Canadian Journal of Fisheries and Aquatic Sciences* 51 (supplement 1): 310-329.
- Waples, R.S., and J. Drake. 2004. Risk-benefit considerations for marine stock enhancement: a Pacific salmon perspective. Pages 260-306 in *Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities*. Blackwell, Oxford.
- Waples, R. S., and J. Drake. 2004. Risk-benefit considerations for marine stock enhancement: a Pacific salmon perspective. pp. 260-306 in K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svåsand, eds. *Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities*. Second Edition, Blackwell, Oxford.
- Waples, R.S., and P.E. Smouse. 1990. Gametic disequilibrium analysis as a means of identifying mixtures of salmon populations. *American Fisheries Society Symposia* 7: 439-458.
- Waples, R.S., and D. Teel. 1990. Conservation genetics of Pacific salmon. I. Temporal changes in allele frequency. *Conservation Biology* 4: 144-156.
- Waples, R.S., D.J. Teel, J.M. Meyers, and A.R. Marshall. 2004. Life-history divergence of Chinook salmon: Historic contingency and parallel evolution. *Evolution* 58: 386-403.
- Waples, R.S., M. Masuda, and J. Pella. 2006. *SalmonNb*: A program for computing cohort-specific effective population sizes (N_b) in Pacific salmon and other semelparous species using the temporal method. *Molecular Ecology Notes (in press)*.
- Weir, B. S. 1996. *Genetic Data Analysis II*. Sinaur Associates Inc., Sunderland, MA.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*. 38:1358-1370.
- Wright, S. 1969. *Evolution and genetics of populations II. The theory of gene frequencies*. University of Chicago Press, Chicago.

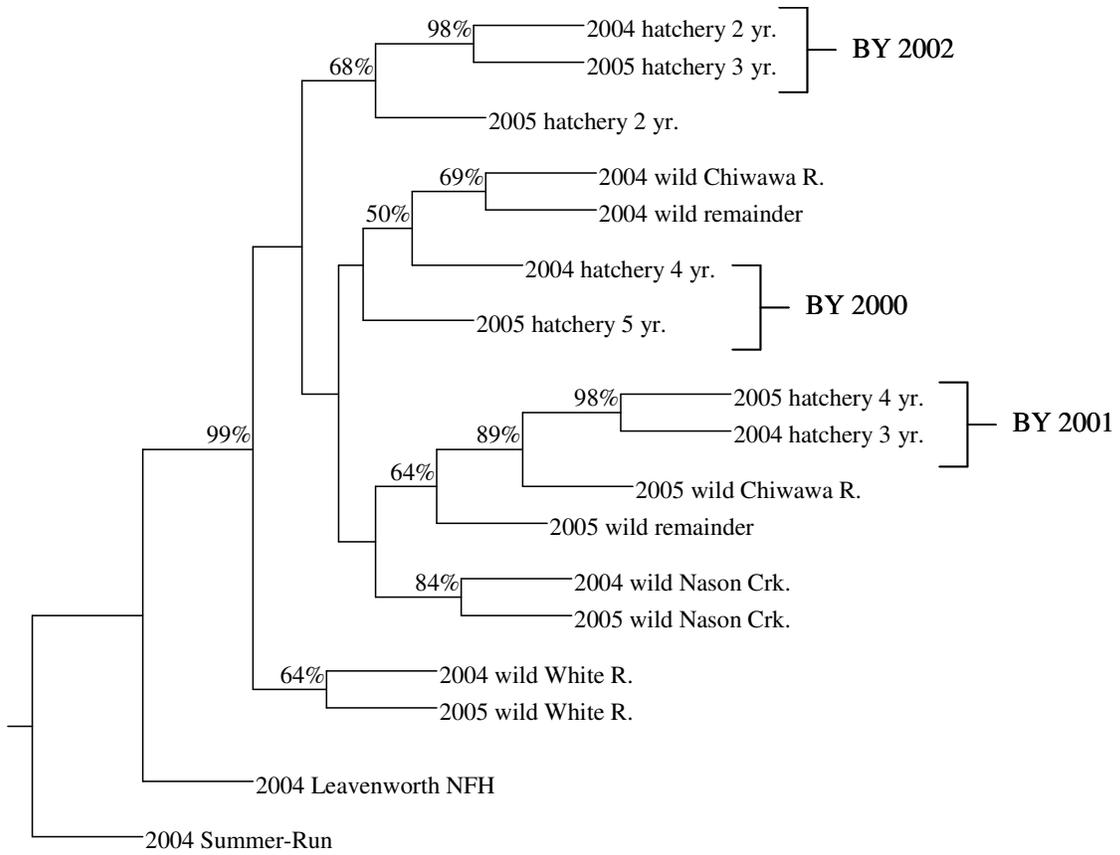
Figure Legends

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

Figure 2: Neighbor-joining phenogram based on Cavalli-Sforza (1967) cord distance units among the 2004 and 2005 Wenatchee River spring-run, and rooted using the 2004 summer-run Chinook salmon as an out group. The phenogram was constructed using data from 11 microsatellite loci with PHYLIP v. 3.6b (Felsenstein 1989). For 1000 bootstrap replicates, node values $\geq 50\%$ are shown. Individuals were grouped as 2, 3, 4, or 5 year old hatchery (CWT, adipose fin-clip, or scale pattern), or wild origin (according to carcass recovery location). Brood years (BY) of Chiwawa R. hatchery fish are indicated. Fish identified as summer-run were removed from each group prior to constructing the phenogram.

Figure 3 - Total number of naturally spawning hatchery (solid squares) and wild (open diamonds) spring Chinook salmon within Wenatchee R. drainage from 1989 to 2005 based on carcass recoveries from all tributaries above Tumwater Dam. The Chiwawa R. hatchery program was started in 1989 and the first return of hatchery fish occurred in 1993.





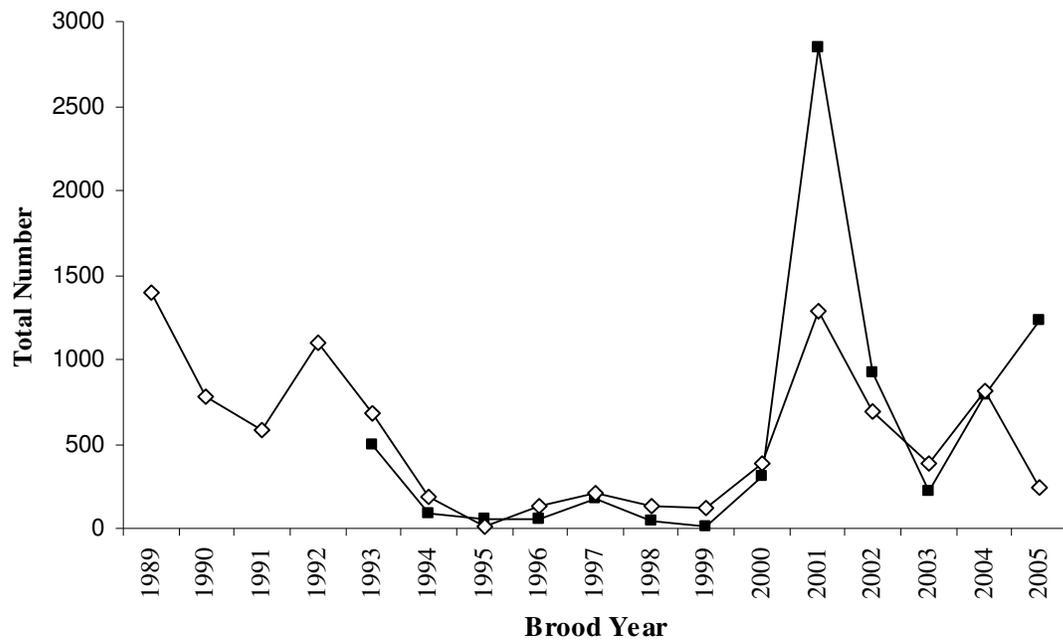


Table Captions

Table 1 - Thermocycler conditions and references for 11 microsatellite loci used to evaluate the 2004-05 Wenatchee River spring-run Chinook. 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 annealing temperature (T_m °C) for 40s, 72 °C for 40s, and a final extension cycle of 60 °C for 45 min.

Table 2: Matrix of pair-wise F_{ST} (Weir and Cockerham 1984) values for 2004 Leavenworth National fish Hatchery (LNFH), and Wenatchee R. spring- and summer-run Chinook salmon grouped according to origin (Hatchery vs. Wild). The White R. sample includes adults collected during 2004-5 and juveniles collected during 2005. 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 $\alpha = 0.05$. 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.). Collection date is 2004 unless otherwise noted.

Table 3: Estimates of effective number of breeders (N_b) for 1989-2005 Chiwawa R. hatchery breeders and naturally spawning spring Chinook salmon based on unequal sex ratio (SR) and variance in reproductive success (VRS). Spawner-recruit data for three generations (1989-2000) was used to calculate inbreeding effective size (N_{eI}) of the total population since supportive breeding began in 1989. Only wild-origin fish were available for captive breeding in 1989-93. Generation intervals of 4 years (i.e.- 1989-92 = generation 1) were used to calculate N_{eI} . Supportive breeding was not carried out in 1995 and 1999. Numbers of female (F) and male (M) hatchery breeders used and estimated numbers of naturally spawning fish are shown.

Table 4: Estimates of inbreeding N_e (Waples 2002a and 2006) for Chiwawa R. hatchery and naturally spawning Wenatchee R. spring Chinook salmon from 1989 to 2000.

Table 5 – Estimated effective number of breeders ($N_{b(i)}$) that produced each year i of fish using the linkage disequilibrium (LD) and temporal methods (Waples 2006a and Waples et al. 2006, respectively) for Chiwawa R. hatchery and naturally spawning spring Chinook salmon collected during 2004 and 2005. The temporal estimate of N_e was calculated as the sum of the harmonic mean of temporal pair-wise $N_{b(i)}$ estimates multiplied by the estimated generation interval (g) for hatchery and wild fish ($g = 3.5$, and 4.0, respectively). Harmonic mean of pair-wise $N_{b(i)}$ estimates are shown. Inf. = infinity.

Table 1 - Thermocycler conditions and references for 11 microsatellite loci used to evaluate the 2004-05 Wenatchee River spring-run Chinook. 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 annealing temperature (T_m °C) for 40s, 72 °C for 40s, and a final extension cycle of 60 °C for 45 min.

Locus Name	MgCl₂ (mM)	T_m (°C)	References
Oke4 ^a	1.75	54	Buchholz et al. 1999
Ogo4 ^b	1.75	60	Olsen, Bentzen, and Seeb 1998
Ots2M	2.00	60	Greig and Banks 1999
Ots3 ^c	1.75	48	Banks et al. 1999
Ots10M ^d	1.75	54	Greig and Banks 1999
Ots519NWFSC ^a	1.75	54	Naish and Park, 2002
Ots104 ^c	1.75	48	Nelson and Beacham 1999
Ots201b ^e	2.00	60	Banks <i>unpublished</i>
Ots211 ^b	1.75	60	Grieg, Jacobson, and Banks 2003
Ots213 ^d	1.75	54	Grieg, Jacobson, and Banks 2003
Ssa408 ^e	2.00	60	Cairney, Taggert, and Hoyheim 2000

^{a-e} Superscripts on locus names indicate loci pairs duplexed in the same PCR reaction.

Table 2: Matrix of pair-wise F_{ST} (Weir and Cockerham 1984) values for 2004 Leavenworth National fish Hatchery (LNFH), and Wenatchee R. spring- and summer-run Chinook salmon grouped according to origin (Hatchery vs. Wild). The White R. sample includes adults collected during 2004-5 and juveniles collected during 2005. 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 $\alpha = 0.05$. 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.). Collection date is 2004 unless otherwise noted.

Groups	Spring-Run								
	Wild				Chiwawa R. Hatchery			LNFH	Summer-Run
	White	Chiwawa	Nason	Remainder	2 Yr. old	3 Yr. old	4 Yr. old		
1994 Chiwawa Hatchery	0.019*	0.003	0.011*	0.003*	0.025*	0.008*	0.010*	0.018*	0.110*
Wild White R.		0.026*	0.027*	0.019*	0.031*	0.031*	0.036*	0.024*	0.094*
Wild Chiwawa R.			0.016*	0.001*	0.019*	0.013*	0.008*	0.016*	0.084*
Wild Nason Crk.				0.009*	0.037*	0.017*	0.018*	0.018*	0.091*
Wild Remainder					0.017*	0.010*	0.009*	0.012*	0.088*
Hatchery 2 Yr. old						0.023*	0.030*	0.019*	0.092*
Hatchery 3 Yr. old							0.013*	0.013*	0.084*
Hatchery 4 Yr. old								0.021*	0.099*
LNFH Spring									0.079*

Table 3: Estimates of effective number of breeders (N_b) for 1989-2005 Chiwawa R. hatchery breeders and naturally spawning spring Chinook salmon based on unequal sex ratio (SR) and variance in reproductive success (VRS). Spawner-recruit data for three generations (1989-2000) was used to calculate inbreeding effective size (N_{eI}) of the total population since supportive breeding began in 1989. Only wild-origin fish were available for captive breeding in 1989-93. Generation intervals of 4 years (i.e.- 1989-92 = generation 1) were used to calculate N_{eI} . Supportive breeding was not carried out in 1995 and 1999. Numbers of female (F) and male (M) hatchery breeders used and estimated numbers of naturally spawning fish are shown.

Year	Chiwawa R. hatchery fish										Naturally spawning fish										Prop.			Overall N_e^j			
	No. of		SR ^b		VRS ^c				Recruits	Estimated #		SR ^b		VRS ^c				Recruits	Contrib. ^f			Combined	R _i /R _t				
	F	M	$P_{(w)}^a$	N_b	N_b/N	N_{bf}	N_{bm}	$N_{b(Demo)}^d$	$N_{b(Demo)/N}$	(R _t) ^e	F	M	$P_{(h)}^a$	N_b	N_b/N	N_{bf}	N_{bm}	$N_{b(Demo)}^d$	$N_{b(Demo)/N}$	(R _t) ^e	N_T^g	(X _{hi})	(X _{wi})		$N_{b(i)}$	Recruits ^h	(X _i)
1989	17	11	1.0	27	0.95	15.1	5.0	15.1	0.54	533	613	779	0	1372	0.99	418.6	430.0	848.5	0.61	415	1420	0.56	0.44	88	947	0.77	
1990	12	6	1.0	16	0.89	10.6	2.8	8.7	0.49	18	346	429	0	766	0.99	236.3	236.7	473.0	0.61	50	793	0.27	0.73	215	68	0.06	
1991	19	13	1.0	31	0.96	16.8	6.0	17.6	0.55	39	251	334	0	573	0.98	171.4	184.1	355.0	0.61	26	617	0.60	0.40	88	65	0.05	
1992	39	39	1.0	78	1.00	34.6	17.9	47.2	0.60	23	491	608	0	1087	0.99	335.3	335.8	671.1	0.61	128	1177	0.15	0.85	1045	152	0.12	147
1993	48	46	1.0	94	1.00	42.5	21.1	56.5	0.60	214	531	637	0.42	1158	0.99	362.6	351.6	714.1	0.61	296	1262	0.42	0.58	462	511	0.41	
1994	6	5	0.64	11	0.99	5.3	2.3	6.4	0.58	12	125	155	0.32	277	0.99	153.6	85.6	219.8	0.58	45	291	0.21	0.79	159	57	0.05	
1995	0	0	0	0	--	0	0	--	--	0	23	35	0.86	56	0.96	15.7	19.3	34.6	0.60	77	58	0.00	1.00	58	77	0.06	
1996	4	14	0.44	12	0.69	3.5	6.4	9.1	0.51	142	72	110	0.28	174	0.96	49.2	60.7	108.7	0.60	461	200	0.24	0.76	159	603	0.48	522
1997	71	40	0.29	102	0.92	62.9	18.4	56.9	0.51	2825	175	214	0.46	385	0.99	119.5	118.1	237.6	0.61	1430	499	0.66	0.34	235	4255	0.58	
1998	20	27	0.28	46	0.98	17.7	12.4	29.2	0.62	1083	81	97	0.25	177	0.99	55.3	53.5	108.8	0.61	780	226	0.58	0.42	122	1863	0.25	
1999	0	0	0	0	--	0	0	--	--	0	48	84	0.10	122	0.93	32.8	46.4	76.8	0.58	21	133	0.00	1.00	133	21	0.00	
2000	11	19	0.3	28	0.93	9.7	8.7	18.4	0.61	320	282	406	0.45	666	0.97	192.6	224.1	414.3	0.60	883	718	0.27	0.73	319	1203	0.16	490
2001	241	130	0.3	338	0.91	213.6	59.7	186.6	0.50	inc.	1788	2342	0.69	4056	0.98	1220.9	1292.9	2511.7	0.61	inc.	4501	inc.	inc.	--	inc.	inc.	--
2002	43	28	0.28	68	0.96	38.1	12.9	38.4	0.54	inc.	787	826	0.57	1612	1.00	537.4	456.0	986.7	0.61	inc.	1684	inc.	inc.	--	inc.	inc.	--
2003	46	48	0.44	94	1.00	40.8	22.0	57.2	0.61	inc.	248	355	0.37	584	0.97	169.3	196.0	363.4	0.60	inc.	697	inc.	inc.	--	inc.	inc.	--
2004	133	82	0.39	203	0.94	117.9	37.7	114.2	0.53	inc.	491	1117	0.49	1364	0.85	335.3	616.6	868.7	0.54	inc.	1823	inc.	inc.	--	inc.	inc.	--
2005	131	139	0.33	270	1.00	116.1	63.8	164.8	0.61	inc.	818	654	0.83	1454	0.99	558.6	361.0	877.2	0.60	inc.	inc.	inc.	inc.	--	inc.	inc.	--
Harmonic mean ^k				0.93	14.8	10.3	24.3	0.56						0.96	114.1	124.2	237.9	0.60									

Table 3 (con't.):

^a The proportion of wild fish used as breeders ($P_{(w)}$), and proportion of hatchery-origin fish on spawning grounds ($P_{(h)}$)

^b Effective number of breeders based only on unequal sex ratio (SR). Regarded as a maximum estimate of N_b .

^c Effective number of breeding females and males (N_{bf} and N_{bm} , respectively) based on the mean and variance of progeny number for Yakima R. hatchery and wild spring Chinook (Schroder et al. 2005).

^d Effective number of breeders based on unequal SR and variation in reproductive success data.

^e Total number of adult spawners in subsequent years produced by adults spawning in year i . Incomplete (inc.) returns.

^f Proportional contribution of hatchery (X_{hi}) and wild (X_{wi}) adult spawners in subsequent years produced by adults spawning in that year. Some data is unavailable due to incomplete (inc.) returns.

^g Total number (N_T) of hatchery and naturally-spawning adults combined.

^h Combined hatchery and wild recruits in next generation produced by adults spawning in that year. Some data is unavailable due to incomplete (inc.) returns.

ⁱ Overall proportion of recruits produced by hatchery and naturally-spawning fish for a given year.

^j Inbreeding effective size (Ryman and Laikre 1991) that incorporates the correction for overlapping generations by Waples (2002 and 2006)

^k Harmonic means do not include 1995 and 1999 hatchery, or 1995 wild data.

Table 4: Estimates of inbreeding N_e (Waples 2002a and 2006) for Chiwawa R. hatchery and naturally spawning Wenatchee R. spring Chinook salmon from 1989 to 2000.

		Captive breeding program					
		Yes				No	
		Hatchery		spawning N_e		Wild^d	
G^a	Years	N_T^b	N_e	N_T^b	N_e	N_e	N_e
1	1989-92	156	37	3851	2683	147	2776
2	1993-96	164	83	1688	572	522	328
3	1997-00	251	178	1388	989	490	629
Harmonic mean N_e/N_T		0.39		0.52		0.34	

^a Generation interval (G) assumed to span 4 yr. (Chapman et al. 1995; A. Murdoch, unpub.).

^b The total number of spawners in a generation (N_T).

^c Hatchery and naturally spawning fish.

^d Wild population segment only.

Table 5 – Estimated effective number of breeders ($N_{b(i)}$) that produced each year i of fish using the linkage disequilibrium (LD) and temporal methods (Waples 2006a and Waples et al. 2006, respectively) for Chiwawa R. hatchery and naturally spawning spring Chinook salmon collected during 2004 and 2005. The temporal estimate of N_e was calculated as the sum of the harmonic mean of temporal pair-wise $N_{b(i)}$ estimates multiplied by the estimated generation interval (g) for hatchery and wild fish ($g = 3.5$, and 4.0 , respectively). Harmonic mean of pair-wise $N_{b(i)}$ estimates are shown. Inf. = infinity.

Yr. Born	Chiwawa R. hatchery					Naturally spawning fish						
	N^a	S^b	LD Estimate		Temporal Estimate		N^c	S^b	LD Estimate		Temporal Estimate	
			$N_{b(i)}$	(95% CI)	$N_{b(i)}$	N_e			$N_{b(i)}$	(95% CI)	$N_{b(i)}$	N_e
2000	30	460.4	23.1	(13.4-36.1)	40.8		688	925.8	226.3	(118.7-457.5)	116.8	
2001	371	1103.9	79.5	(48.1-124.3)	63.9		4130	493.1	623.6	(178.7-inf.)	311.8	
2002	71	763.8	20.2	(12.0-30.7)	40.2		1613	9.9	inf.		1683.0	
2003	94	292.2	19.5	(10.9-31.6)	69.5	176	603	--	--		--	971
Harmonic mean			25.5		50.4				--		242.7	

Chapter 5 -- 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. In this report, we provide preliminary results for questions (1) and (2) for the 2004 parental spawning year. We are using fractional assignment methods and a sample of subyearling parr trapped in Nason Creek and the Chiwawa and White Rivers in fall of 2005 and a sample of smolts trapped in the lower Wenatchee River near Monitor in 2006 to estimate the relative fitness of hatchery and natural origin fish, and evaluate how weight, run timing, and age contribute to these differences. We also conducted computer simulations to evaluate the effectiveness of the fractional assignment methods. Based on our preliminary results, both male and female hatchery fish produced fewer progeny per parent when spawning naturally than did natural fish, particular when progeny were counted at the smolt stage. Differences in age structure and to a lesser degree weight and run timing were responsible for a portion of the difference in fitness between hatchery and wild fish. Male size and age had a large influence on fitness, with older and larger males selectively favored. Male run time had a smaller but still significant effect on fitness, with earlier returning fish favored. Female size had a significant effect on fitness, but the effect was much smaller than the effect of size on male fitness. Additional variables that are likely to affect fitness, including spawning location and spawning time, have been measured but not yet analyzed and will be included in subsequent reports.

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 (NRC 1996). For example, supplementation projects, in which adult hatchery fish are planned to spawn naturally to augment a population's abundance, have become common throughout the Columbia River Basin (Williams et al. 2003). However, little direct data are available on the beneficial or harmful influence hatchery production has on the natural production of Chinook salmon (ISAB 2003, 2005).

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 (Reisenbichler & McIntyre 1977; Ford 2002). 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 (Moberg et al. 2005). Determining the relative reproductive success of hatchery fish that stray from traditional hatchery programs is also important. Stray hatchery fish can often obscure the status of natural populations because their reproductive success is unknown (McClure et al. 2003), 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 (Lynch & O'Hely 2001; Ford 2002). 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.

The 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. In this report, we provide preliminary results for questions (1) and (2) for the 2004 parental spawning year.

Methods

Sampling - Parental sampling was conducted as described in Chapter 1. The first planned sampling of progeny for this project occurred in spring of 2006, when smolts produced from the 2004 spawning year were captured in the lower Wenatchee River near Monitor. We also took advantage of another ongoing sampling program in the Wenatchee River tributaries to obtain samples from 2004 broodyear parr collected from the Chiwawa River, Nason Creek, and White River in fall/winter of 2005. We conducted a preliminary parentage analysis of both the 2005 and 2006 juvenile samples.

Juvenile Chinook tissue samples were collected from rotary smolt traps located on the lower Wenatchee River (rkm 9.6), Chiwawa River (rkm 1.0) and Nason Creek (rkm 0.8).

All smolt traps are located downstream from the majority of spawning habitat for each of the respective watersheds.

Fish were removed from the trap at a minimum every morning and placed in an anesthetic solution of MS-222. Fish were identified to species and counted. Non-target species were allowed to fully recover in fresh water prior to being released in an area of calm water downstream from the smolt trap. Target species were held in separate live boxes when needed for mark/recapture efficiency trials conducted in the evening.

Mark/recapture efficiency trials were conducted throughout the trapping season. Fish for the mark/recapture trials were marked by clipping the tip of either the upper or lower lobe of the caudal fin. Whenever possible, fin clips (0.5 cm²) also served as DNA samples and placed on blotter paper. All tissue samples were sent to NWFSC for analysis. Samples sizes and collection dates are reported in Table 1.

Table 1-- 2004 brood year juvenile Chinook DNA collection summary (NP = naturally produced; H = hatchery).

Year	Life stage	Origin	Trap	Collection dates		N	% Total
2005	Subyearling	NP	Nason	6 Oct	12 Nov	574	61.9
		NP	Chiwawa	27 Sep	6 Nov	576	5.2
2006	Yearling	NP	Nason	10 Mar	23 Apr	315	65.2
		H	Chiwawa	18 Apr	30 Apr	2,000	20.5
		NP	Chiwawa	7 Mar	25 Jun	708	14.2
		NP	Wenatchee	9 Feb	2 Jun	635	100.0

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 2). 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 2). 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 MgCl₂, 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 2) 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 2 -- 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 annealing temperature (T_m °C) for 40s, 72 °C for 40s, and a final extension cycle of 60 °C for 45 min.

Locus Name	MgCl₂ (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.

Parentage assignment – 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). Individuals with missing data at more than 1 locus were excluded from the analysis, resulting in 2594 analyzed parents out of 2616

total. The analyzed progeny population consisted of 975 parr sampled from the Wenatchee tributaries in 2005 (574 from Chiwawa River and 401 from Nason Creek), and 1490 smolts trapped in 2006 (558 from the Monitor trap in the Lower Wenatchee, 194 from Nason Creek, and 738 from the Chiwawa River). 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 (Δ LOD) was calculated. The simulation function of the FAMOZ program was used to generate expected distributions of Δ LOD scores for correct and incorrect assignments. As an alternative method of fitness estimation, we also used the FAMOZ program output to fractionally assign progeny to the 20 most likely parent pairs in proportion to their likelihoods (see below).

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 (Sancristobal & Chevalet 1997; Gerber et al. 2000).

In an initial analysis of 196 Chiwawa parr conducted previously, we discovered that the rate of successful assignment of progeny to parents was lower than we originally anticipated (Murdoch et al. 2006). In addition, we found that progeny of wild parents appeared to be assigned to parents more readily than were the progeny of hatchery parents, apparently due to a higher degree of genetic relatedness among hatchery origin fish compared to wild origin fish. Ultimately, we intend to address this issue by genotyping additional loci (see our 2007 proposal and statement of work -- <http://www.cbfwa.org/solicitation/components/forms/Proposal.cfm?PropID=160>). In the meantime, we have attempted to address this issue through analyses of further simulations and by using fractional assignment of progeny to parents (Devlin et al. 1988; Smouse & Meagher 1994).

We expected that fractional assignment of progeny would reduce or eliminate the bias in fitness estimation caused by differences in progeny 'assignability' between wild and hatchery origin fish. In situations where multiple parent pairs are compatible with some offspring, fractional assignment methods also provide a statistically robust way to estimate selection gradients (e.g., Morgan & Conner 2001; Nielsen et al. 2001). For these preliminary analyses, we focused on a simple version of fractional assignment in which an individual progeny is divided amongst parent pairs based on the conditional probabilities they are the true parents, assuming equal prior probability of parentage. In

order to make the analyses computationally tractable, only the twenty most likely parent pairs for each offspring were included in the analysis.

In order to explore the effects of alternative methods of estimating fitness, we used the 2004 Wenatchee spring Chinook parents to simulate 10000 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 and offspring were either assigned to single pairs of parents (LOD threshold method) or were fractionally assigned to the most likely 20 pairs of parents in proportion to their relatively likelihood (fractional assignment method). The assignments were then 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.

Selection and fitness analysis – All statistical analyses were conducted using the general linear model (GLM) function in the SYSTAT v11 (Systat Software Inc) computer package. We estimated the effects of the following traits (all measured at Tumwater Dam) on fitness: weight, run time, age, and origin (hatchery or wild). Lengths were also measured at Tumwater Dam, but length and weight were so highly correlated that there appeared to be little point in measuring selection on both traits. Weight was cube root transformed prior to standardization, and run time was converted to ordinal days. Traits were standardized within each sex by subtracting the mean and dividing by the standard deviation. Age 2 males were excluded from some analyses, and in those cases traits were restandardized after excluding the age 2 males. Absolute fitness (progeny counts) within sexes was converted to relative fitness by dividing by the mean fitness. The effects of age, origin, weight and run timing on fitness and standardized linear selection gradients were estimated using ANCOVA.

Results

Simulation results

As expected, when fitness was estimated using the true (known) progeny counts from the simulations, there were no significant differences in fitness among ages or between hatchery and natural origin spawners (Table 3). In contrast, when simulated offspring were assigned to a single pair of parents based on their LOD score, there were highly significant differences in “fitness” between hatchery and natural origin fish (Table 3), consistent with the previous simulations results reported by Murdoch et al. (2006). Finally, when progeny were fractionally assigned to parents in proportion to the parent pairs’ likelihoods, relative fitness of hatchery and natural origin fish was again close to the expected value of 1.0, although some of the differences were statistically significant at the $p < 0.05$ level (Table 2).

The actual fitness estimates (standardized mean progeny counts based on fractional assignments) are reported in Table 4. When progeny were counted as parr, we found no significant difference in progeny/parent for hatchery and natural fish if the comparison were made within age classes, although hatchery fish produced fewer sampled progeny/parent in all age classes. For males, if all ages were combined hatchery fish produced significantly fewer progeny/parent than wild fish, due at least in part to a higher fraction of younger males among hatchery fish compared to wild fish. When progeny were counted at the smolt stage, hatchery fish produced significantly fewer progeny/parent within both sexes and within the four year old age class (Table 4).

For males, although age, weight and run time significantly influenced fitness, hatchery fish remained significantly less fit than wild fish even after taking these factors into account (Table 5, Table 6). For males, weight had a strong effect on relative fitness (Table 5, Table 6, Figure 1), and run time had a relative minor effect (Table 5, Table 6, Figure 2).

Table 3 -- Simulated fitness estimates (mean progeny numbers) by origin, sex, and age.
10000 simulated progeny -- true progeny counts

Sex	Age	Wild				Hatchery		
		N	Mean	SD	H/W ¹	N	Mean	SD
Male	2	0	na	na	na	594	5.436	2.379
	3	27	5.185	2.481	1.06	705	5.508	2.47
	4	387	5.478	2.455	0.95	90	5.189	2.203
	all	417	5.475	2.457	1.00	1,415	5.454	2.411
Female	all	350	16.783	4.271	0.97	253	16.308	4.16

10000 simulated progeny -- single pair assignments using LOD threshold

Sex	Age	Wild				Hatchery		
		N	Mean	SD	H/W ¹	N	Mean	SD
Male	2	0	na	na	na	594	3.221	2.368
	3	27	4.037	2.377	0.99	705	3.983	2.281
	4	387	4.597	2.285	.78***	90	3.567	2.375
	all	417	4.576	2.291	.79***	1,415	3.634	2.345
Female	all	350	12.843	3.931	.77***	253	9.941	5.041

10000 simulated progeny -- fractional assignment of progeny

Sex	Age	Wild				Hatchery		
		N	Mean	SD	H/W ¹	N	Mean	SD
Male	2	0	na	na	na	594	5.476	2.325
	3	27	5.156	2.277	1.08	705	5.554	2.297
	4	387	5.637	2.508	1.14*	90	6.443	2.804
	all	417	5.621	2.496	0.99	1,415	5.578	2.405
Female	all	350	16.527	4.226	.95*	253	15.726	4.415 *

p < 0.05, *** p < 0.001

For females, the ANCOVA results indicated significant effects of origin, weight and run timing when progeny were counted as smolts (Table 7, Table 8). Selection on female weight was considerably weaker than selection on male weight. Age was not included in the female analyses since 97% of the females were four year olds.

Table 4 -- Actual fitness estimates for the 2004 brood natural spawners, based on parr counts (top) or smolt counts (bottom)

975 parr sampled from Chiwawa and Nason Creek -- fractional assignments

Sex	Age	Wild			H/W	p-value	Hatchery		
		N	Mean	SD			N	Mean	SD
Male									
	2	--	--	--	--	--	626	0.221	0.495
	3	28	1.059	2.031	0.68	0.39	740	0.717	1.317
	4	403	2.481	3.435	0.81	0.12	96	1.998	2.502
	all	435	2.381	3.368	0.25	<0.001	1,491	0.59	1.256
Female									
	all	369	1.035	1.361	0.88	0.21	268	0.909	1.167

1490 smolts -- fractional assignments

Sex	Age	Wild			H/W	p-value	Hatchery		
		N	Mean	SD			N	Mean	SD
Male									
	2	--	--	--	--	--	626	0.318	0.502
	3	28	0.842	1.186	0.83	0.54	740	0.7	1.01
	4	403	2.495	3.117	0.55	<0.001	96	1.375	1.491
	all	435	2.42	3.165	0.24	<0.001	1,491	0.58	0.917
Female									
	all	369	1.226	1.362	0.55	<0.001	268	0.677	0.955

Table 5 -- Effects of origin, age, weight and run time, for males, based on parr counts

parameter	estimate	p-value
CONSTANT	1.02	
Effect of hatchery origin	-0.218	0.018
Effect of age 1.1	0.383	0.005
Origin by age	-0.016	0.857
run timing	-0.147	0.003
weight	0.86	<0.001

Notes: Ages 3 and 4 only.

Table 6 -- Effects of origin, age, weight and run time, for males, based on smolt counts

parameter	estimate	p-value
CONSTANT	0.909	
Hatchery origin	-0.3	<0.001
Age 1.1	0.37	0.002
Hatchery origin*age 1.1	0.145	0.073
run timing	-0.148	0.001
weight	0.791	<0.001

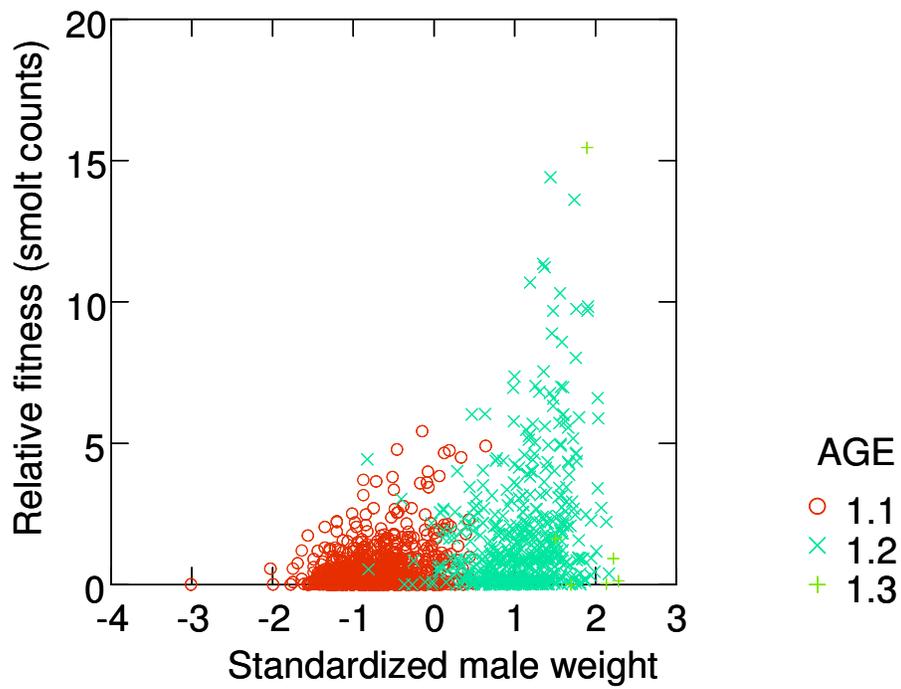


Figure 1 -- Relationship between male weight and relative fitness, based on smolt counts

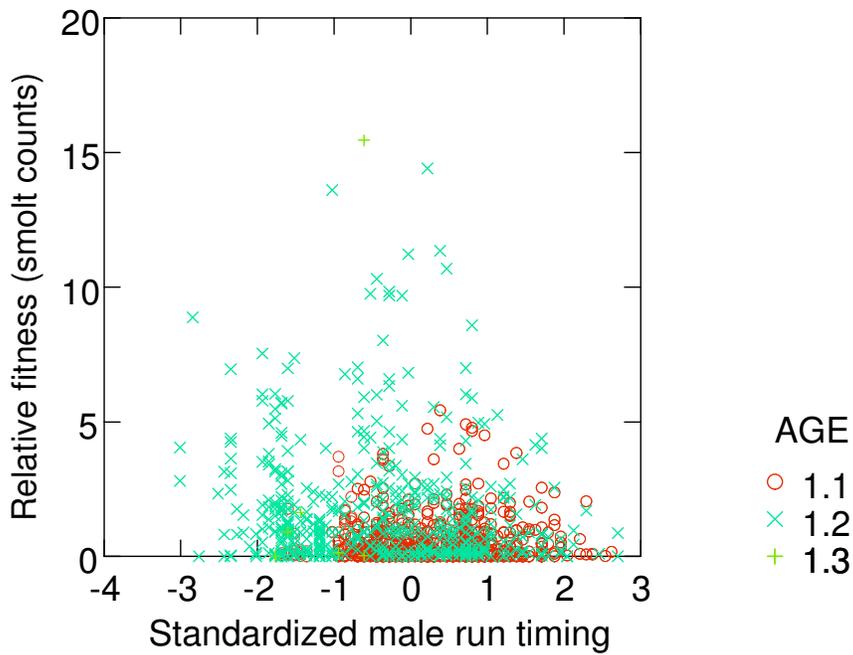


Figure 2 -- Relationship between male run timing and relative fitness, based on smolt counts

Table 7 -- Effects of origin, weight and run time, for females, based on parr counts

parameter	estimate	p-value
CONSTANT	0.97	
hatchery origin	-0.079	0.14
run timing	0.031	0.562
weight	0.111	0.033

Table 8 -- Effects of origin, weight and run time, for females, based on smolt counts

parameter	estimate	p-value
CONSTANT	0.957	
hatchery origin	-0.251	0
run timing	-0.145	0.004
weight	0.189	0

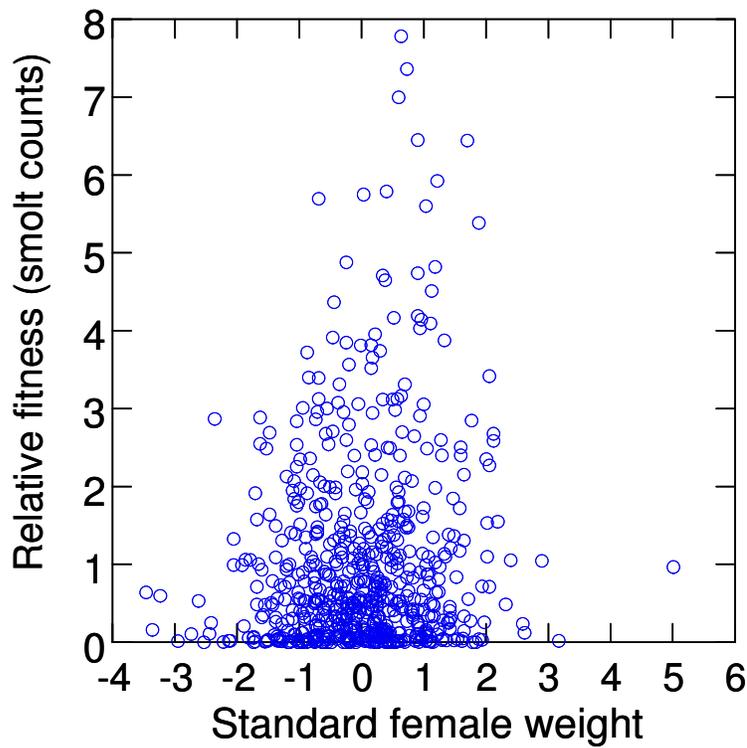


Figure 3 -- Relationship between female weight and relative fitness, based on smolt counts

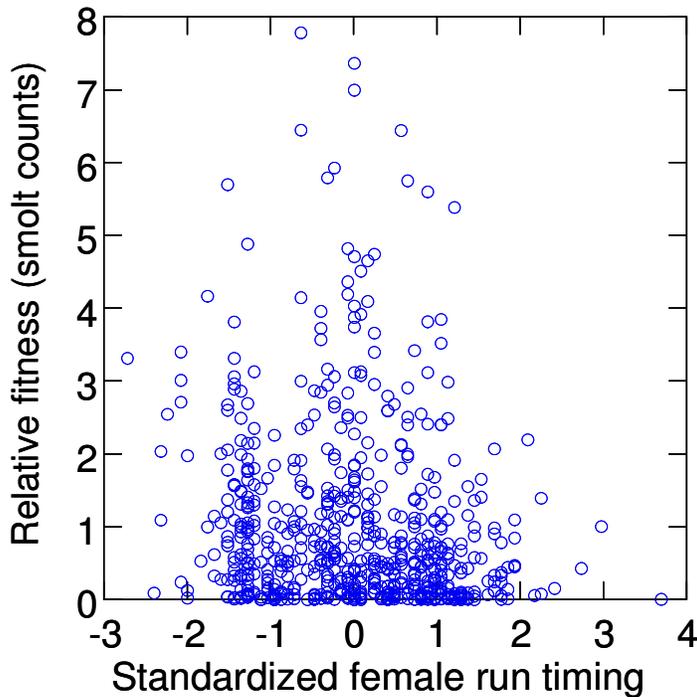


Figure 4 -- Relationship between female run timing and relative fitness, based on smolt counts

Discussion

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 (Murdoch et al. 2006). We discussed several possible causes of this difference, and concluded that difficulty in assigning offspring whose parents were hatchery fish was probably related to low effective population size of the hatchery broodstock (see also Chapter 4). We also suggested that one potential workaround for this problem was to use fractional assignment of progeny in order estimate relative fitness. Fractional assignment does not require identification of a single likely parent pair for an individual offspring, but instead apportions an offspring to multiple potential parent pairs in proportion to their likelihoods. In this report, we therefore concentrated on testing and applying the fractional assignment method in order to determine if we could obtain unbiased estimates of the relative fitness of hatchery origin fish despite our inability to accurately identify a single set of parents for many putative offspring of hatchery origin parents.

We tested the fractional assignment method by using the observed 2004 parent genotypes to simulate a large number of offspring under the assumption that all fish had equal relative fitness. The simulated offspring and real parents were then analyzed to test for fitness differences among origins or age classes. If the fractional assignment method worked as predicted, the estimated fitness using the simulated offspring of hatchery and

natural origin fish should be the same. In practice, we did indeed find that when the fractional assignment method was used to estimate fitness using the simulated offspring, mean fitness differed little by age or origin (Table 3). Although in several cases hatchery and natural origin fitness differed statistically ($p < 0.05$), the absolute level of the differences were quite small and not in any consistent direction. In contrast, when single assignment was used, the estimated fitness of hatchery fish was significantly biased downward (Table 3). Based on these simulation results, we conclude that the fractional assignment method has the potential to produce useful estimates of relative fitness of hatchery fish compared to wild fish, despite the differences in offspring “assignability” between the two types of fish.

With some confidence that the fractional assignment method produces reasonable results, we then used it to evaluate the effects of hatchery/wild origin, age, weight and run timing on relative fitness of the 2004 natural spawners. In these analyses the parental samples consisted of the spring Chinook salmon sampled at Tumwater Dam that were not used for hatchery broodstock. The offspring sample consisted of subyearling parr collected in fall of 2005 in traps in the Chiwawa River and Nason Creek, and smolts collected in 2006 the same two rivers and at the Monitor Trap in the Lower Wenatchee River. Absolute fitness was measured as the number of offspring assigned to an individual (can be fractional) and relative fitness was simply absolute fitness divided by the mean fitness. Overall, the mean relative fitness of hatchery fish was only 25% that of wild fish for males, and 55-88% that of wild fish for females, depending on whether fitness was measured at the parr or smolt stage (Table 4). For males, a large part of the low relative fitness of hatchery fish was explained by age structure. In particular, the hatchery males that returned in 2004 were predominately two and three year old fish, whereas the wild males were predominately four year olds (Chapter 1). Regardless of origin, the three year old fish had relatively low fitness compared to the four year olds, and the two year old fish had very low fitness (Table 4). After taking into account variation in fitness due to age, size and run timing, hatchery fish still produced significantly fewer progeny/parent than wild fish (Tables 5-8), however. In future reports we will explore the relationship between additional traits, including spawning location, spawning time, and redd micro-habitat characteristics (see Chapters 1 - 3) in order to further explore the causes of the fitness differences between hatchery and natural fish.

In addition to hatchery origin, we found significant relationships between relative fitness and size and run timing for both males and females. Selection on size was stronger for males than females, consistent with results from coho salmon (Ford et al., unpublished data). Strong selection on size apparently explains most if not all of the differences in fitness between three and four year old males (Table 4). Indeed, the ANCOVA results indicate that although three year old males have poor fitness due to their small size, they are actually significantly more fit compared to four year olds than would be expected based on their size alone (positive coefficients for the age 3 effect in Tables 5 and 6). For both males and females, earlier run time was mildly favored, although visual inspection of the data suggests the possibility of stabilizing selection on run timing (Figure 2, Figure 4). Further analyses of selection on run timing will be presented in future reports.

These results are preliminary, and it would be premature to draw any firm conclusions from them regarding the effectiveness of the Wenatchee supplementation program. However, if confirmed, our results would indicate that it will be necessary to take into account the observed differences in fitness between hatchery and natural fish when evaluating the risks and benefits of the supplementation program. The differences in fitness between hatchery and natural fish we observed are consistent with estimates of hatchery fish relative fitness from other species (reviewed by Berejikian & Ford 2004), and might be expected to grow larger when fitness is estimated at later progeny stages (e.g., Leider et al. 1990; e.g., Kostow et al. 2003).

References

- Berejikian, B., and M. J. Ford. 2004. Review of relative fitness of hatchery and natural salmon. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-61, 28 p.
- Devlin, B., K. Roeder, and N. C. Ellstrand. 1988. Fractional paternity assignment: theoretical development and comparison to other methods. *Theoretical and Applied Genetics* **76**:369-380.
- Ford, M. J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology* **16**:815-825.
- Gerber, S., P. Chabrier, and A. Kremer. 2003. FaMoz: a software for parentage analysis using dominant, codominant and uniparentally inherited markers. *Molecular Ecology Notes* **3**:479-481.
- Gerber, S., S. Mariette, R. Streiff, C. Bodenes, and A. Kremer. 2000. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. *Molecular Ecology* **9**:1037-1048.
- ISAB. 2003. Review of salmon and steelhead supplementation. Pages 1-205, Portland.
- ISAB. 2005. Monitoring and evaluation of supplementation projects.
- Kostow, K., A. Marshall, and S. Phelps. 2003. Naturally spawning hatchery steelhead contribute to smolt production but experience low reproductive success. *Transactions of the American Fisheries Society* **132**:780-790.
- Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. *Aquaculture* **88**:239-252.
- Lynch, M., and H. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. *Conservation Genetics* **2**:363-378.

- McClure, M. M., E. E. Holmes, B. L. Sanderson, and C. E. Jordan. 2003. A large-scale, multispecies status assessment: anadromous salmonids in the Columbia River Basin. *Ecological Applications* **13**:964-989.
- Meagher, T. R., and E. Thompson. 1986. The relationship between single parent and parent pair genetic likelihoods in genealogy reconstruction. *Theoretical Population Biology* **29**:87-106.
- Mobrand, L. E., J. Barr, L. Blankenship, D. E. Campton, T. T. P. Evelyn, T. A. Flagg, C. V. W. Mahnken, L. W. Seeb, P. R. Seidel, and W. W. Smoker. 2005. Hatchery reform in Washington State: principles and emerging issues. *Fisheries* **30**:11-33.
- Morgan, M. T., and J. K. Conner. 2001. Using genetic markers to directly estimate male selection gradients. *Evolution* **55**:272-281.
- Murdoch, A., T. Pearsons, T. Maitland, M. Ford, and K. Williamson. 2006. Monitoring the reproductive success of naturally spawning hatchery and natural spring Chinook salmon in the Wenatchee River. BPA Project No. 2003-039-00. Bonneville Power Administration, Portland, Oregon. Department of Energy, Bonneville Power Administration.
- Nielsen, R., D. Mattila, P. Clapham, and P. Palsboll. 2001. Statistical approaches to paternity analysis in natural populations and applications to the North American humpback whale. *Genetics* **157**:1673 - 1682.
- NRC 1996. Upstream: salmon and society in the Pacific Northwest. National Academy Press, Washington DC.
- Reisenbichler, R. R., and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board of Canada* **34**:123-128.
- Sancristobal, M., and C. Chevalet. 1997. Error tolerant parent identification from a finite set of individuals. *Genetical Research* **70**:53-62.
- Smouse, P. E., and T. R. Meagher. 1994. Genetic analysis of male reproductive contributions in *Chamaelirium luteum* (L.) Gray (Liliaceae). *Genetics* **136**:313-322.
- Williams, R., J. Lichatowich, P. Mundy, and M. Powell. 2003. Integrating artificial production with salmonid life history, genetic, and ecosystem diversity: a landscape perspective. Trout Unlimited.

Appendices

Appendix A. Daily number of spring Chinook observed at Tumwater Dam during trapping in 2006 (PM = precocious male).

Date	Natural				Hatchery				Unknown			Daily Total
	Females	Males	Jacks	PM	Females	Males	Jacks	PM	Females	Males	Jacks	
06/17/2006	1											1
06/18/2006												0
06/19/2006	1						2					3
06/20/2006	2					1						3
06/21/2006		3				5	3					11
06/22/2006	2	1				5	5					13
06/23/2006	2					2	4					8
06/24/2006	3	1				9					1	14
06/25/2006	10	9				17	13			1		50
06/26/2006	23	11				47	28			2	1	112
06/27/2006	18	6				29	30	1		2	3	89
06/28/2006	26	14				48	31	3		1	1	124
06/29/2006	18	5				35	18	1		1	2	81
06/30/2006	3	8	1			30	20	1		1		64
07/01/2006	14	21	1			59	39	6		1		141
07/02/2006	14	13				54	35	5		1		122
07/03/2006	23	30				87	36	3	1	2		182
07/04/2006	26	28				82	45	4		1		186
07/05/2006	11	18				57	31	10				127
07/06/2006	16	8	1			47	28	7	2	2		111
07/07/2006	4	1				21	12	5				43
07/08/2006	3	10	1			17	8	7	1			47
07/09/2006	16	11				33	21	12	2			95
07/10/2006	16	12				53	22	12	1			116
07/11/2006	17	7				40	21	23	1			109
07/12/2006	15	6	1			49	15	17				103
07/13/2006	3	3	1			17	12	12	2			50
07/14/2006	2	3				6	6	10	1			28
07/15/2006	2	2				13	2	2	3			24
07/16/2006	3	5				13	5	8	1			35
07/17/2006	4	3				4	2	5	3			21
07/18/2006	1					6	1	4	3			15
07/19/2006		1				2	3		16			22
07/20/2006		1	1			5	1	1	4			13
07/21/2006	2					4	1	2	52			61

Date	Natural				Hatchery				Unknown			Daily Total
	Females	Males	Jacks	PM	Females	Males	Jacks	PM	Females	Males	Jacks	
07/22/2006	1				4	4	3	23				35
07/23/2006	3				1			15				19
07/24/2006		1			6	2		8	1			18
07/25/2006	2				1			3				6
07/26/2006				1	4		1	13				19
07/27/2006	1		1		2		1	33				38
07/28/2006							1	6				7
07/29/2006					1							1
07/30/2006								2				2
07/31/2006					1							1
08/01/2006												0
08/02/2006												0
08/03/2006												0
08/04/2006												0
08/05/2006		1						4		1		6
Totals	308	243	8	1	917	506	167	200	16	9	1	2376

Appendix B. Spring Chinook spawn timing in the upper Wenatchee River Basin in 2006.

Date	Stream					Daily Total	Cumulative Total
	Nason	Chiwawa	Wenatchee	Little Wenatchee	White		
08/01/2006	1	0	0	0	0	1	1
08/07/2006	0	2	0	0	0	2	3
08/08/2006	0	0	0	0	1	1	4
08/09/2006	0	2	0	0	0	2	6
08/10/2006	2	5	0	0	0	7	13
08/11/2006	0	4	0	0	0	4	17
08/12/2006	0	3	0	0	0	3	20
08/14/2006	2	15	0	0	0	17	37
08/15/2006	1	9	0	0	0	10	47
08/16/2006	0	1	0	0	0	1	48
08/17/2006	2	11	0	1	3	17	65
08/18/2006	0	10	0	0	2	12	77
08/21/2006	9	32	0	3	7	51	128
08/22/2006	4	27	0	0	1	32	160
08/23/2006	1	0	0	2	0	3	163
08/24/2006	10	20	0	0	6	36	199
08/25/2006	4	21	0	0	3	28	227
08/28/2006	13	10	0	4	1	28	255
08/29/2006	3	25	0	0	2	30	285
08/30/2006	14	0	3	0	0	17	302
08/31/2006	3	23	0	3	1	30	332
09/01/2006	16	33	0	0	0	49	381
09/04/2006	5	14	0	0	0	19	400
09/05/2006	24	15	9	0	0	48	448
09/06/2006	0	0	0	5	0	5	453
09/07/2006	9	3	0	0	1	13	466
09/08/2006	12	0	6	0	1	19	485
09/11/2006	5	8	0	0	1	14	499
09/12/2006	6	3	2	0	0	11	510
09/13/2006	0	0	0	2	0	2	512
09/14/2006	0	1	0	0	0	1	513
09/15/2006	0	0	4	0	1	5	518
09/18/2006	3	0	0	0	0	3	521
09/19/2006	2	0	0	0	0	2	523
09/20/2006	0	0	0	1	0	1	524
09/21/2006	0	0	2	0	0	2	526
09/25/2006	1	0	1	0	0	2	528
Total	152	297	27	21	31	528	528

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

River (<i>Tributary</i>)	Reach	River kilometer
Chiwawa River		
Mouth to Grouse Creek	C1	0 – 19.5
<i>Big Meadow Creek</i>		0 – 1.5
Grouse Creek to Rock Creek CG	C2	19.5 – 32.2
<i>Chikamin Creek</i>		0 – 1.0
<i>Rock Creek</i>		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
<i>Napeaqua River</i>		
Napeaqua R. to Grasshopper Meadows	H3	18.3 – 21.5
<i>Panther Creek</i>		
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
<i>Chiwaukum Creek</i>		
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		
	<i>N</i>	Mean	SD	<i>N</i>	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		
	<i>N</i>	Mean	SD	<i>N</i>	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		
	<i>N</i>	Mean	SD	<i>N</i>	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