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

Andrew R. Murdoch
Todd N. Pearsons
Travis W. Maitland

Washington Department of Fish and Wildlife
600 Capitol Way North
Olympia, WA 98501-1091

and

Michael Ford
Kevin Williamson

National Oceanographic and Atmospheric Administration
Northwest Fisheries Science Center
Conservation Biology Division
2725 Montlake Blvd. East, Seattle, WA 98112

BPA Project No. 2003-039-00

April 2005

Executive Summary

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

Hatcheries have been increasingly asked to contribute to conserving natural salmon populations, as well as continuing to produce fish to mitigate for lost harvest opportunities. However, 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 on the Wenatchee River we will use a DNA-based pedigree analysis to (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. DNA was extracted and analyzed for all spring Chinook trapped at Tumwater Dam or released as adults into the natural environment in 2004. Results will not be available until after smolts are sampled in 2006.

Spawning ground surveys in the upper Wenatchee River basin are essential for understanding spawning distribution and redd microhabitat of hatchery and naturally produced fish. In 2004, spring Chinook redds were distributed similarly to that of years past. A total of 491 redds were found upstream of Tumwater Dam, of which the female origin was identified on 290 redds. Based on redd counts, the survival of adult and jack spring Chinook from Tumwater Dam to the spawning grounds was estimated at 83%. Microhabitat variables were measured on 186 redds, which included 42 and 144 redds constructed by hatchery and natural origin females, respectively. No differences were found in the estimated age composition or the proportion of hatchery fish of the spawning population compared to the expected population sampled at Tumwater Dam. A difference in the spawning distribution was found between hatchery and natural origin fish. No difference in spawn timing was detected.

Visual observations, PIT tag detections, and snorkel surveys were used to determine composition of adult hatchery and natural origin spring Chinook salmon and abundance of precocious males on redds. Hatchery and natural origin females were paired on redds with hatchery and natural origin males in proportions that were not significantly different from the proportions of hatchery and natural origin males available to spawn naturally ($P>0.05$). This suggested that there was not strong selection for assortative pairing by hatchery or natural origin salmon. However, differences in the spatial distribution of hatchery and natural fish may have contributed to the smaller size of the male fish paired with hatchery females. The estimated number of hatchery and natural origin precocious males that potentially contributed to natural spawning was 9 and 43, respectively. The low relative abundance of precocious males observed on the spawning grounds suggests that the majority of the precocious males observed at Tumwater Dam do not successfully migrate to the major spawning areas or die before spawning. The precocity rate for juveniles released from Chiwawa Ponds in 2004 that migrated downstream and survived to pass Tumwater Dam was calculated as 0.4%.

Table of Contents

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. The project is intended to last until 2012 in order to evaluate two entire spring Chinook salmon generations.

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

Description of Project Area

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

Historical river discharge monitored by the United States Geological Survey (USGS gauging station number 12462500 at river km 9.4) reported a 41-year mean monthly summer low discharge of 23 m^3/s and a mean monthly spring peak discharge of 257 m^3/s. Of the total river discharge, the Little Wenatchee River (15%) and White River (25%) are the only tributaries that feed Lake Wenatchee (Mullan et al. 1992). Other primary

tributaries of the Wenatchee River below the lake are Nason Creek (18%), Chiwawa River (15%) and Icicle Creek (20%) (Mullan et al. 1992).

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

History of Artificial Propagation

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

More recently, a supplementation program was initiated in 1989 on the Chiwawa River as part of the Rock Island Migration Agreement between Chelan County Public Utility District and the fishery management parties (RISPA 1989). The program is designed to mitigate for smolt mortality as a result of the operation of Rock Island Hydroelectric Project and has a production level goal of 672,000 yearling smolts. Currently, the program is operated under the Rock Island Habitat Conservation Plan and has established a goal for the program to increase the abundance of the naturally spawning population while maintaining the genetic integrity and long-term fitness of the stock (CCPUD 2002). However, low escapement to the Chiwawa River has limited smolt production and the mean number of smolts released since 1991 has been 101,843 (1989-2002 brood).

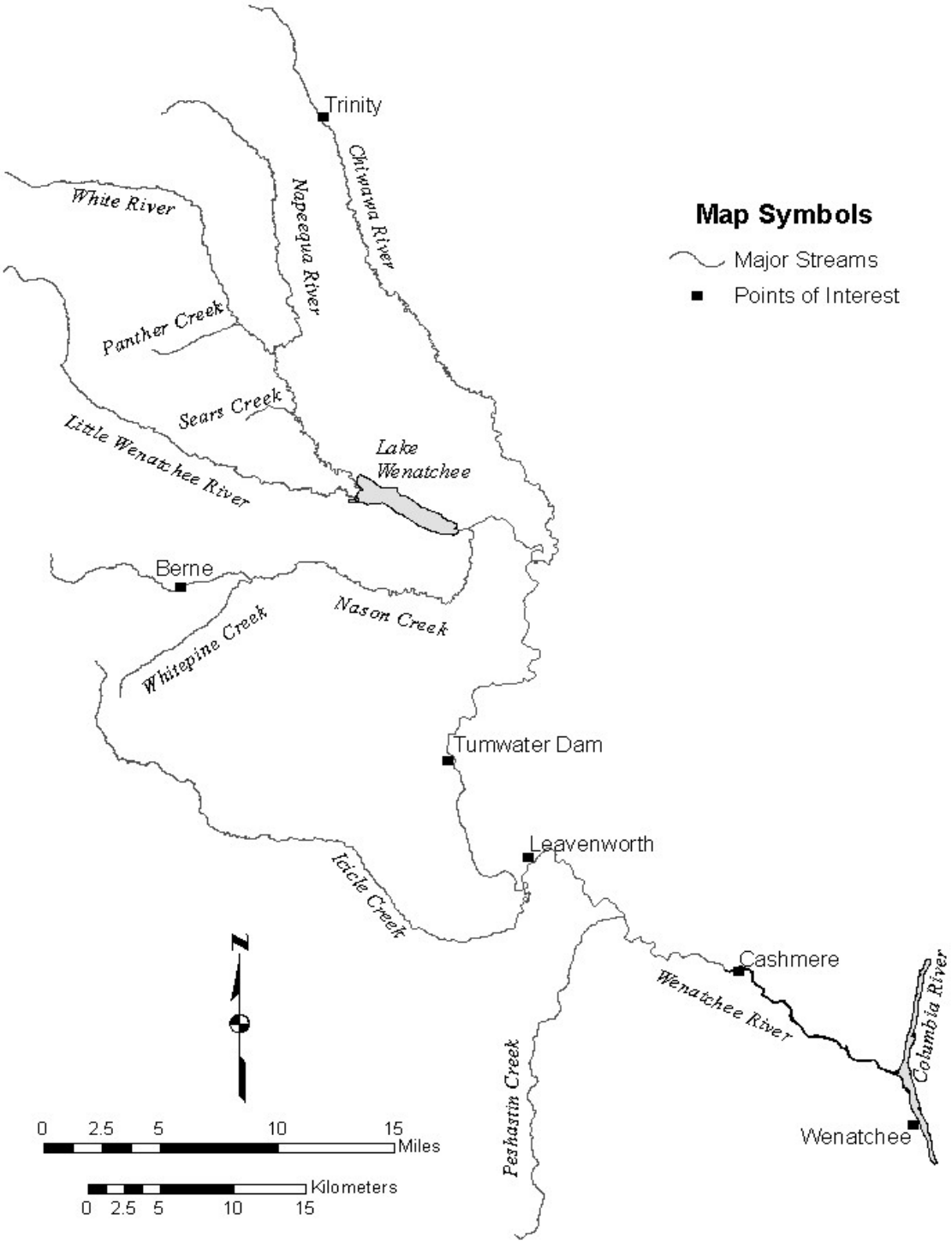

Figure 1. Map of Wenatchee River Basin and spring Chinook spawning tributaries.

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.

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.

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.

Chapter 1

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

Abstract

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

Introduction

Hatcheries can change the demographics of salmonids populations (Carmichael and Messmer 1995, Olson et al. 2004). These changes may result from loss of within population genetic variation or domestication effects in the hatchery environment (Busack and Currens 1995). Quantifying differences in phenotypic traits of hatchery and natural origin salmonids can provide explanations for differences that may be observed through genetic analysis of relative reproductive success (Kostow et al. 2003; McLean et al. 2003). Resolving differences, of lack thereof, in phenotypic traits provides 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 the also the progeny. In addition, the proportion of eggs retained in post-spawned females was examined to assess any differences in egg deposition of hatchery and natural origin female spring Chinook.

Methods and Materials

Adult Trapping

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

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

Broodstock for the Chiwawa spring Chinook program was collected at Tumwater Dam (only hatchery fish with CWT) or a weir located on the Chiwawa River 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 spawned.

Biological Sampling

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

Similar biological data was 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. 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

Run timing of hatchery and natural origin fish were compared a Kruskal-Wallis Test (Zar 1999). Body weights and length (fork and POH) of hatchery and wild fish by age class and sex were compared using two sample t-tests. Sex ratios by age of hatchery and naturally produced adult spring Chinook were compared with a Chi-square test using a Yates (1934) correction for continuity to prevent inflating the probability of committing a Type I error.

Fecundity and egg weight of hatchery and naturally produced females of the same age were compared using two sample t-tests. A linear regression was performed using fish size (POH) and fecundity for both, hatchery and wild fish. Using the regression model, the estimated fecundity for all females examined for egg retention was calculated and used to determine the proportion of eggs retained. The proportion of eggs retained in hatchery and wild carcasses found on the spawning grounds was compared using a two sample t-test of arcsine square root transformed data to meet the assumption of normally distributed data (Zar 1999).

Results and Discussion

Run timing

The trap was operated between 1 May and 9 August 2004. The trap operated passively from 1 May to 22 June due to low fish passage. During this time period, personnel checked the trap and sampled fish several times daily. Active trapping occurred during the day between 23 June and 9 August, and was passively operated only during night when fish passage was low. On several occasions the trap did not operate, due to mechanical failures and maintenance procedures. On 6 and 7 May, the denile was out of operation due to a shaft bearing failure dealing with the supply pump. It was back in service on 8 May after being fitted with a bearing that was more suitable for continuous operation. No fish were trapped during this period. On 28 May, the main ladder slide gate was opened for one hour in an attempt to flush debris and sediment, which had accumulated in the ladder and was affecting water levels in the primary collection chamber. During this period, fish may have traveled past the dam without being trapped and sampled. On 4 June, the entire ladder was dewatered for 10 h while Chelan PUD crews manually removed the sediment build up behind the main ladder slide gate. Some fish may have passed through before the ladder was dewatered and during the initial opening of the gate.

Sediment accumulation was a problem throughout the trapping season. An interim solution to the problem included opening the main ladder slide gate 4-6 cm and flushing the sediment out with a high pressure hose. This process needed to be performed 3 to 4 times each week. During the month of December, Chelan PUD crews installed a removable picket barrier below the main ladder slide gate. This will allow us to completely open the gate in order to flush debris and sediment, without allowing passage upstream of the trap.

A total of 2,261 spring Chinook and 635 minijack (age-2) fish were counted at Tumwater Dam, including nine spring Chinook that were counted on videotapes after trapping had ended (Figure 2). 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 1,332 hatchery adults, 888 natural adults, 32 unknown origin, and 635 hatchery minijacks. Naturally produced spring Chinook were captured at Tumwater Dam between May 18 and August 28 (103 days). Hatchery spring Chinook were captured at Tumwater Dam between 20 May and 11 August (84 days). In addition, hatchery minijack Chinook (31.7% adipose clipped and 68.3% adipose present) were trapped from 10 June to 3 August (Figure 2, Appendix A). No naturally produced minijacks were observed during trapping.

Passage timing of all (age-3 and age-4) hatchery and naturally produced spring Chinook was similar ($P = 0.26$, Table 1). Age-5 fish were not included in this analysis due to low abundance. A closer examination of passage timing based on age and origin was conducted because of observed differences in the age composition of the hatchery and origin fish. No difference was detected in the passage timing at Tumwater Dam of age-2,

age-3, and age-4 hatchery fish ($P = 0.79$). However, natural origin age-4 fish had a significantly earlier run timing than age-3 natural origin fish ($P < 0.001$). Differences were also detected in the passage timing of age-3 hatchery and natural origin fish ($P < 0.01$). Age-4 natural origin fish had a significantly earlier run timing than hatchery age-4 fish ($P < 0.01$, Figure 3). Run timing differences observed in the Wenatchee Basin are similar to those found in the Yakima Basin. Knudsen et al. (2005) reported that adults had an earlier run timing than jacks, but no difference was detected in the run timing of hatchery and natural origin jacks. Furthermore, natural origin Yakima spring Chinook had an earlier run timing than hatchery origin fish.

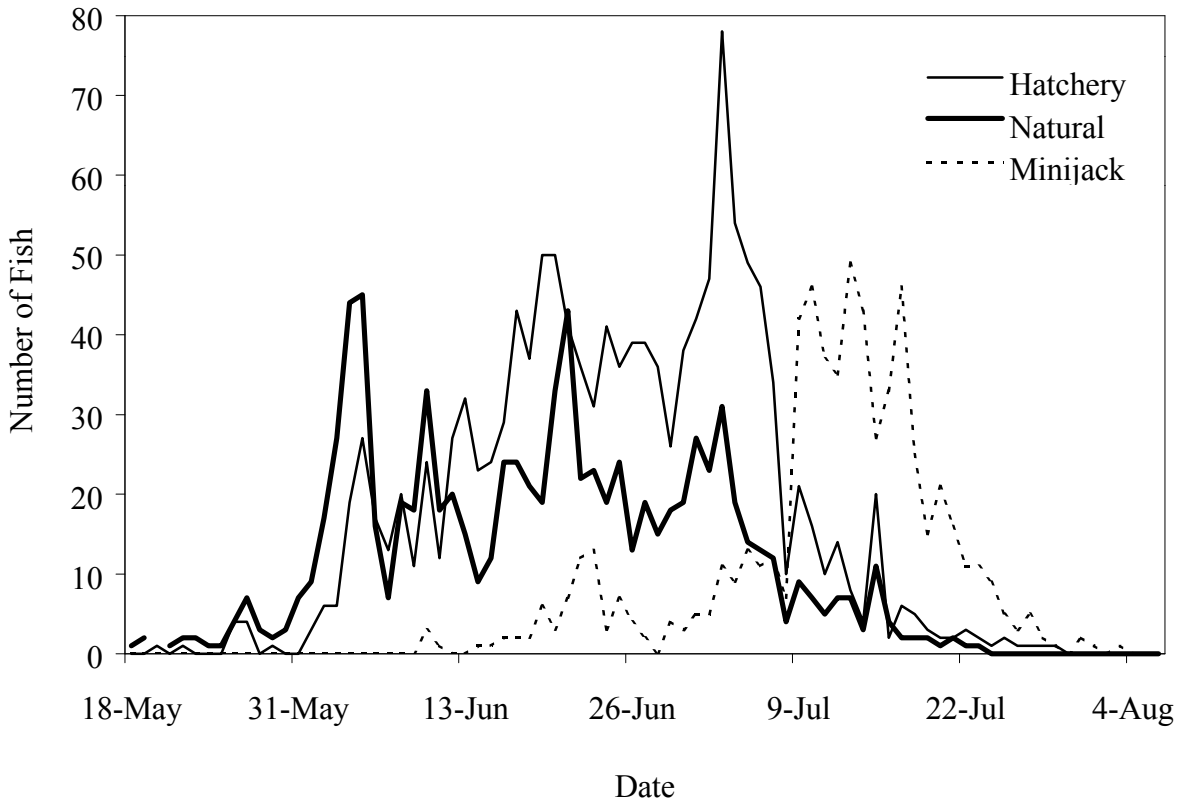

Figure 2. Run timing of adult hatchery and naturally produced spring Chinook and minijack Chinook at Tumwater Dam in 2004.

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

Origin/Age	Cumulative Run Timing		
	10%	50%	90%
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

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

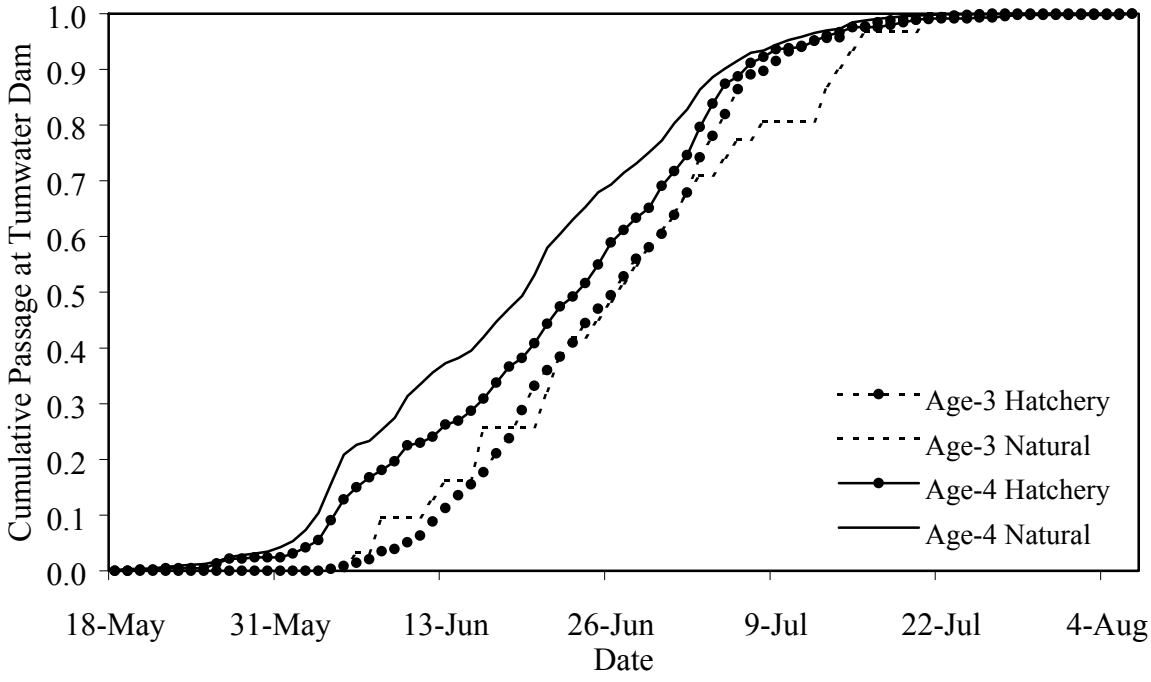

Figure 3. Cumulative passage of age-3 and age-4 spring Chinook at Tumwater Dam in 2004.

Age Composition

Ages were determined through scale samples for 1,273 and 888 hatchery and natural adults respectively (Table 2). A significant difference was found in the age and sex composition of hatchery and natural origin fish ($\chi^2 = 1134$, df = 5, $P<0.001$). In addition, all 635 hatchery minijack fish were scale sampled and determined to be age-2 fish. A

difference in age composition of hatchery fish was attributed to the number of hatchery fish released. The Chiwawa spring Chinook Program did not release any 1999 brood fish. Hence, all age-5 hatchery fish migrating upstream of Tumwater Dam would be considered strays. Lack of age-5 fish in the natural population was the result of low escapement in 1999. A comparison of age composition by brood year will be conducted once all age classes have returned.

Table 2. Age composition of Wenatchee River spring Chinook sampled at Tumwater Dam in 2004.

Origin	Total Age			*N*
	3	4	5	
Hatchery	64.1%	35.4%	0.5%	1,273
Natural	3.5%	95.2%	1.3%	888
All	39.2%	60.0%	0.8%	2,161

Sex Ratio

Analysis of sex ratios was limited to age-4 fish because all age-5 hatchery fish are strays. Sex determination at Tumwater Dam was based on morphological characteristics early in the year without secondary sexual characteristics and may not be accurate. A comparison of the sex determined at Tumwater Dam to those fish subsequently recovered on the spawning grounds and during hatchery spawning found that sex determination was correct 96.6 % for female and 82.2% for males. After correction, the male to female ratio of the age-4 natural and hatchery fish was 0.8 to1.0 and 0.3 to 1.0, respectively (Table 3). Age-4 hatchery fish had significantly lower proportion of males than age-4 natural origin fish ($\chi^2 = 44.01$, $P < 0.001$). A lower proportion of older aged males suggest that hatchery males mature at an earlier age than natural origin males. For example, the number of hatchery age-2 and age-3 males sampled at Tumwater Dam was much greater than natural origin fish. When all age classes from each brood year has been sampled at Tumwater Dam, age at maturity and sex ratios of hatchery and natural origin fish will be compared.

Table 3. The estimated number of male and female age-4 spring Chinook counted at Tumwater Dam and the corrected number based on known sex.

Origin	Sex	Tumwater Dam	Corrected Number
Hatchery	Male	115	107
	Female	343	351
Natural	Male	438	374
	Female	407	471

Size-at-Age

Differences in size were detected between age-3 and age-4 hatchery and natural origin fish (Table 4). Both male and female age-3 and age-4 hatchery fish were significantly greater in fork length and weight than natural origin fish (two sample t-tests, $P<0.05$). Differences in size may be attributed to differences in size at release. Naturally produced yearling spring Chinook smolts typically range in length between 90 and 100 mm, while hatchery fish are released at a length between 135 and 140 mm (WDFW, unpublished data). Interestingly, these findings were opposite of what was observed in the Yakima Basin where hatchery origin fish were smaller than natural origin (Knudsen et al. 2005).

Data collected in previous years as part of the hatchery program and from the spawning grounds was used to determine if the differences in length observed in 2004 were present since the hatchery program was initiated in 1989. Data sets for natural (1991 – 2004) and hatchery (1997 – 2004) were initially analyzed separately using a one-way ANOVA, regardless of gender. Not all years were used in the analysis because hatchery fish were not produced every year (i.e., 1995 or 1999) or due to small sample size as a result of low escapement. No difference was detected in the POH of natural origin fish (df = 6, F = 1.82, P = 0.09) or hatchery fish (df = 3, F = 0.16, P =0.92). The number of age-4 fish sampled in 1997, 2001, 2002, and 2004 was large enough to examine the differences between year, gender, and origin. An ANOVA was conducted on the four years of data and significant differences were found for gender, origin, and the interaction term gender × year (Table 5). In all years, hatchery fish were greater in length than natural origin fish (Figure 4). This trend was consistent for both females and males, except in 2004 (Figure 5).

Table 4. Mean fork length (SD) and weight (SD) at age for Wenatchee River spring Chinook sampled at Tumwater Dam in 2004.

Origin	Sex	*N*	Age-3	*N*	Age-4	*N*	Age-5
				Fork length (cm)			
Hatchery	Male	821	52.9 (5.9)	115	80.2 (6.6)	2	98.0 (1.4)
	Female	5	62.2 (4.9)	343	79.6 (4.5)	4	82.8 (8.4)
	All	826	53.0 (6.0)	458	79.7 (5.1)	6	87.8 (10.3)
Natural	Male	31	50.7 (5.4)	438	78.5 (6.5)	5	91.6 (4.8)
	Female	0		407	77.9 (4.0)	7	91.3 (5.7)
	All	31	50.7 (5.4)	845	78.3 (5.5)	12	91.4 (5.1)
				Weight (g)			
Hatchery	Male	821	1.76 (0.66)	115	5.49 (1.40)	2	9.10 (0.42)
	Female	5	2.85 (0.75)	343	5.51 (0.98)	4	6.15 (1.84)
	All	826	1.77 (0.66)	458	5.50 (1.10)	6	7.13 (2.10)
Natural	Male	31	1.52 (0.56)	438	5.33 (1.27)	5	7.86 (1.01)
	Female	0		407	5.29 (0.81)	7	8.22 (1.77)
	All	31	1.52 (0.56)	845	5.31 (1.07)	12	8.08 (1.46)

Table 5. Analysis of variance table for POH of age-4 hatchery and natural origin Chiwawa spring Chinook collected for broodstock or on the spawning grounds in 1997, 2001, 2002, and 2004.

Effect	SS	df	MS	*F*	*P*
Intercept	2898639	1	2898639	138935.6	0.000
Gender	177	1	177	8.5	0.004
Year	154	3	51	2.5	0.060
Origin	334	1	334	16.0	0.000
Gender × Year	516	3	172	8.2	0.000
Gender × Origin	30	1	30	1.5	0.227
Origin × Year	61	3	20	1.0	0.400
Gender × Origin × Year	148	3	49	2.4	0.070
Error	42185	2022	21		

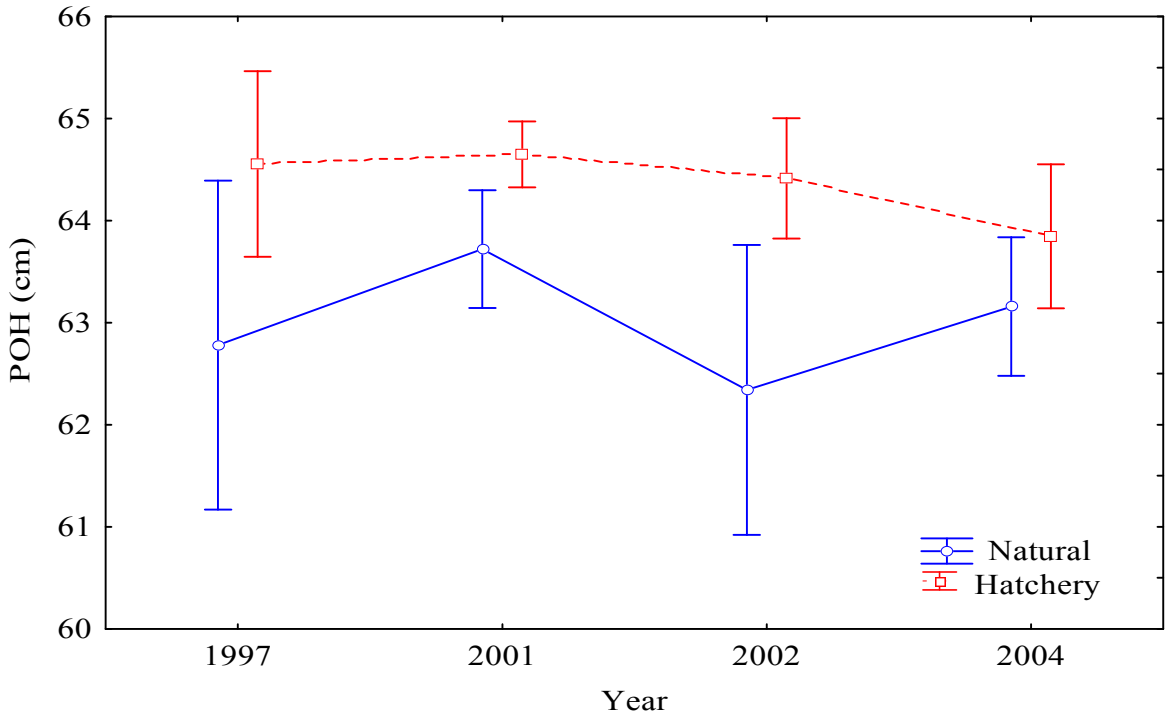

Figure 4. Mean post-orbital to hypural plate length of age-4 Chiwawa spring Chinook sampled on the spawning grounds and as broodstock. Vertical bars denote 95% confidence intervals.

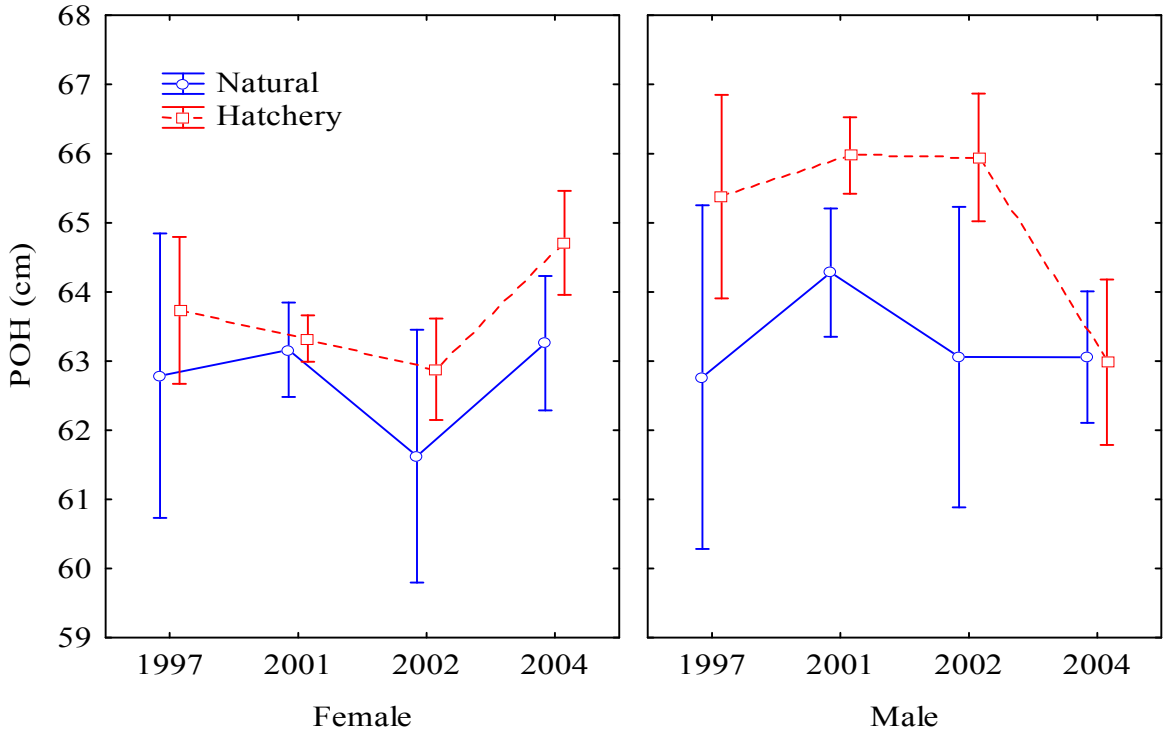

Figure 5. Mean post-orbital to hypural plate length of male and female age-4 Chiwawa spring Chinook sampled on the spawning grounds and as broodstock. Vertical bars denote 95% confidence intervals.

Fecundity and Egg Weight

A total of 293 spring Chinook were collected and held at Eastbank Fish Hatchery for broodstock in 2004. Age and origin was determined through scale analysis and CWT decoding for 193 and 93 hatchery and wild fish, respectively (Table 6). No age-5 hatchery fish were present because broodstock was not collected in 1999 due to low escapement. Hence, the age-5 component of the naturally produced fish was also lower than expected due to low escapement.

Table 6. Age composition of Chiwawa spring Chinook at Eastbank Fish Hatchery in 2004.

Origin	Total Age			*N*
	3	4	5	
Hatchery	37.3%	62.7%	0.0%	193
Natural	4.3%	92.5%	3.2%	93
All	26.6%	72.4%	1.0%	286

Fecundity was determined for 83 hatchery and 37 naturally produced age-4 female spring Chinook. The mean (standard deviation, SD) fecundity of the hatchery and naturally produced females was 4,676 (901) and 4,833 (747), respectively. Mean egg weight (SD) of the hatchery fish was 0.22 (0.03) g and 0.21 (0.03) g for the naturally produced fish. No difference was found between in the mean fecundity ($P = 0.36$) and egg weight ($P = 0.33$) of hatchery and naturally produced age-4 fish. However, the slopes of the fecundity regression lines were significantly different (df = 116, $t = -1.607$, $P < 0.05$). The fecundity of hatchery fish at a given length was lower than that of natural fish (Figure 6). These results explain the reason in which hatchery fish are significantly larger in length, but do not have a greater fecundity.

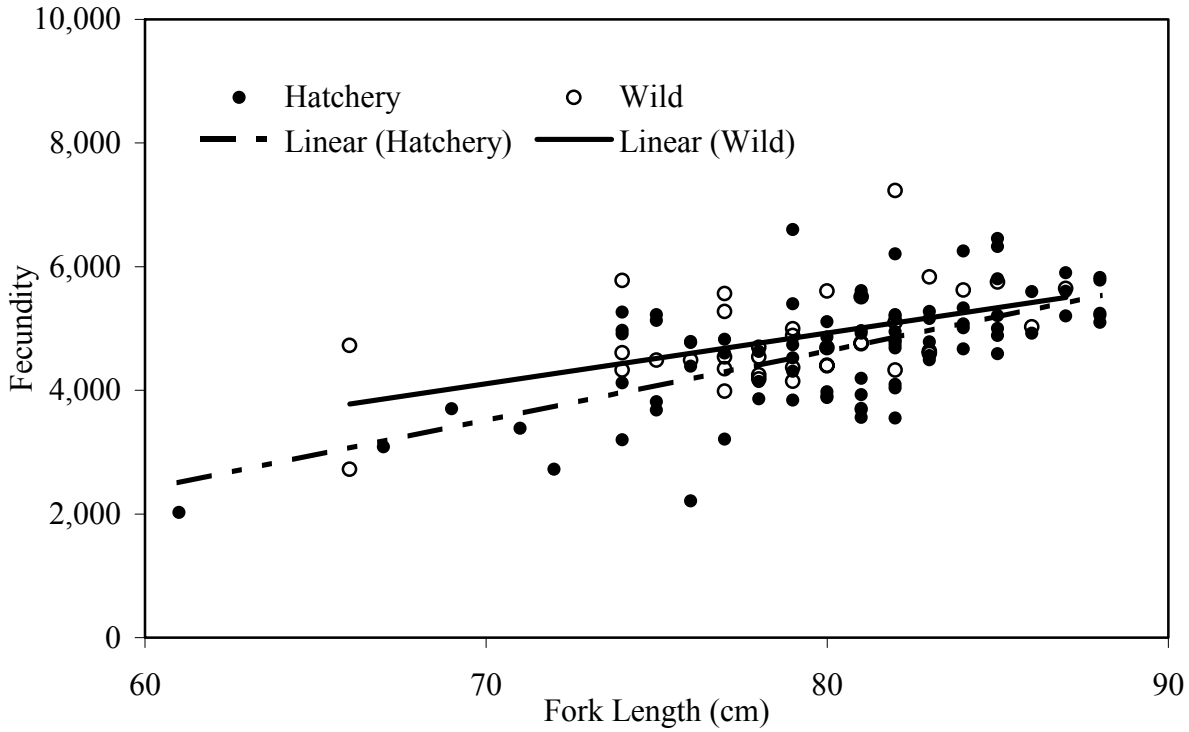

Figure 6. The relationship between fecundity and fork length of age-4 hatchery and natural origin Chiwawa spring Chinook.

Egg Retention

A total of 44 hatchery and 97 naturally produced fish were examined to determine the number of eggs retained in the body cavity after spawning (Table 7). Fecundity of females examined for egg retention was estimated using a linear regression model ($r^2 = 0.53$, $P < 0.001$) derived from hatchery broodstock (Table 8). The estimated mean (SD) proportion of eggs retained for hatchery and naturally produced fish was 0.01 (0.04) and 0.003 (0.01), respectively. No difference was detected in the proportion of eggs retained between hatchery and naturally produced fish ($P = 0.25$).

Table 7. Number of female spring Chinook examined and the mean number of eggs retained in the body cavity after spawning in 2004.

Stream	Hatchery			Naturally produced		
	N	Mean	SD	*N*	Mean	SD
Chiwawa	22	63	255	32	13	53
Nason	14	37	75	56	12	42
Wenatchee	6	10	6	3	2	4
White	2	10	13	5	5	11
Little Wenatchee	0	--	--	1	8	--

Table 8. Estimated mean fecundity of female spring Chinook examined after spawning in 2004.

Stream	Hatchery			Naturally produced		
	N	Mean	SD	*N*	Mean	SD
Chiwawa	22	4,575	487	32	4,241	434
Nason	14	4,450	645	56	4,094	531
Wenatchee	6	4,431	514	3	4,629	687
White	2	3,770	280	5	4,471	401
Little Wenatchee	0			1	3,571	0

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 1,989 adults and jacks and 635 hatchery minijacks (Table 9).

Table 9. Distribution of spring Chinook detected at Tumwater Dam in 2004.

Origin	Below Tumwater Dam		Above Tumwater Dam			
	Fallback	Eastbank Hatchery	Prespawn Mortality	Chiwawa Weir	Spawning Grounds	Total
Hatchery	11	148	2	48	1,124	1,333
H. minijack	0	0	0	0	635	635
Natural	0	4	7	93	792	896
Unknown	0	0	0	0	32	32

Summary and Conclusions

Adult trapping at Tumwater Dam was successful this year, however modifications have been scheduled that should minimize or eliminate sediment accumulation. Operation of the trap will also be modified to ensure trap related mortality (*N*=3) is reduced or eliminated. PIT tag retention by adult spring Chinook was not as high as anticipated (85%). Additional training on proper technique and placement will be conducted early in the next field season. In 2005, a larger PIT tag (20mm) will be used for adults, while the standard 12mm PIT tag will be used for jacks and precocious males. The 20mm PIT tags should also increase the probability of detection on the spawning grounds.

Differences in size at return examined over time could prove useful in detecting affects of hatchery fish on size at return of natural origin fish. Data suggests that the size of natural origin fish has not changed since the hatchery fish have been present. Size at age of hatchery origin spring Chinook salmon adults in the Tucannon River was 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. 2005). Differences observed in the Wenatchee Basin may be because of the larger size disparity of hatchery and natural origin smolts.

In 2004, the natural escapement and hatchery production levels affected the differences in age distribution of hatchery and natural origin fish. In future years, direct comparisons between brood years (all age classes) instead of run year would provide insight into the age at return differences in hatchery and natural origin fish.

Acknowledgements

The Bonneville Power Administration provided funding for this project. We thank Jonathan McCloud, the administrator for this project, for supporting our unique contracting requirements. We would like to thank Chelan County Public Utility District and the Yakama Nation for providing the expertise and funding for the design and construction of the Tumwater Dam fish trapping facilities. We would also like to thank Clint Deason, Nathan Smeltzer, Brain Johanson, Nathan Dietrich, and Sara Carani for their assistance in operation of Tumwater Dam fish trap and collecting data from all fish trapped. Mike Tonseth and the hatchery personnel at Eastbank Complex provided the data on the hatchery broodstock. John Sneva of the WDFW Scale Lab read all the scale samples. Leavenworth National Fish Hatchery and the Mid-Columbia Fisheries Resource Office (USFWS) personnel provide DNA samples of fish outplanted in Peshastin Creek.

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.

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, 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.

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.

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.

Zar, J.H. 1999. Biostatistical Analysis, 4th edition. Prentice Hall, Upper Saddle River, New Jersey.

Chapter 2

Estimating the reproductive success of naturally spawning hatchery and natural origin spring Chinook in the Wenatchee River Basin

Abstract

Hatcheries have been increasingly asked to contribute to conserving natural salmon populations, as well as continuing to produce fish to mitigate for lost harvest opportunities. However, 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 on the Wenatchee River we will use a DNA-based pedigree analysis to (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. DNA was extracted and analyzed for all spring Chinook trapped at Tumwater Dam or released as adults into the natural environment in 2004. Results will not be available until after smolts are sampled in 2006.

Introduction

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

A key biological uncertainty about the effects of hatchery production on natural populations is the degree to which hatchery produced fish can reproduce in the natural environment. Accurately measuring the relative biological causes for 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, the 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 impeding) the recovery of salmon populations. Determining the relative reproductive success of hatchery fish that stray from traditional hatchery

programs is also important. Stray hatchery fish can often mask the status of natural populations because their reproductive success is unknown, and may lead to reduced short and long-term natural productivity due to genetic deterioration of the natural population as a result of interbreeding between naturally produced fish and some hatchery strays. By directly quantifying the reproductive success of stray hatchery fish in the natural environment relative to that of fish from the natural population, the viability of natural populations receiving substantial stray hatchery fish can be much more accurately evaluated.

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.

Methods

Sampling sites - Washington Department of Fish and Wildlife (WDFW) personnel obtained fin clips from 2,975 spring-run Chinook as they were being passed over the Tumwater Dam fish weir from May to August 2004 (See Chapter 1). These samples represent adult spring-run fish returning to spawn in the tributaries of the Wenatchee River, such as, the Chiwawa River, Nason Creek, and the White River. Personnel of WDFW also provided dried fin-clip samples from 350 Leavenworth National Fish Hatchery (Carson stock) spring-run Chinook adults that had been out-planted from Peshastin Creek in 2004 and 192 Wenatchee R. summer-run Chinook adults collected during 2004.

Initial selection of microsatellite loci used for parentage assignment - 'Preparental' simulations were run with the software program PAPA 2.0 (Duchesne et al. 2002) prior to the collection of potential parents to provide a statistical basis for the choice of loci to be genotyped for parentage assignment. The PAPA software package performs parental allocation based on likelihood methods and comprises simulators that permit statistical assessments of allocation accuracy (Duchesne et al. 2002). The breeding likelihood (Sancristobal and Chevalet 1997) is defined as follows: given an offspring's genotype, the likelihood of a parental pair of genotypes [i.e.- two parents selected from a pool of putative parents] is defined as the probability of that parental pair producing that offspring's genotype. Simulations to ascertain the minimum number of microsatellite loci required to correctly assign parentage were carried out based on a sub-sample of 96 adults collected during the 2004 Wenatchee River spring-run spawning migration. The sub-sample of fish was genotyped as detailed in the following section. The 14

microsatellite loci used to analyze the sub-set of adults included the 11 loci in Table 1 as well as Ots208b (Grieg et al. 2003), Oki23mmbl (Spidle, A.P., T.P. Quinn, and P. Bentzen,P., unpublished), and Omy1011 (unpublished locus used in Bentzen et al. 2001). The allelic frequencies of the sub-sample were provided as input for PAPA to generate pseudo-parents. The simulations are a three step process. First, pseudo-collected and uncollected parental genotypes are generated. Second, the two sets of parental genotypes are combined to breed/create pseudo-offspring. Third, pseudo-offspring are allocated to parental pairs belonging to the pseudo-collected parents only. The simulations were evaluated using sexed and non-sexed conditions of the parents, and assumed a 2% genotyping error rate. Sexed and non-sexed parent simulations used 500 each of pseudo-sires and pseudo-dams, and 1000 pseudo-parents, respectively. For the purposes of the simulations a closed system was assumed. Namely, that all putative parental fish would be sampled and that zero uncollected parents are present. Multiple simulations using each set of parameters were run, and the summarized results of each set of simulations were compared to determine the minimum number of loci required to correctly allocate a juvenile's genotype to a putative parental pair of genotypes.

Microsatellite genotyping - Genomic DNA was extracted from fin clips using a QIAge DNA tissue extraction kit, eluted into a 96-well sample plate, and quantified using a FL_X 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^{o}C until needed. Unused portions of fin-clips have been appropriately cataloged and stored. Individuals were genotyped at 11 previously developed di- and tetranucleotide repeat microsatellite loci: Ots3, Ots104, Ots201b, Ots211, Ots213, Ots2M, Ots10M, OtsD9, Oke4, Ogo4, and Ssa408 (references provided in Table 1). The growth hormone pseudogene marker (GH-Ψ) (Du et al. 1993) was used to identify the genetic sex of each individual. Microsatellite alleles were amplified by polymerase Chain reaction (PCR) assays using 15 ng of genomic DNA, 1.75 or 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM of each PCR primer, 0.25 Units of T_{aq} DNA polymerase (Promega Biosciences, San Luis Obispo, California), 20 mM Tris (pH 8.5) and 50 mM KCl in 10 μl volumes. The forward primer of each PCR primer pair was labeled with a fluorescent phosphoamidite (FAM, NED, PET, or VIC). Tetrad® thermal cyclers (MJ Research, San Francisco, CA) were programmed with the conditions, shown in Table 1, which permitted pairs of loci to be co-amplified (duplexed) into single PCR reactions. Each set of PCR conditions included a lengthy final extension cycle used to "fill-in" the +A nucleotide additions T_{aq} 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).

Characterization of microsatellite loci – Allele frequencies, the total number of observed alleles, expected heterozygosity (H_e) under Hardy-Weinberg equilibrium (HWE), observed heterozygosity (H_o), and F_{IS} values (and their 95% confidence intervals) for each of the 11 microsatellite loci were calculated using the program GENETIX version

4.05 (Belkhir et al. 2000, available at http://www.University-montp2.fr/~genetix/genetix.htm). Pair-wise comparisons of loci for linkage disequilibrium were made by estimation of exact P-values by the Markov chain method (Guo and Thompson 1992) as implemented in GENEPOP (dememorization steps 1000; 50 batches; 1000 iterations per batch). Sequential Bonferroni adjustments to α were applied when appropriate for simultaneous tests to decrease the chance of erroneously rejecting null hypotheses (Rice 1989).

Results

Initial selection of microsatellite loci used for parentage assignment – As the number of microsatellite loci used by the simulation was increased from seven to eleven, the proportion of pseudo-offspring correctly allocated to the true pseudo-parents also increased (Table 1). Once nine loci were used, greater than 98% of the offspring were consistently allocated to non-sexed parents in the simulations. Correct allocation appeared was maximized by using 11 loci and by establishing the sex of the pseudo-parents (data not shown).

Microsatellite genotyping – To date 2,975 alleged spring-run and 192 known summer-run Chinook salmon adults collected during the 2004 spawning migration have been preliminarily genotyped up to 11 microsatellite loci and the genetic sex marker, growth hormone psuedogene. Due to a faulty DNA primer produced by a vendor, one locus, Oke4, has not been typed in every fish examined. Approximately 800 spring-run adults remain that need to be typed at Oke4.

Characterization of microsatellite loci – Characterization of microsatellite loci was performed using genotype data from 92 adult spring-run fish that had been typed for at least nine out of 11 loci. The remaining samples are ongoing quality control checks. The observed number of alleles per locus (n) ranged from four (OtsD9) to 31 (Ots104), and mean expected and observed heterozygosity over all 11 microsatellite loci (0.744 and 0.763, respectively) were in close agreement with each other (Table 2). No significant ($P<0.0045$, after Bonferroni correction) deviations from Hardy-Weinberg equilibrium were detected (Table 3). Analysis of linkage disequilibrium detected a significant ($P<0.0045$) association of alleles in only four out of 55 (~7%) pair-wise comparisons of loci (Table 4).

Discussion

Initial selection of microsatellite loci used for parentage assignment - A sub-set of 11 microsatellite loci (Table 2) were chosen that would allow a high degree of accuracy (>98%) to correctly allocate a sampled juvenile back to the pair of parental genotypes most likely to have produced that offspring's multilocus genotype. Indeed, greater than 98% accuracy was obtained even when only nine loci were used. A marker of genetic sex, the growth hormone psuedogene (Du et al. 1993), was also included for the data

collection of the adult samples. While it is not mandatory to determine the sex of each putative parent in order to carry out parentage assignment of offspring, doing so adds power to the allocation procedures implemented by PAPA 2.0. Using two additional polymorphic loci in addition to the sex marker should provide an advantage by maintaining a high degree of accuracy of parentage allocation since, unlike the simulations of a closed system, not every putative parent in the Wenatchee R. spring-run Chinook population can be sampled (i.e.- the presence of unsampled adults decreases the probability that you can find both parents of a given offspring).

Microsatellite genotyping – While genotypic data has been collected on 2,975 alleged spring-run and 192 known summer-run Chinook adults some work remains to be performed. Individuals that had not been successfully genotyped for all 11 loci will be reanalyzed to fill in gaps in its multilocus genotype. Similarly, any sample from which genomic DNA was not successfully extracted (and could not be genotyped) will be re-extracted and analyzed. The 350 spring-run Chinook adults that had been out-planted from Peshastin Creek have only been extracted. Quality control measures to insure consistency of genotype scoring across the entire dataset and to verify the accuracy of genotyping are currently being employed.

Characterization of microsatellite loci – The 11 selected microsatellite loci generally meet the assumptions (Hardy-Weinberg equilibrium and no Linkage Disequilibrium) for assignment testing and are thus suitable for parentage assignment. The genetic loci used to assign parentage must meet several criteria, otherwise assignment based on data from these markers will be less accurate. First, microsatellites must exhibit enough diversity (numbers of alleles and genotypic variation within the population) to provide adequate information needed to exclude non-parental fish. Second, the alleles of each genetic locus must be inherited independently of alleles of other loci. Since data from each locus is considered to be an independent test of parentage, transmission of an allele at one locus to the next generation must occur independently of the alleles of another locus. Failure of this condition to be met between two loci is called Linkage Disequilibrium.

Conclusions

The 11 selected microsatellite loci are suitable for use in parentage assignment since they appear to be easily typed and appear to be segregating independently. Depending upon how the set of microsatellite loci actually perform with respect to correctly assigning parentage of offspring more loci may need to be evaluated and added to those already chosen. This may be the case if a subset of the originally selected loci provides poor resolving power needed to exclude any given relationship based on the allele frequencies in the spring-run population. We are currently conducting more extensive simulations using the data collected in 2004 to determine if more loci will be necessary or not.

The data collected and analyses performed, to date, are preliminary. Genotypic data is still being gathered for adults collected in 2004 and appropriate quality control must be performed to assure genotyping consistency and accuracy. Missing data, and/or

irregularities in the actual scoring of microsatellite alleles will increase the error rate of assigning offspring to sampled adults. Once a full and accurate data set is available for the 2004 adult fish, further in depth analyses of potential population structure and the capacity of the loci for parentage assignment will be performed.

Summer-run Chinook – Prior to conducting a parentage analysis on the spring-run population, we will use genetic mixture analysis of the adults sampled in 2004 to identify summer-run Chinook that may have been inadvertently included in our samples. The sample set of alleged spring-run Chinook salmon adults that had been passed over the Tumwater Dam fish weir in 2004 likely contains some summer-run fish, especially in the latter part of the season. Since the tail end of the spring-run adult migration period over laps with the beginning of the summer-run adult migration, fish from both seasonal runs are likely to have been sampled, although the staff operating the weir attempted to only sample spring-run fish. Because our focus is on the spring-run population, fish positively identified as summer-run Chinook will be removed from the dataset prior to parentage analysis.

References

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., et al. 2000. GENETIX, program for Windows™ for the genetic analysis of populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, University of Montpellier II, Montpellier (France).

Bentzen, P., J.B. Olsen, J.E. McLean, and T.P. Quinn. 2001. Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. The Journal of Heredity 92 (2): 1278-136.

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.

Cairney, M., J.B. Taggert, 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.

Du, Shao Jun, R.H. Devlin, C.L. Hew. 1993. Genomic structure of growth hormone genes in Chinook salmon (*Oncorhynchus tshawytscha*): Presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-PSI. DNA and Cell Biology 12 (8): 739-751.

Duchesne, Pierre, Marie-Helene Godbout, and Louise Bernatchez. 2002. PAPA (package for the analysis of parental allocation): a computer program for simulated and real parental allocation. Molecular Ecology Notes 2: 191-193.

Greig, C., Davis P. Jacobson, Michael A. Banks. 2003. New tetranucleotide microsatlellites for fine-scale discrimination among endangered Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology Notes 3: 376-79.

Greig, C., 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.

Naish, K.A., 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.

Olsen, J.B., P. Bentzen, J.E. Seeb. 1998. Characterization of seven microsatellite loci derived from Pink salmon. Molecular Ecology 7: 1087-1089.

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.

Sancristobal, M. and C. Chevalet. 1997. Error tolerant parent identification from a finite set of individuals. Genetical Research 70: 53-62.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution. 38**:**1358-1370.

Table 1- Simulation results from PAPA 2.0 (Duchesne, et al. 2002) for initial choice of microsatellite loci for use in parentage analysis. Simulations were based on the following parameters: 1000 pseudo-collected parents (non-sexed), zero uncollected parents, 200 pseudo-offspring generated at each iteration, and a genotyping error rate of 2% across all loci examined. Definitions for decisions regarding allocations allocation are: (*i*) correct- allocated parent is a true parent of pseudo-offspring, (*ii*) ambiguous- more than one parental pair reached the maximum likelihood of having produced a given off-spring, (*iii*) null- all parental pairs have zero likelihood of having produced a given off-spring. Specific sets of loci (A-E) used in the simulations are listed below the table.

		Type of decisions regarding allocations			**Correctness among allocated pseudo-offspring**		
# Loci	**Choice of Loci**	**Correct**	**Ambiguous**	**Nulls**	**2 correct adults**	**1 correct adult**	**0 correct adults**
7	A	0.950	0.050	0.000	0.863	0.095	0.042
8	B	0.967	0.033	0.000	0.924	0.066	0.010
9	C	0.983	0.017	0.000	0.912	0.071	0.017
11	D	0.995	0.005	0.000	0.960	0.035	0.005
11	E	1.000	0.000	0.000	0.980	0.020	0.000

Choice of loci:

A) Ogo4 Oke4 Ots104 Ots10M Ots2M Ots211 Ots213

B) Ogo4 Oke4 Ots104 Ots10M Ots2M Ots3 Ots208b Ots213

C) Ogo4 Oke4 Ots104 Ots10M Ots2M Ots3 OtsD9 Ssa408 Ots208b

D) Ogo4 Oke4 Ots104 Ots10M Ots2M Ots3 OtsD9 Ssa408 Ots201b Ots208b Ots211

E) Ogo4 Oke4 Ots104 Ots10M Ots2M Ots3 OtsD9 Ssa408 Ots201b Ots208b Ots213

Table 2 - Thermocycler conditions and references for 11 microsatellite loci and one sex-specific locus (GH-Ψ) used to genotype 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 ^{o}C for 150 seconds, amplification cycles of 95 ^{o}C for 40s, X ^{o}C annealing temperature for 40s, 72 ^{o}C for 40s, and a final extension cycle of 60 ^{o}C for 45 min.

Duplexed Microsatellite Loci Pairs	Annealling temperature (^{o}C)	[Mg^{+2}] (mM)	# PCR Amplification Cycles	Reference
Ots2M GH-□	60	2.00	40	Greig and Banks 1999 Du et al. 1993
Ogo4 Ots211	60	1.75	32	Olsen, Bentzen, and Seeb 1998 Grieg, Jacobson, and Banks 2003
Ots213 Ots10M	54	1.75	32	Grieg, Jacobson, and Banks 2003 Greig and Banks 1999
Ssa408 Ots201b	60	2.00	40	Cairney, Taggert, Hoyheim 2000 Grieg, Jacobson, and Banks 2003
Ots3 Ots104	48	1.75	40	Banks et al. 1999 Nelson and Beacham 1999
Oke4 OtsD9	54	1.75	32	Buchholz et al. 1999 Naish and Park, 2002

Table 3: Population genetic statistics of 11 microsatellite loci for the 2004 Wenatchee River Spring-Run Chinook adults. Observed number of alleles (n), expected and observed heterozygosities (H_e and H_o, respectively), and Hardy-Weinberg equilibrium (F_{IS}, Weir & Cockerham 1984) are shown. The 95% confidence intervals for F_{IS} were calculated by bootstrapping 500 times using the software package GENETIX4.05 (Belkhir et al. 2000). The p-values for were calculated using the software package GenePop3.4 (Raymond and Rousset 1995). This data was calculated using a subset of 92 individuals.

Msat Locus	n	He	Ho	FIS (95% CI)	P-value
Ogo4	12	0.843	0.911	0.075 (-0.155 - 0.001)	0.217
Ots10M	5	0.536	0.554	-0.028 (-0.217 - 0.139)	0.523
Ots211	21	0.923	0.900	0.030 (-0.040 - 0.085)	0.095
Ots213	25	0.905	0.891	0.020 (-0.041 - 0.076)	0.006
Ots2M	5	0.552	0.549	0.010 (-0.176 - 0.206)	0.537
Oke4	7	0.595	0.554	0.075 (-0.059 - 0.206)	0.302
Ots104	31	0.954	0.955	0.005 (-0.036 - 0.051)	0.072
Ots201b	25	0.918	0.922	0.001 (-0.059 - -0.057)	0.218
Ots3	7	0.620	0.692	-0.111 (-0.234 - 0.017)	0.064
OtsD9	4	0.451	0.630	0.054 (-0.102 - 0.186)	0.104
Ssa408	20	0.885	0.837	0.060 (-0.031 - 0.152)	0.472
All 11 Loci		0.744	0.763	0.005 (-0.030 - 0.026)	0.005

Table 4: Pair-wise comparisons of Linkage Disequilibrium (LD) for 11 microsatellite loci used to genotype the 2004 Wenatchee River Spring-Run Chinook adults. Out of 55 pair-wise comparisons only four (~7%) indicated non-independent assortment of alleles. Pair-wise comparisons were made using the software package GenePop v3.04 (Raymond and Rousset 1995). Statistical significance ($p<0.0045$) of Chi-squared (X^2) values was evaluated using a correction for multiple comparisons (Rice 1989). The p-value for significant non-independent assortment of alleles between two loci was determined by dividing α (0.05) by the total number of loci (11) being compared.

Locus pair	X^2	df	P-value
Ogo4 & Oke4	Infinity	2	$p<0.0001$
Ots213 & Oke4	Infinity	2	$p<0.0001$
Ots2M & Oke4	Infinity	2	$p<0.0001$
Ogo4 & Ots3	Infinity	2	$p<0.0001$

Chapter 3

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

Abstract

Spawning ground surveys in the upper Wenatchee River basin were used to evaluate spawning distribution and redd microhabitat characteristics of hatchery and naturally produced fish. In 2004, the composite population of spring Chinook redds were distributed similarly to that of years past. A total of 491 redds were found upstream of Tumwater Dam, of which the female origin was identified on 290 redds. Based on redd counts, the survival of adult and jack spring Chinook from Tumwater Dam to the spawning grounds was estimated at 83%. Natural origin fish spawned in a greater number of tributaries and tributary reaches than hatchery origin fish. Hatchery origin fish tended to spawn in an area near the acclimation site or in relatively low elevation portions of tributaries. Microhabitat variables were measured on 186 redds, which included 42 redds and 144 redds constructed by hatchery and natural origin females, respectively. Differences were found in the redd width of hatchery and natural origin females. No differences were found in the estimated age composition or the proportion of hatchery fish of the spawning population compared to the expected population sampled at Tumwater Dam.

Introduction

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

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

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

Methods and Materials

Spawning ground surveys

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

When new redds were found, the origin and fork length of the female was determined if possible. Origin was determined visually by the presence or absence of the adipose fin using binoculars or snorkeling depending on the river conditions. 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 where the female origin was known. If identification of the female origin was not possible, then subsequent attempts were made the next time the stream reach was surveyed. In some cases, it was possible to determine origin via the PIT tag inserted when the fish was sampled at Tumwater Dam. Post spawn guarding females were scanned for PIT tags using an underwater antenna mounted on an extension handle. Using this technique, we were also able to determine the actual fork length of the fish. This data would later be used in developing a correction factor for previously estimated fork lengths.

Carcass surveys

Biological data was recorded from all spring Chinook carcasses encountered during spawning ground surveys. Surveys for carcasses continued after the completion of spawning until no live fish were observed within the reach. A unique GPS waypoint was assigned to every carcass. The PIT tag code of each carcass was recorded. An additional genetic tissue sample was collected from those carcasses without a PIT tag. In addition, the fork and POH length (to the nearest cm), scales, and snouts from adipose fin clipped

or unknown fish was collected. The number of eggs retained in the body cavity was counted for females with an intact body cavity. Finally, each carcass was mark sampled by removing the caudal fin to prevent double sampling.

Redd microhabitat data

Microhabitat characteristics were measured for redds of known female origin, both hatchery and naturally produced. The maximum length and width of the redd was recorded to the nearest 0.1 m. Water depth measurements (nearest cm) were taken at the upstream side of the bowl, the deepest point within the bowl, the upstream side of the tail, the shallowest point of the tail, the downstream side of the tail, and left and right side of the redd (Figure 1). Water velocity (m/s) was measured using a Marsh McBirney Model 2000 or Swoffer Model 2100 flow meter. Water velocity was recorded at the upstream side of the bowl (60% depth), upstream side of the tail (60% depth, surface, bottom), downstream side of the tail (60% depth), and the left and right side of the redd (60% depth). 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.

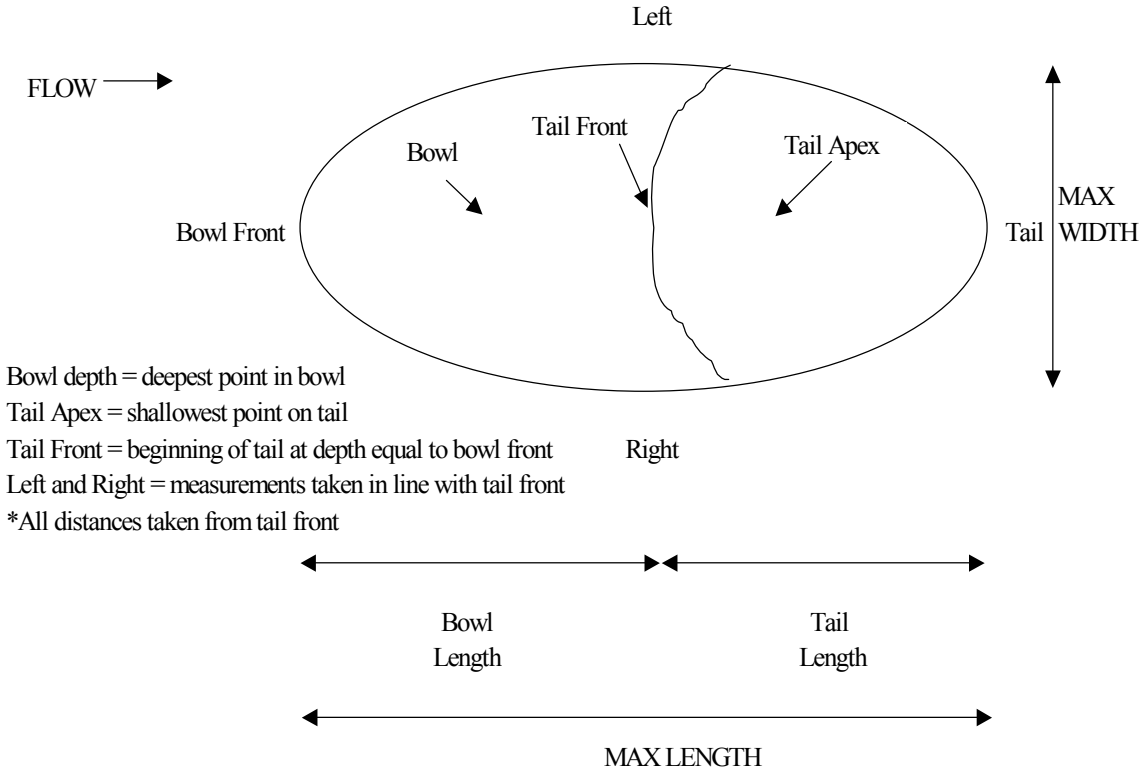

Figure 1. Locations of redd microhabitat characteristic measurements.

Data Analysis

A Chi-squared 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 observed at Tumwater Dam to the spawning population. Differences in the spatial distribution of Chiwawa hatchery and naturally produced fish recovered in the Chiwawa River and Nason Creek were also tested using a Chi-square test. Chi-square test was also used to examine the age compositions of hatchery and natural fish at Tumwater Dam and spawning grounds.

Spawn timing was compared using a Kruskal-Wallis Test. Microhabitat characteristics were statistically compared between redds constructed by hatchery and natural origin fish using a two sample t-test. Correlation analysis was performed to examine the relationship between fish size and redd microhabitat characteristics. All statistical tests were performed at a significance level (α) of 0.05.

Spawning Ground Surveys

Chiwawa River

A total of 241 redds were found in the Chiwawa River basin in 2004. Of those redds, 238 redds (98.8%) were found in the Chiwawa River, while only 3 redds (1.2%) were found in tributaries (i.e., Chikamin and Rock creeks). Redds were made earliest in the higher elevation reaches and progressively later downstream (Table 1). Spawning began the first week of August and continued until second week of September, with peak spawning occurring during the fifth week of August (Appendix B). The origin of the female constructing the redd was determined for 141 redds (58.5%). Of those redds in which origin was determined, 33 were hatchery and 108 were naturally produced (Figure 2).

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

Survey Week	Historical Reach (rkm)						Totals redds
	0-20	20-32	32-37	37-43	43-45	45-51	
07/25	0	0	0	0	0	0	0
08/01	0	3	2	0	0	3	8
08/08	0	20	2	14	1	0	37
08/15	2	10	1	10	4	0	27
08/22	1	33	1	11	11	10	67
08/29	10	40	0	12	5	6	73
09/05	19	5	0	1	0	0	25
09/12	4	0	0	0	0	0	4
09/19	0	0	0	0	0	0	0
09/26	0	0	0	0	0	0	0
Total	36	111	6	48	21	19	241

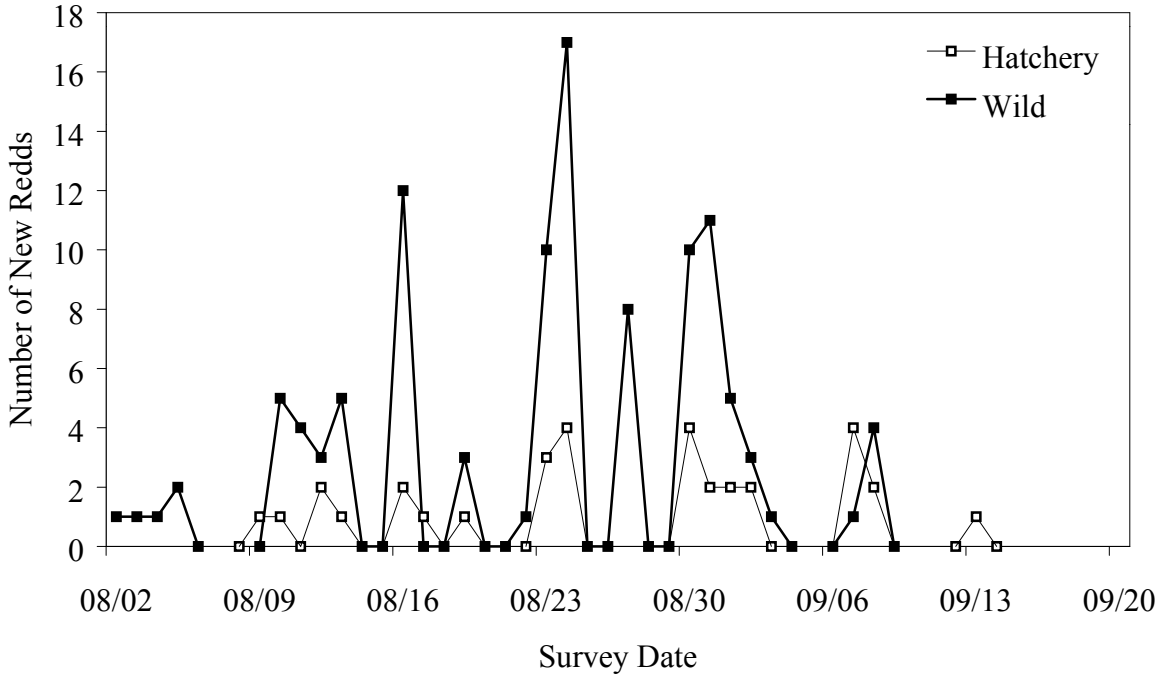

Figure 2. Number of spring Chinook redds found during spawning ground surveys on the Chiwawa River in 2004.

Nason Creek

During surveys on Nason Creek a total 169 redds were found in 2004. The temporal distribution of redds was similar to that observed on the Chiwawa River. Spawning began earliest in the uppermost reaches and progressively later downstream (Table 2). Spawning activity began during the first week of August and continued until the third week of September, with peak spawning occurring in the fifth week of August (Appendix B). The origin of the female constructing the redd was determined for 110 redds (65.1%). Of those redds in which origin was determined, 21 were hatchery and 89 were naturally produced (Figure3).

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

Survey	Historical Reach (rkm)				Total
Week	0-7	7-14	14-22	22-26	redds
07/25	0	0	0	0	0
08/01	0	0	0	2	2
08/08	0	0	2	2	4
08/15	0	0	8	6	14
08/22	0	1	7	5	13
08/29	5	11	31	13	60
09/05	35	16	1	0	52
09/12	10	2	0	0	12
09/19	3	4	5	0	12
09/26	0	0	0	0	0
Total	53	34	54	28	169

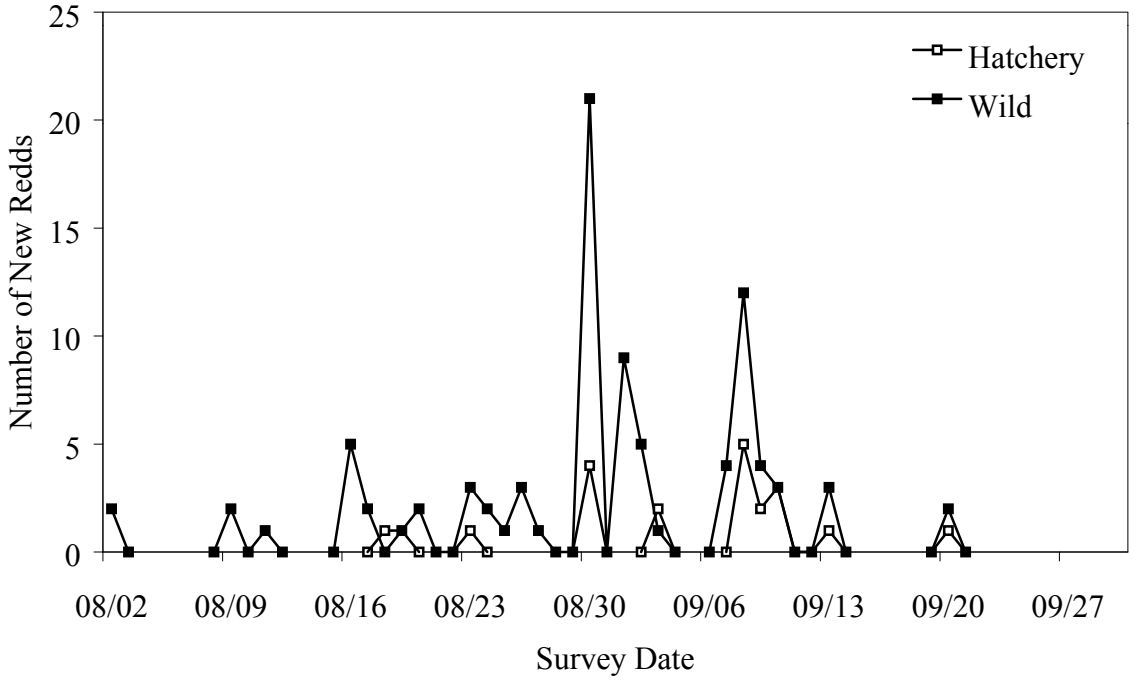

Figure 3. Number of spring Chinook redds found during spawning ground surveys on Nason Creek in 2004.

Upper Wenatchee River

A total of 46 redds were located by raft on the upper Wenatchee River in 2004. Only the two highest elevation reaches (W9 and W10) were surveyed based on historical spring Chinook spawning ground surveys. The temporal distribution of redds was primarily confined to the upper most reach (W10), with only 1 redd found in the lower reach during the last week of active spawning (Table 3). Spawning began the first week of September and continued until the third week of September, with peak spawning occurring during the second week of September (Appendix B). Female origin was determined for 15 redds (32.6%). Of those redds in which origin was determined, 10 were hatchery and 5 were naturally produced.

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

Survey Week	Historical Reach (rkm)		Totals Redds
	59-81	81-90	
08/22	0	0	0
08/29	0	0	0
09/05	0	11	11
09/12	0	26	26
09/19	1	8	9
09/26	0	0	0
Total	1	45	46

White River

Survey crews found a total of 22 redds in the White River basin in 2004. Of those, 20 redds (90.9%) were found in the White River, while 2 redds (9.1%) were found in Panther Creek. Redds were distributed primarily in the reach H3, the two redds found in the uppermost reach were in Panther Creek (Table 4). Spawning activity started during the third week of August and continued until the second week of September, with peak spawning occurring in the first week of September (Appendix B). The origin of the female was determined for 20 redds (90.9%). Of the redds in which origin was determined, 4 were hatchery and 16 were naturally produced.

Table 4. Number of spring Chinook redds found within historical reaches during spawning ground surveys on the White River in 2004.

Survey	Historical Reach (rkm)		Totals
Week	18-22	22-24	redds
08/08	0	0	0
08/15	0	0	0
08/22	5	0	5
08/29	5	0	5
09/05	7	1	8
09/12	3	1	4
09/19	0	0	0
09/26	0	0	0
Total	20	2	22

Little Wenatchee River

A total of 13 redds were found during spawning on the Little Wenatchee River in 2004. 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 first week of September, with peak spawning occurring during the fourth week of August (Appendix B). Female origin was determined for 4 redds (30.8%). Of those redds, it was determined that 1 was 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 2004.

Survey Week	Historical Reach (rkm)			Totals redds
	5-9	9-15	15-21	
08/08	0	0	0	0
08/15	0	4	0	4
08/22	0	2	0	2
08/29	1	4	0	5
09/05	1	1	0	2
09/12	0	0	0	0
09/19	0	0	0	0
09/26	0	0	0	0
Total	2	11	0	13

Carcass Surveys

Chiwawa River

Of the 179 carcasses sampled throughout the Chiwawa River basin, scale analysis determined the proportion of hatchery and naturally produced fish was 43% (*N*=71) and 57% (*N*=93), respectively. Based on a male to female ratio derived from the broodstock of 2.56 to 1, spawning escapement was estimated to be 371 hatchery and 487 naturally produced fish. Of the hatchery fish recovered, 66 snouts containing CWTs were sent to the WDFW CWT lab in Olympia to be extracted and decoded. The abundance of hatchery carcasses was highest in the lowest reach (rkm 0.0-19.1), which was near the acclimation pond, while the naturally produced carcass distribution was more similar to the redd distribution (Figure 4). A large proportion (48%) of the carcasses recovered in reach C1 were age-3 males.

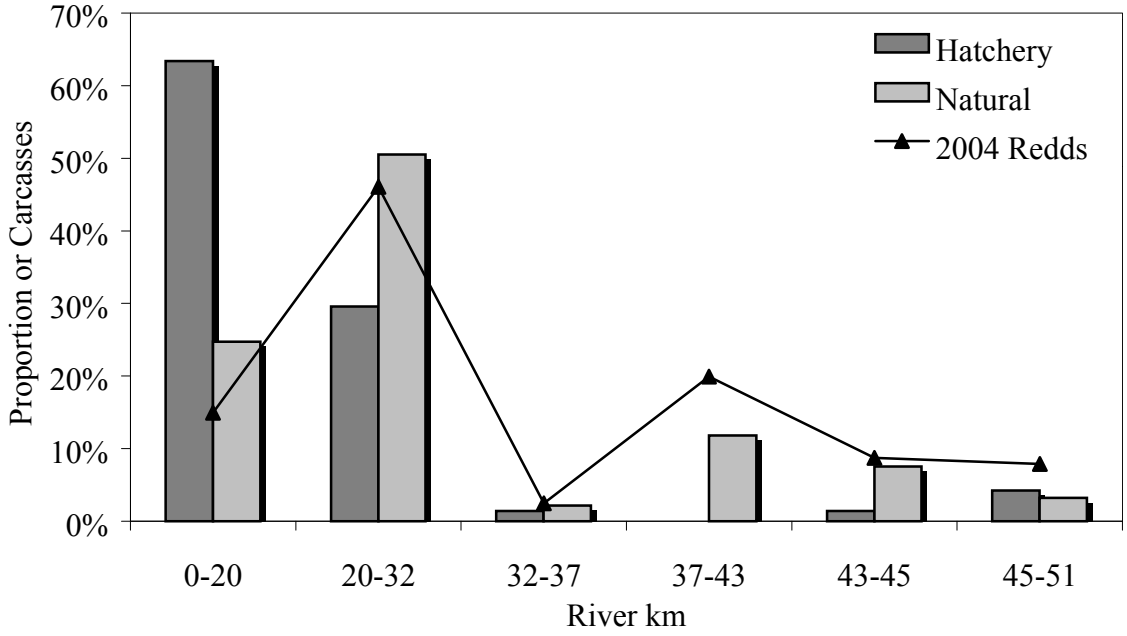

Figure 4. Hatchery and wild spring Chinook carcass distribution in the Chiwawa River in 2004.

Nason Creek

A total of 186 carcasses were recovered in Nason Creek. Scale analysis determined the proportion of hatchery and naturally produced fish was 43% (*N*=78) and 57% (*N*=104), respectively. Of the hatchery fish, 62 snouts containing CWTs were recovered and sent to the WDFW CWT lab to be extracted and decoded. All hatchery fish in Nason Creek were considered strays because no hatchery programs are currently releasing fish into Nason Creek. An estimated 217 hatchery and 290 naturally produced fish spawned in Nason Creek during 2004. The largest proportion of hatchery carcasses were recovered in the lowest reach, while naturally produced carcasses were more evenly distributed (Figure 5).

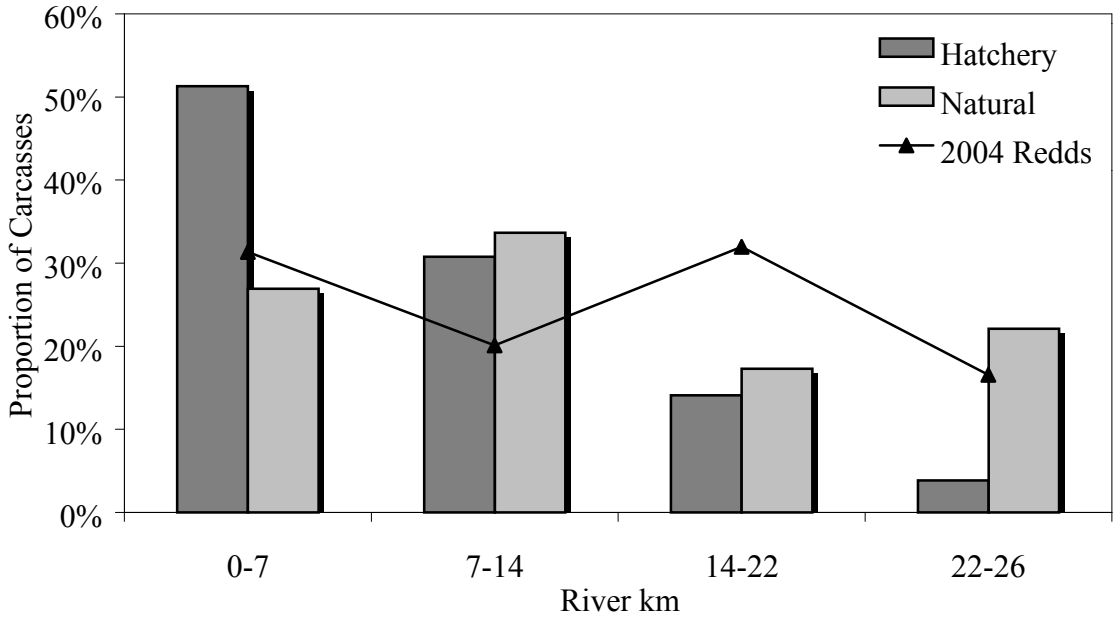

Figure 5. Hatchery and wild spring Chinook carcass distribution in Nason Creek in 2004.

Upper Wenatchee River

In the upper Wenatchee River a total of 23 carcasses were recovered during spawning round surveys. Scale analysis determined the proportion of hatchery and wild fish recovered was 70% (*N*=14) and 30% (*N*=6), respectively. Of the hatchery fish, 14 snouts 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 97 hatchery and 41 wild fish. Carcass distribution of both hatchery and naturally produced fish was similar to redd distribution (Figure 6).

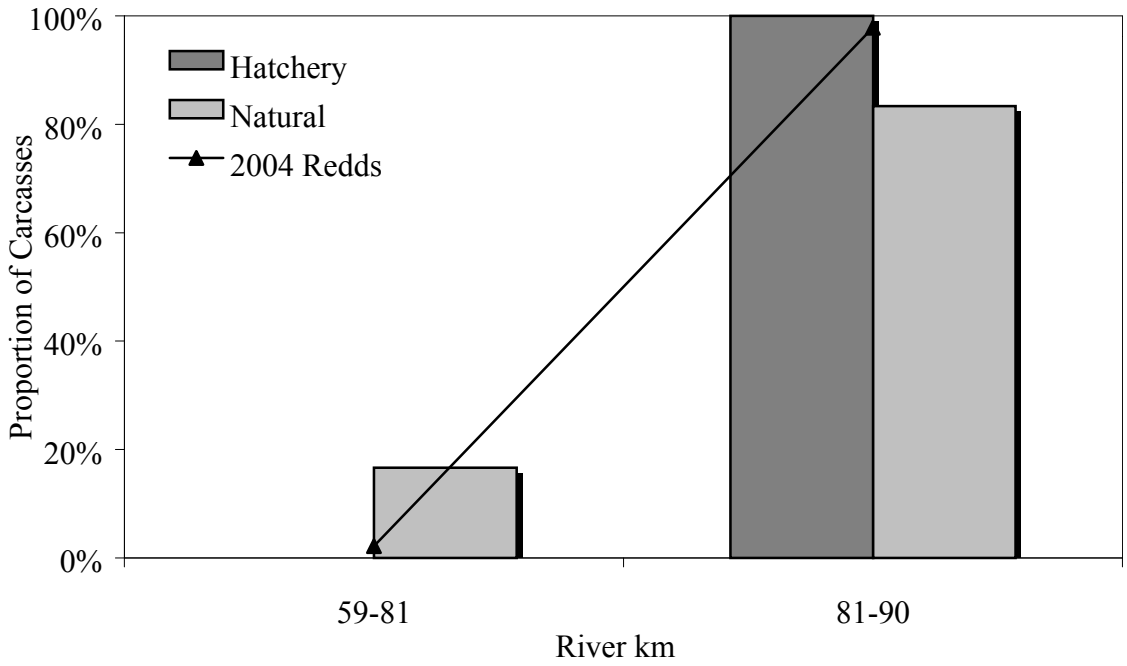

Figure 6. Hatchery and wild spring Chinook carcass distribution in the upper Wenatchee River in 2004.

White River

Of the 13 carcasses recovered in the White River, scale analysis determined the proportion of hatchery and wild fish was 10% (*N*=1) and 90% (*N*=9) respectively. Of the hatchery fish, one snout containing a CWT was 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 7 hatchery and 59 wild fish. Hatchery carcass distribution occurred primarily within the reach where a majority of the redds were located (Figure 7).

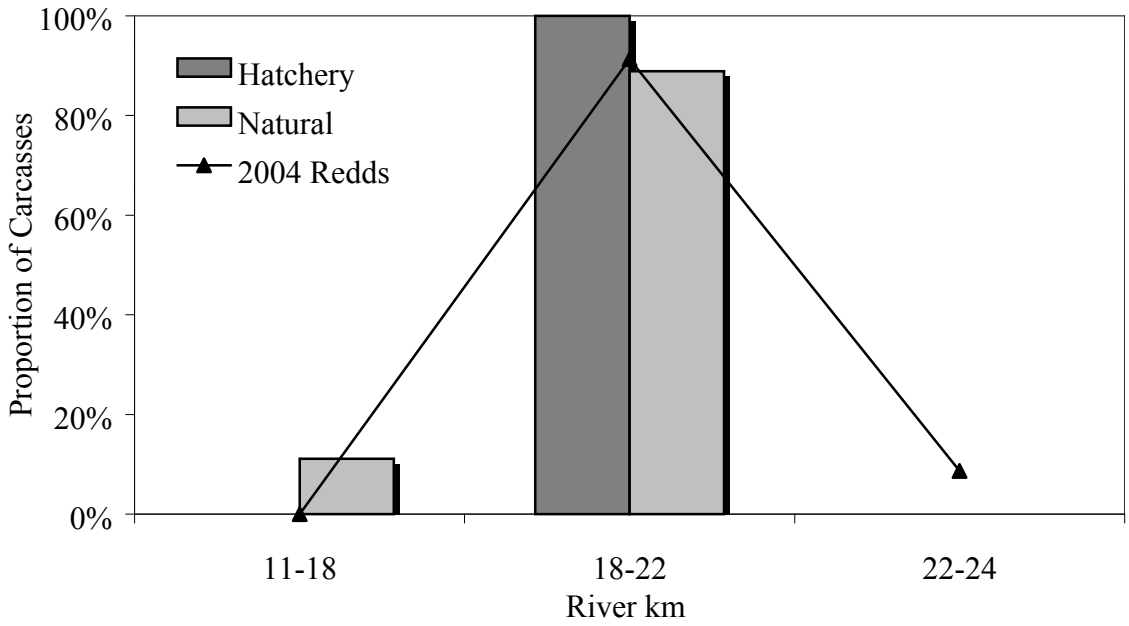

Figure 7. Hatchery and wild spring Chinook carcass distribution in the White River in 2004.

Little Wenatchee River

A single naturally produced female carcass was recovered in the Little Wenatchee River (Figure 8). The estimated spawning population was 39 naturally produced fish.

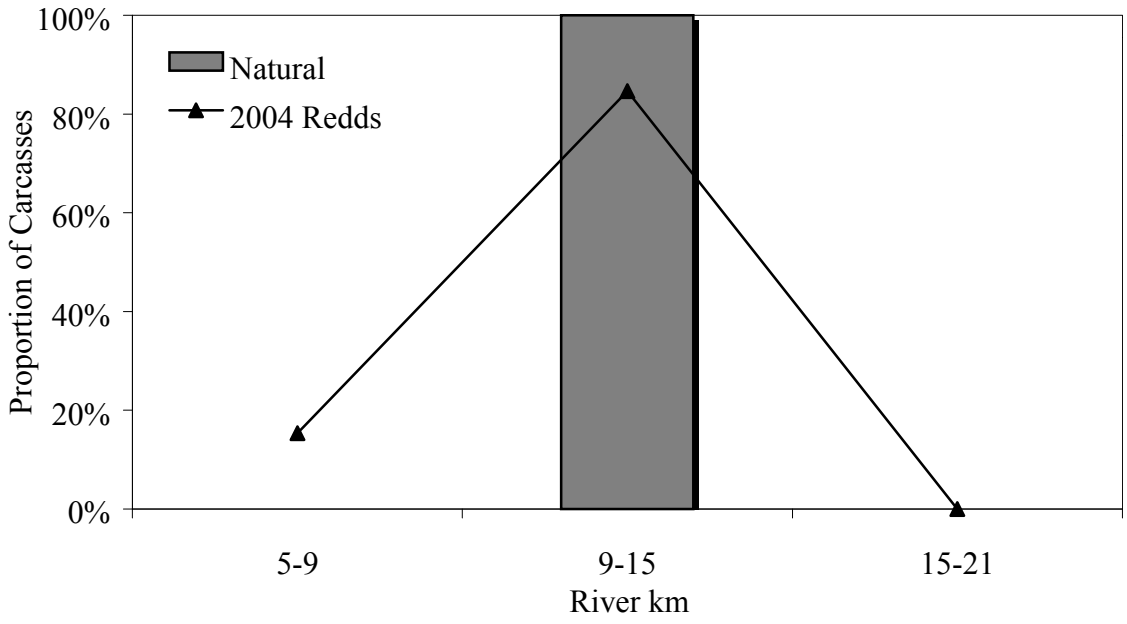

Figure 8. Hatchery and wild spring Chinook carcass distribution in the Little Wenatchee River in 2004.

Spring Chinook Spawning Ground Surveys Downstream of Tumwater Dam

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

Icicle Creek

A total of 30 redds were found during spawning ground surveys. Historically, fish recovered on the Icicle River originate from the Leavenworth National Fish Hatchery (LNFH), which is also located on the Icicle River. Of the16 carcasses sampled, scale analysis determined the proportion of hatchery and naturally produced fish to be 92% (N=12) and 8% (N=1), respectively. The number and composition of the spawning population was estimated at 50 hatchery and 4 naturally produced fish. Of the hatchery fish sampled, 12 snouts containing CWTs were recovered and sent to the WDFW CWT lab in Olympia to be extracted and decoded.

Peshastin Creek

The United State Fish and Wildlife Service (USFWS) released 350 LNFH broodstock into Peshastin and Ingalls creeks in 2004. Genetic tissue samples and biological data were collected by USFWS personnel on all fish prior to release and were subsequently sent to NOAA Fisheries with samples collected from Tumwater Dam. In addition, the fish were externally tagged with a light blue floy tag. CCPUD personnel found 34 redds in Peshastin Creek and Ingalls Creek. Of the 40 carcasses sampled, scale analysis determined that 100% of the spawners were hatchery fish. Of the hatchery fish sampled, 33 snouts containing CWTs were recovered and sent to WDFW CWT lab in Olympia to be extracted and decoded. Estimated spawning escapement was calculated to be 97 hatchery fish.

Spawning Ground Summary

In 2004, 88% of the spring Chinook redds were found upstream of Tumwater Dam. Based on scale samples collected from carcasses, the composition of the Wenatchee River Basin spawning population was 43% hatchery and 57% naturally produced (Table 6). Sampling at Tumwater Dam indicated the proportion of hatchery and natural origin fish available for spawning upstream of Tumwater Dam was 56% and 44%, respectively. The estimated composition of the spawning population upstream of Tumwater Dam was significantly different than the population sampled at Tumwater Dam ($\chi^2 = 55.91$, df = 1, P<0.001).

Table 6. Number of redds and the estimated number of hatchery and wild fish, based on scale samples from carcasses, that spawned in the upper Wenatchee River Basin in 2004.

River	Number of redds	Sample Rate	Number of fish		
			Hatchery	Natural	Total
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
Upper Wenatchee Basin			*692*	*916*	*1,608*
Icicle	30	0.2963	50	4	54
Peshastin	34	0.4124	97	0	97
Total	*555*		*839*	*920*	*1,759*

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

In the Wenatchee Basin, carcass recovery probability was also size dependent (i.e., age-3 = 0.12; age-4 = 0.26; age-5 = 0.31) and the expected and observed age composition of carcasses recovered on the spawning grounds was significantly different than that observed at Tumwater Dam ($\chi^2 = 23.26$, df = 2, $P<0.001$). The mean carcass recovery probability was calculated using the formula provided in Zhou (2002), except the length measurement used was post-orbital to hypural plate (POH) instead of mid-eye to posterior scale (MEPS). Because carcass recovery probabilities were calculated for each age class 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 7). No difference was found between the age composition of fish at Tumwater Dam and the estimated age composition when these corrections were made ($\chi^2 = 0.61$, df = 2, $P=0.74$).

Table 7. 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 carcasses and the recovery probability.

	At Tumwater Dam		Carcasses		Recovery Probability	Estimated Proportion
	N	%	*N*	%		
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

Spawning Distribution

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 during the month of December through February. As a result, returning adults have poor homing fidelity and spawn in varying degrees throughout the basin. Recently, strays from the Leavenworth National Fish Hatchery have also been recovered annually upstream of Tumwater Dam. Analysis was confined to the Chiwawa River and Nason Creek where a majority (85%) of the hatchery fish spawn.

Spawning distribution was determined from carcass recovery location, assuming carcasses were recovered in the same reach in which they spawned. Differences in the distribution of hatchery and natural origin fish were found in both the Chiwawa River ($df = 5$, $\chi^2 = 27.4$, $P < 0.001$) and Nason Creek ($df = 3$, $\chi^2 = 16.19$, $P < 0.001$). Hatchery origin carcasses were found in many of the same reaches as natural fish, but a disproportionately high number of hatchery carcasses were found in the lowest elevation reaches and the reach nearest the hatchery acclimation site.

Spawn Timing

The origin of spawners for individual redds was recorded on those redds as part of the redd characterization study. Analysis of spawn timing was conducted for the hatchery broodstock and the Chiwawa River and Nason Creek spawning populations for reasons previously discussed. Correlation analysis of passage timing and spawning timing for hatchery and natural origin fish collected as broodstock resulted in no significant relationship for hatchery ($r = 0.15$, $P = 0.20$) or natural origin fish ($r = 0.12$, $P = 0.51$). A similar lack of correlation between passage and spawn timing was also reported in the Yakima Basin (Knudsen et al. 2005).

During spawning at the hatchery, no difference in spawn timing was detected between hatchery and natural origin fish (Kruskal-Wallis test $H = 0.05$, $P = 0.83$). Mean spawn timing of hatchery and natural origin fish was identical (Table 8). Based on the origin of the female that was observed spawning (See Redd Microhabitat Characteristics), no correlation was found between the passage date at Tumwater Dam and the spawn date

($r = 0.03$, $P = 0.85$). Spawning in the natural environment begins at the higher elevations and progresses to lower elevations (See this Chapter).

As previously discussed, the spatial distribution of hatchery and natural origin fish in the Chiwawa River and Nason Creek were different. The difference in spatial distribution and subsequently the elevation of spawning locations required that the influence of elevation be controlled in the analysis. A frequency distribution for both the redd and carcass data was used to identify clusters that could be analyzed separately (Table 8). Carcasses generally provided a broader range of elevations for the analysis, regardless no difference in spawn timing was found within a cluster. Based on similar results from the analysis of spawn timing at the hatchery and the spawning ground, both redds and carcasses, no difference in spawn timing was detected between hatchery and natural origin fish. Knudsen et al. (2005) reported that Yakima hatchery spring Chinook spawned earlier at the hatchery, but using carcasses no consistent difference was found in on the spawning grounds.

Table 8. Summary of spawn timing analysis for spawning clusters in the Chiwawa River and Nason Creek in 2004.

Stream/method	Spawning cluster elevation (m)		Sample size		KW Statistic	*P*
	Lower	Upper	Hatchery	Natural		
Chiwawa/Redds	729	739	13	17	1.41	0.24
	775	814	16	80	0.09	0.76
Chiwawa/Carcass	607	610	16	7	0.50	0.48
	668	673	15	4	0.33	0.56
	727	737	27	40	1.58	0.21
	775	804	11	40	0.62	0.43
Nason/Redds	606	613	13	22	0.07	0.79
	665	686	8	41	0.57	0.45
Nason/Carcass	605	615	45	36	0.02	0.90
	663	680	27	40	0.57	0.45
	730	746	3	23	0.13	0.72

Overall, the mean spawn timing of hatchery fish in both the Chiwawa River and Nason Creek was later than that of natural origin fish (Table 9). The difference in the mean spawning was attributed to hatchery fish spawning at lower elevations and subsequently later than natural origin fish spawning at higher elevations.

Table 9. Mean spawn timing of hatchery and natural origin fish in the Wenatchee River Basin in 2004.

Location/Method	Hatchery Origin			Natural Origin		
	Mean	Lower 95%CI	Upper 95% CI	Mean	Lower 95%CI	Upper 95% CI
Hatchery	30 Aug	29 Aug	01 Sep	30 Aug	28 Aug	02 Sep
Chiwawa/Redds	27 Aug	24 Aug	29 Aug	22 Aug	21 Aug	24 Aug
Nason/Redds	04 Sep	01 Sep	08 Sep	30 Aug	29 Aug	01 Sep
Chiwawa/Carcass	08 Sep	06 Sep	09 Sep	06 Sep	04 Sep	07 Sep
Nason/Carcass	10 Sep	08 Sep	12 Sep	07 Sep	05 Sep	08 Sep

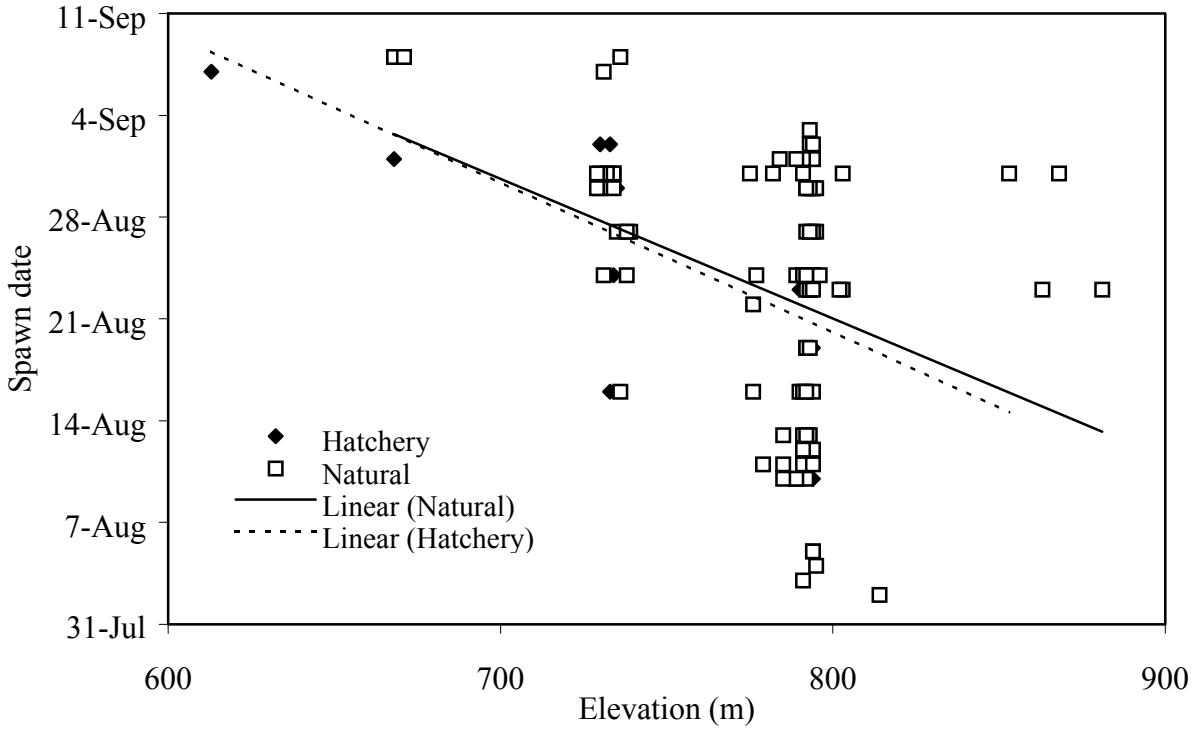

Figure 9. Relationship between elevation and hatchery and natural origin fish spawning in the Chiwawa River in 2004.

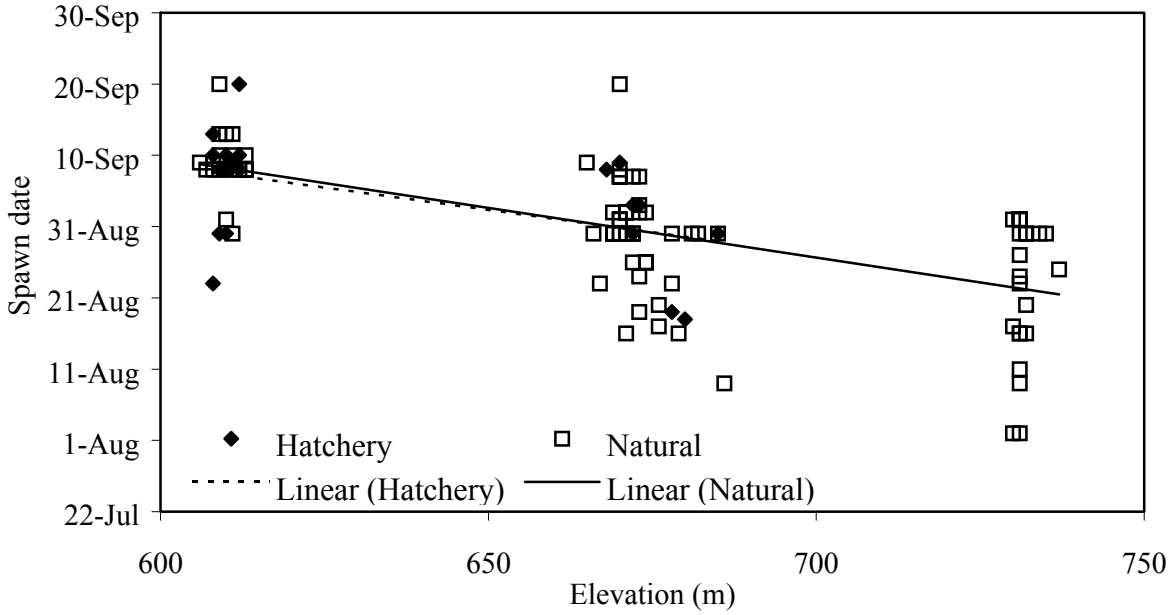

Figure 10. Relationship between elevation and hatchery and natural origin fish spawning in the Nason Creek in 2004.

Survival to Spawning

Overall survival from Tumwater Dam to spawning was 83% (1,608/1,948). The estimated number of fish by origin and age was calculated from carcasses recovered during spawning ground surveys and estimated proportion in each age class (Table 10). Differences were detected in the age composition of hatchery fish ($\chi^2 = 6.65$, df = 2, $P<0.04$) at Tumwater Dam and the estimated spawning population, but not in natural origin fish ($\chi^2 = 0.09$, df = 2, $P=0.96$). Differences in the age composition of hatchery fish are due to the high proportion of age-3 fish in the estimated population. This difference may be the result of the formula used to calculate the estimated age class proportion. In the future, a basin specific formula will be developed. However, until an equation to estimate carcass recovery probability has been developed for the Wenatchee Basin, survival of hatchery and natural origin fish from Tumwater Dam to spawning is assumed equal.

Table 10. Age and origin of Wenatchee Basin spring Chinook at Tumwater Dam. The composition of the spawning population was derived from carcasses and estimated based on carcass recovery probability (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
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

Redd Microhabitat Characteristics

Spring Chinook redd microhabitat variables were measured on 186 redds (90 Chiwawa, 80 Nason, 7 Wenatchee, 5 White, 1 Panther Creek, and 3 Rock Creek). Of those redds, we determined that 42 and 144 were constructed by hatchery and wild fish, respectively. Several fall freshets in the Wenatchee Basin occurred during spawning ground surveys and a majority of redds were not measured at discharges similar to when the redd was constructed. All tributaries, except Panther Creek a tributary of the White River, have discharge gauging stations. The change in river discharge for all redds was calculated using the mean daily discharge on the day the redd was constructed and the day when the redd was measured (Table 10). Only spring Chinook redds in which the difference in discharge was ± 10% were included in the analysis. Of those redds, 58 were located in the Chiwawa Basin (including Rock Creek), 7 in Nason Creek, and 1 in Panther Creek (Appendix D).

Average redd water depth was the calculated from water depth measurements recorded at the left and right side of the redd and the upstream side of the bowl and tail. Bowl depth was calculated by subtracting the average depth from the maximum depth of the bowl. Differences between redds constructed by hatchery females ($N = 18$) and natural origin female spring Chinook ($N = 48$) were found only in the redd width and area (Table 11). Natural origin fish constructed redds greater in width and subsequently area than hatchery fish. Significant, but weak correlations were found between female fork length and area ($r = -0.28$, $P < 0.03$), bowl depth ($r = -0.26$, $P < 0.04$), and water velocity ($r = -0.36$, $P < 0.01$) measured at the upstream side of the bowl.

Table 10. Summary river discharge differences from the date spring Chinook redds were constructed and measured for microhabitat variables in the Wenatchee River Basin in 2004.

Change in river discharge (range)	Origin of female (*N*)	
	Hatchery	Natural
> -50%	2	11
-11% thru - 49%	12	56
-1% thru - 10%	11	24
0% thru +10%	7	24
+11% thru +49%	2	9
> +50%	8	20

Table 11. Two sample t-t test results for spring Chinook redd microhabitat variables measured in the Wenatchee River Basin in 2004.

Variable	*T*	*P*	Hatchery			Natural		
			Mean	Lower 95% CI	Upper 95% CI	Mean	Lower 95% CI	Upper 95% CI
Female length (cm)	0.31	0.76	75.2	71.3	79.0	74.6	72.7	76.5
Mean water depth (m)	-1.19	0.24	0.37	0.32	0.41	0.39	0.37	0.42
Redd area (m^2)	-2.32	0.02	13.8	9.5	18.1	18.9	16.7	21.2
Redd length (m)	-1.41	0.17	5.6	4.8	6.4	6.2	5.8	6.5
Redd width (m)	-2.86	0.01	2.9	2.4	3.5	3.8	3.5	4.2
Tail height (m)	-1.42	0.16	0.12	0.10	0.15	0.14	0.12	0.17
Bowl depth (m)	-0.47	0.64	0.12	0.08	0.16	0.13	0.34	0.52
Water Velocity								
Front of tail	-0.95	0.35	0.30	0.22	0.39	0.34	0.31	0.38
Front of bowl	-1.46	0.15	0.44	0.33	0.55	0.52	0.47	0.57

Summary and Conclusions

The spawning distribution of the upper Wenatchee River Basin spring Chinook population was similar to that observed in previous years (Mosey and Murphy 2002). Natural origin fish spawned in a greater number of tributaries and tributary reaches than hatchery origin fish. Hatchery origin fish tended to spawn in an area near the acclimation site or in relatively low elevation portions of tributaries. Thus, it is possible that reproductive success of hatchery and natural origin fish could differ because of differences in spawning location. No difference in spawn timing was found in the natural or hatchery environment.

Carcass recovery location will continue to be used to assess the spatial distribution with each river. Once sufficient carcass recovery probability data has been collected, a basin specific equation will be developed and used to estimate the age composition of the spawning population. When the age composition of the hatchery and natural origin fish are different, precise estimates of the spawning population may be needed to explain differences in reproductive success. Differential survival of hatchery and natural fish could mask the actual estimates of reproductive success.

The sampling design for measuring redd microhabitat will be changed to ensure adequate sample size of hatchery and natural origin redds within a given reach. Several reaches in both the Chiwawa and Nason Creeks will be selected for redd characterization. Sampling will not occur when discharge is greater than 10% of the redd completion date. These changes should ensure a larger sample size and more comparable data.

Acknowledgements

The Bonneville Power Administration provided funding for this project. We thank Jonathan McCloud, the administrator for this project, 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

Chandler, G.L., and T.C. Bjornn. 1988. Abundance, growth, and interactions of juvenile steelhead relative to time of emergence. Trans. Am. Fish. Soc. 117:432-443.

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.

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. Can. J. Fish. Aquat. Sci. 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.

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.

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

Chapter 4

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

Abstract

Visual observations, PIT tag detections, and snorkel surveys were used to determine composition of adult hatchery and natural origin spring Chinook salmon and abundance of precocious males on redds. Hatchery and natural origin females were paired on redds with hatchery and natural origin males in proportions that were not significantly different from the proportions of hatchery and natural origin males available to spawn naturally ($P>0.05$). This suggested that there was not strong selection for assortative pairing by hatchery or natural origin salmon. However, differences in the spatial distribution of hatchery and natural fish may have contributed to the smaller size of the male fish paired with hatchery females. The estimated number of hatchery and natural origin precocious males that potentially contributed to natural spawning was 9 and 43, respectively. The low relative abundance of precocious males observed on the spawning grounds suggests that the majority of the precocious males observed at Tumwater Dam do not successfully migrate to the major spawning areas or die before spawning. The precocity rate for juveniles released from Chiwawa Ponds in 2004, that migrated downstream and survived to pass Tumwater Dam was calculated as 0.4%.

Introduction

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

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

supplementation hatchery are precocious males and some of these fish are observed on the spawning grounds approximately four months after they are released from acclimation sites (Larsen et al. 2004). Preliminary results from the YKFP indicate that precocial males sired a significant number of offspring in an experimental spawning channel that contained anadromous males and females (Young, WDFW, unpublished data). Age 1+ precocious males may migrate downstream, but generally do not reach the ocean. These fish are undesirable because of the potential for negative ecological and genetic impacts to natural fish, and because they are an undesirable fishery product. For example, a high incidence of precociously maturing males will lead to direct ecological interactions with native conspecifics and other non-target species of concern. Also, age structure, sex ratio and, potentially, other phenotypic characters of the spawning population will be altered. Precocity and other forms of residualism in hatchery fish is an expression of the genotype x environment interaction. To the extent that the phenomenon has in part a genetic basis and is coupled with changes in the reproductive potential of individuals within the hatchery population as a whole, high precocity or residualism is a source of domestication selection. In this study, we will examine if hatchery precocious males are (1) produced by the hatcheries in question, (2) observed on the spawning grounds, and (3) contribute genetic material to future generations (i.e., progeny attributed to unknown male parentage).

Methods and Materials

Snorkel Surveys

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

Chinook salmon that are on or associated with active redds were counted and identified to origin, sex, and size while snorkeling. Surveys were conducted weekly and lasted throughout the spawning season. Active redds (the presence of an anadromous fish) were found by floating downstream in an inflatable raft or by walking. When a salmon redd was observed, we determined if an anadromous salmon was present. If a salmon was 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. Anadromous fish were distinguished from precocious males based on size. Anadromous fish are generally greater than 400 mm and

precocials are generally less than 200 mm. Females were distinguished from males by the mouth shape and the condition of the caudal fin. Males have a kype and females have a white band on the margin of the caudal fin from digging a redd. After a redd was snorkeled, it was flagged and numbered for subsequent redd measurements.

Data Analysis

The age and origin of fish observed on the spawning grounds was compared to expected proportions at Tumwater Dam using Chi-square analysis. Relationships between female fork length and male fork length were analyzed using linear regression analysis. Assortative pairings of hatchery and natural origin fish was examined using an ANCOVA with female length as a covariate.

Results and Discussion

A total of 104 redds (21.2%) snorkeled in the upper Wenatchee River Basin during spawning ground surveys (Table 1). Water clarity limited snorkeling on the Chiwawa River, which contained the greatest number of redds in the Wenatchee Basin. Of the 20 redds snorkeled on the Chiwawa River, 2 hatchery and 9 naturally produced precocious fish were observed on three redds. Water clarity was excellent in Nason Creek and 73 redds were snorkeled. In Nason Creek, 1 redd was found with 2 naturally produced precocious fish. We did not observe any age 0+ precocious males in any of the surveys. The upper Wenatchee River conditions permitted minimal snorkel surveying opportunities. On the White River, we were unable to perform any snorkel surveys due to low water clarity. Although, snorkeling was conducted on the two redds in Panther Creek, in which no precocious males were found.

Table 1. Precocious males found during spawning ground surveys on the upper Wenatchee River basin in 2004.

Stream	Redds snorkeled	Number of Precocious fish		Number of precocious fish per redd		
		Hatchery	Natural	Hatchery	Natural	Total
Chiwawa	20	2	7	0.10	0.35	0.45
Nason	73	0	2	0.0	0.27	0.03
White (Panther)	2	0	0	0.0	0.0	0.0
Upper Wenatchee	9	0	0	0.0	0.0	0.0
Total Upper Basin	104	2	9	0.019	0.087	0.106

Using the mean number of precocious males per redd and the total numbers of redds, an estimated 9 hatchery and 43 natural precocious males potentially contributed gametes during spawning. None of the 635 precocious males sampled at Tumwater Dam were detected or recovered on the spawning grounds. The mark rate of the 2002 brood Chiwawa spring Chinook was 36.4%. The mark rate of the precocious fish sampled at

Tumwater Dam was 31.7%. Chi-square analysis resulted in no difference between the number of marked precocious fish at Tumwater Dam and the expected number from the hatchery population (χ^2 with Yates correction = 2.95, df = 1, P = 0.09). Hence, assuming all precocious fish sampled at Tumwater Dam were from the Chiwawa Ponds and all precocious fish migrated below Tumwater Dam, the precocity rate of the 2002 brood Chiwawa spring Chinook was 0.4% (149,668 fish released in 2004). The carcass recovery probability of age-2 fish is likely very low. The mean (standard deviation, SD) size of the age-2 fish sampled at Tumwater Dam was 210 (16) mm. Zhou (2002) reported that no fish tagged less than 350 mm was recovered over 11 years in the Salmon River, Oregon. Thus, carcass surveys likely underestimate the contribution of precocious males and necessitate the need for snorkel surveys.

Single pairings were observed at the same rate (50%) as multiple male pairings (Table 2). Based on the number and origin of male fish (excluding age-2 and age-3 fish) expected on the spawning grounds, no difference was found in the proportion of hatchery or natural males observed with hatchery (χ^2 with Yates correction = 0.53, df = 1, P = 0.46) or natural females (χ^2 with Yates correction = 3.69, df = 1, P = 0.08). Age-2 and age-3 males were not included in the analysis due to the inability of determining origin based solely on observations (Table 3).

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

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

Table 3. Summary of pairings of hatchery and natural origin spring Chinook on selected redds in the upper Wenatchee River Basin in 2004. Age-3 fish were not included in the analysis because origin could not be determined on the spawning grounds.

Female origin	Male origin	Observed proportions of adult males on the spawning grounds	Expected proportions of adult males on the spawning grounds
H	H	0.28	0.19
	W	0.72	0.81
W	H	0.11	0.19
	W	0.89	0.81

The fork length of the male (age-3 included) tended to increase as the fork length of the female increased. Linear regression analysis was performed separately for hatchery and natural origin females. A significant but weak relationship was found for natural origin females ($r^2 = 0.05$, $F = 4.55$, $P < 0.04$), but not for hatchery females ($r^2 = 0.06$, $F = 2.11$, $P = 0.17$). Assortative mating was examined for hatchery and natural origin females separately, using male fork length as the covariate. Significant differences were found in the fork length of males that spawned with hatchery and natural origin females (Figure 1, df = 1 $F = 4.73$, $P = 0.03$). The probable cause why hatchery females spawned with smaller males than natural origin females was that most hatchery fish spawned in the lower reaches of the streams. Of those hatchery fish that spawned, age-3 males (N=742) were in greater abundance than age-4 and age-5 males (N=89).

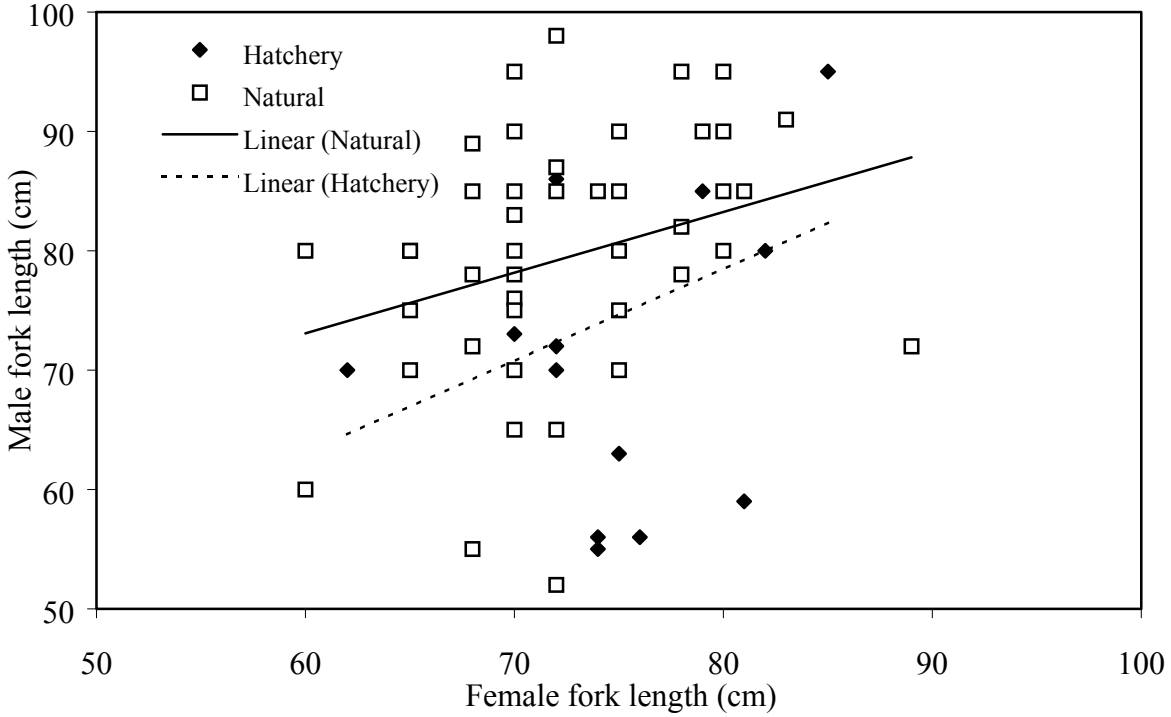

Figure 1. Relationship between female fork length and male fork length for hatchery and natural origin female spring Chinook in the upper Wenatchee Basin in 2004.

Summary and Conclusions

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

Data collected in 2004 suggests that mate selection in the Wenatchee Basin is random. Although, hatchery females did spawn with smaller males than natural origin females presumably due to the differences in the spatial distribution of hatchery and natural origin fish. These data will be used in conjunction with the DNA pedigree analysis (See Chapter 2), which should also provide information regarding mate selection. The sex ratio of males to females (excluding age-2 males) was 2.0:1.0 at Tumwater Dam. Based on the number of males observed on the spawning grounds the male to female ratio was 1.98:1.0. These data suggest that survival of males and female to spawning is equal and males only spawn with a single female.

References

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.

Mullan, J.W., K.R. Williams, G. Rhodus, T.W. Hillman, and J.D. McIntryre. 1992. Productions and Habitat of Salmonids in Mid-Columbia River Tributary Streams. U.S. Fish and Wildlife Service

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.

Zhou, S. 2002. Size-dependent recovery of Chinook salmon in carcass surveys. Transactions of the American Fishery Society 131:1194-1202.

Appendix A. Daily number of spring Chinook observed at Tumwater Dam during trapping in 2004 (F=female, M=male, J=jack, P=precocious male).

Date	Natural			Hatchery				Unknown			Daily Total
	F	M	J	F	M	J	P	F	M	J	
05/18/04	1										1
05/19/04		2									2
05/20/04				1							1
05/21/04		1									1
05/22/04	1	1		1							3
05/23/04	2										2
05/24/04	1										1
05/25/04		1									1
05/26/04	2	2		3	1						8
05/27/04	2	5		1	3						11
05/28/04	1	2									3
05/29/04	1	1			1						3
05/30/04	2	1									3
05/31/04	3	4									7
06/01/04	2	7		2	1						12
06/02/04	8	9		6				1			24
06/03/04	12	15		3	3			2			35
06/04/04	19	25		9	7	3			1		64
06/05/04	21	24		12	10	5		1	1		74
06/06/04	4	11	1	10	2	5		1			34
06/07/04	2	5		6	2	5			1		21
06/08/04	9	8	2	3	6	11					39
06/09/04	13	5		7	1	3					29
06/10/04	8	25		9	4	11	3		1		61
06/11/04	11	7		2		10	1				31
06/12/04	9	10	1	4	2	21			1		48
06/13/04	9	5	1	10	2	20				1	48
06/14/04	4	4		4		19	1				32
06/15/04	8	4		7	1	16	1		1		38
06/16/04	12	9	3	8	2	19	2	2			57
06/17/04	15	9		11	3	29	2	1			70
06/18/04	18	3		11	3	23	2		1		61
06/19/04	9	10		6	2	42	6				75
06/20/04	15	18		11	3	36	3				86
06/21/04	27	14	2	13	4	24	7				91
06/22/04	12	9	2	10	6	20	12	1			72
06/23/04	11	11	1	5	3	23	13		1		68

06/24/04	10	9		10	2	29	3	1			64
06/25/04	11	12	1	13	2	21	7				67
06/26/04	8	4	1	15	4	20	4				56
06/27/04	9	9	1	9	2	28	2				60
06/28/04	6	8	1	10		26			1		52
06/29/04	5	12	1	6	2	18	4				48
06/30/04	7	11	1	15	4	19	3				60
07/01/04	16	10	1	12	3	27	5	1			75
07/02/04	12	10	1	12	1	34	5		2		77
07/03/04	9	21	1	16	8	54	11				120
07/04/04	9	10		13	9	32	9				82
07/05/04	6	7	1	15	4	30	13		1		77
07/06/04	5	7	1	6	1	39	11			1	71
07/07/04	4	8		9	2	23	12	2		1	61
07/08/04	1	2	1	5		5	7				21
07/09/04	3	6		5	1	15	42				72
07/10/04	3	4			2	14	46				69
07/11/04	2	3		1	1	8	37				52
07/12/04	4	3		4	1	9	35			1	57
07/13/04	4	1	2	3	1	4	49				64
07/14/04		2	1	3		1	43				50
07/15/04	3	7	1	3	1	16	27				58
07/16/04		3	1			2	33				39
07/17/04	1	1				6	46				54
07/18/04		2		1	1	3	25		1	1	34
07/19/04	1	1		1	1	1	15				20
07/20/04		1			2		21				24
07/21/04	1		1	1		1	16				20
07/22/04		1				3	11				15
07/23/04						2	11				13
07/24/04						1	9		1		11
07/25/04				1		1	5				7
07/26/04						1	3				4
07/27/04		1			1		5				7
07/28/04				1			2				3
07/29/04							1				1
07/30/04											
07/31/04							2				2
08/01/04							1				1
08/02/04											

08/03/04							1				1
08/04/04											
08/05/04											
08/06/04				1							1
08/07/04											
08/08/04											
08/09/04											
08/10/04	1*										1*
08/11/04				1*							1*
08/12/04											
08/13/04											
08/14/04	1*										1*
08/15/04	2*										2*
08/16/04											
08/17/04											
08/18/04											
08/19/04	2*										2*
08/20/04											
08/21/04											
08/22/04											
08/23/04											
08/24/04											
08/25/04											
08/26/04											
08/27/04											
08/28/04	2*										2*
Total	422	443	31	367	128	838	635	13	14	5	2,896

* Video recorded counts, sex not determined.

Appendix B. Spawn timing in the upper Wenatchee River Basin in 2004.

Date	Nason	Chiwawa	Wenatchee	Little Wenatchee	White	Daily total	Cumulative total
08/02/2004	2	2	0	0	0	4	4
08/03/2004	0	1	0	0	0	1	5
08/04/2004	0	1	0	0	0	1	6
08/05/2004	0	2	0	0	0	2	8
08/06/2004	0	1	0	0	0	1	9
08/07/2004	0	0	0	0	0	0	9
08/08/2004	0	0	0	0	0	0	9
08/09/2004	2	2	0	0	0	4	13
08/10/2004	0	11	0	0	0	11	24
08/11/2004	2	4	0	0	0	6	30
08/12/2004	0	5	0	0	0	5	35
08/13/2004	0	15	0	0	0	15	50
08/14/2004	0	0	0	0	0	0	50
08/15/2004	0	0	0	0	0	0	50
08/16/2004	5	18	0	0	3	26	76
08/17/2004	2	2	0	0	0	4	80
08/18/2004	1	0	0	0	2	3	83
08/19/2004	2	7	0	0	0	9	92
08/20/2004	4	0	0	4	0	8	100
08/21/2004	0	0	0	0	0	0	100
08/22/2004	0	1	0	0	0	1	101
08/23/2004	4	20	0	1	3	28	129
08/24/2004	3	37	0	1	0	41	169
08/25/2004	2	0	0	0	0	2	171
08/26/2004	3	0	0	0	0	3	174
08/27/2004	1	9	0	0	3	13	187
08/28/2004	0	0	0	0	0	0	187
08/29/2004	0	0	0	0	0	0	187
08/30/2004	36	26	0	0	2	64	252
08/31/2004	1	21	0	4	1	27	279
09/01/2004	11	13	0	0	1	25	304
09/02/2004	7	9	0	0	3	19	323
09/03/2004	5	4	0	1	0	10	333
09/04/2004	0	0	0	0	0	0	333
09/05/2004	0	0	0	0	0	0	333
09/06/2004	0	0	0	0	0	0	333
09/07/2004	4	7	0	0	4	15	348
09/08/2004	20	17	0	1	0	38	386

09/09/2004	10	1	11	0	0	22	408
09/10/2004	18	0	13	1	0	32	440
09/11/2004	0	0	0	0	0	0	440
09/12/2004	1	0	0	0	0	1	441
09/13/2004	9	1	0	0	0	10	451
09/14/2004	0	0	1	0	0	1	452
09/15/2004	1	3	0	0	0	4	456
09/16/2004	0	0	0	0	0	0	456
09/17/2004	1	0	12	0	0	13	469
09/18/2004	0	0	0	0	0	0	469
09/19/2004	0	1	0	0	0	1	470
09/20/2004	5	0	0	0	0	5	475
09/21/2004	0	0	0	0	0	0	475
09/22/2004	1	0	8	0	0	9	484
09/23/2004	6	0	1	0	0	7	491
09/24/2004	0	0	0	0	0	0	491
09/25/2004	0	0	0	0	0	0	491
09/26/2004	0	0	0	0	0	0	491
09/27/2004	0	0	0	0	0	0	491
09/28/2004	0	0	0	0	0	0	491
09/29/2004	0	0	0	0	0	0	491
09/30/2004	0	0	0	0	0	0	491
Total	169	241	46	13	22	491	491

Appendix C. Spring Chinook spawning reaches in the upper Wenatchee River Basin

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

Appendix D. Spring Chinook redd microhabitat variables measured in the Wenatchee river Basin in 2004.

	Hatchery			Natural		
Variable	N	Mean	SD	N	Mean	SD
		Chiwawa River Reach C1A				
Bowl Front Depth	5	0.39	0.10	2	0.32	0.11
Bowl Depth	5	0.06	0.03	2	0.04	0.02
Redd Depth	5	0.35	0.09	2	0.34	0.05
Tail Depth	5	0.14	0.04	2	0.14	0.01
Bowl Front Velocity	5	0.34	0.13	2	0.42	0.03
Tail Front Bottom Velocity	5	0.28	0.11	2	0.31	0.04
Distance to Cover	5	2.74	2.29	2	3.50	2.12
Distance to Nearest Redd	5	5.84	7.62	1	0.00	
Tail Substrate Boulder	5	6.00	5.48	2	0.00	0.00
Tail Substrate Cobble	5	42.00	17.89	2	60.00	28.28
Tail Substrate Gravel	5	39.00	14.32	2	35.00	21.21
Tail Substrate Sand	5	13.00	9.75	2	5.00	7.07
Female Fork Length	5	80.60	7.99	2	85.50	14.85
		Chiwawa River Reach C1C				
Bowl Front Depth	1	0.34				
Bowl Depth	1	0.08				
Redd Depth	1	0.32				
Tail Depth	1	0.06				
Bowl Front Velocity	1	0.61				
Tail Front Bottom Velocity	1	0.41				
Distance to Cover	1	0.50				
Distance to Nearest Redd	1	3400.00				
Tail Substrate Boulder	1	2.00				
Tail Substrate Cobble	1	10.00				
Tail Substrate Gravel	1	80.00				
Tail Substrate Sand	1	8.00				
Female Fork Length	1	72.00				
		Chiwawa River Reach C2A				
Bowl Front Depth	4	0.55	0.21	14	0.55	0.11
Bowl Depth	4	0.15	0.04	14	0.12	0.03
Redd Depth	4	0.46	0.14	14	0.47	0.09
Tail Depth	4	0.16	0.01	14	0.21	0.09
Bowl Front Velocity	4	0.66	0.18	14	0.53	0.18
Tail Front Bottom Velocity	4	0.48	0.24	14	0.39	0.08
Distance to Cover	4	3.40	3.06	14	4.27	3.22
Distance to Nearest Redd	4	31.38	59.13	14	78.02	92.85
Tail Substrate Boulder	4	0.00	0.00	14	0.00	0.00
Tail Substrate Cobble	4	5.00	0.00	14	7.86	6.11
Tail Substrate Gravel	4	82.50	8.66	14	78.57	16.81
Tail Substrate Sand	4	12.50	8.66	14	13.57	12.47
Female Fork Length	4	75.25	3.77	14	76.00	5.39
		Chiwawa River Reach C2C				
Bowl Front Depth	1	0.34				
Bowl Depth	1	0.11				
Redd Depth	1	0.29				
Tail Depth	1	0.12				

Bowl Front Velocity	1	0.48				
Tail Front Bottom Velocity	1	0.18				
Distance to Cover	1	7.60				
Distance to Nearest Redd	1	180.00				
Tail Substrate Boulder	1	0.00				
Tail Substrate Cobble	1	10.00				
Tail Substrate Gravel	1	80.00				
Tail Substrate Sand	1	10.00				
Female Fork Length	1	76.00				
		Chiwawa River Reach C3				
Bowl Front Depth				1	0.48	
Bowl Depth				1	0.15	
Redd Depth				1	0.39	
Tail Depth				1	0.11	
Bowl Front Velocity				1	0.36	
Tail Front Bottom Velocity				1	0.20	
Distance to Cover				1	5.60	
Distance to Nearest Redd				1	0.30	
Tail Substrate Boulder				1	0.00	
Tail Substrate Cobble				1	75.00	
Tail Substrate Gravel				1	20.00	
Tail Substrate Sand				1	5.00	
Female Fork Length				1	77.00	
		Chiwawa River Reach C4				
Bowl Front Depth				14	0.46	0.12
Bowl Depth				14	0.18	0.08
Redd Depth				14	0.36	0.08
Tail Depth				14	0.09	0.06
Bowl Front Velocity				14	0.52	0.15
Tail Front Bottom Velocity				14	0.35	0.11
Distance to Cover				12	7.03	7.06
Distance to Nearest Redd				7	1.51	1.18
Tail Substrate Boulder				14	0.00	0.00
Tail Substrate Cobble				14	45.00	25.65
Tail Substrate Gravel				14	47.50	23.84
Tail Substrate Sand				14	7.50	6.72
Female Fork Length				14	73.14	6.81
		Chiwawa River Reach C5				
Bowl Front Depth	2	0.54	0.03	6	0.47	0.06
Bowl Depth	2	0.15	0.04	6	0.15	0.08
Redd Depth	2	0.42	0.06	6	0.38	0.04
Tail Depth	2	0.10	0.06	6	0.11	0.05
Bowl Front Velocity	2	0.51	0.28	6	0.69	0.17
Tail Front Bottom Velocity	2	0.27	0.08	6	0.38	0.14
Distance to Cover	2	0.00	0.00	6	3.55	3.81
Distance to Nearest Redd						
Tail Substrate Boulder	2	0.00	0.00	6	0.33	0.82
Tail Substrate Cobble	2	42.50	24.75	6	63.33	18.62
Tail Substrate Gravel	2	47.50	17.68	6	30.50	16.54
Tail Substrate Sand	2	10.00	7.07	6	5.83	5.85
Female Fork Length	2	63.50	2.12	6	71.83	5.88

		Chiwawa River Reach C6			
Bowl Front Depth	1	0.34	5	0.38	0.15
Bowl Depth	1	0.43	5	0.08	0.06
Redd Depth	1	0.30	5	0.37	0.12
Tail Depth	1	0.07	5	0.16	0.03
Bowl Front Velocity	1	0.66	5	0.52	0.21
Tail Front Bottom Velocity	1	0.37	5	0.28	0.07
Distance to Cover	1	3.20	5	1.70	2.73
Distance to Nearest Redd	1	150.00	3	43.33	51.32
Tail Substrate Boulder	1	0.00	5	0.00	0.00
Tail Substrate Cobble	1	50.00	5	27.00	31.94
Tail Substrate Gravel	1	40.00	5	55.00	30.41
Tail Substrate Sand	1	10.00	5	18.00	13.51
Female Fork Length	1	72.00	5	73.60	6.23
		Nason Creek Reach N3A			
Bowl Front Depth	1	0.40	1	0.25	
Bowl Depth	1	0.07	1	0.06	
Redd Depth	1	0.35	1	0.26	
Tail Depth	1	0.05	1	0.08	
Bowl Front Velocity	1	0.36	1	0.68	
Tail Front Bottom Velocity	1	0.28	1	0.54	
Distance to Cover	1	75.00	1	7.80	
Distance to Nearest Redd	1	5.00	1	16.00	
Tail Substrate Boulder	1	0.00	1	0.00	
Tail Substrate Cobble	1	30.00	1	80.00	
Tail Substrate Gravel	1	70.00	1	15.00	
Tail Substrate Sand	1	0.00	1	5.00	
Female Fork Length	1	75.00	1	70.00	
		Nason Creek Reach N3B			
Bowl Front Depth			2	0.58	0.18
Bowl Depth			2	0.11	0.08
Redd Depth			2	0.49	0.06
Tail Depth			2	0.12	0.01
Bowl Front Velocity			2	0.34	0.13
Tail Front Bottom Velocity			2	0.15	0.01
Distance to Cover			2	9.00	8.49
Distance to Nearest Redd			2	60.00	14.14
Tail Substrate Boulder			2	0.00	0.00
Tail Substrate Cobble			2	45.00	49.50
Tail Substrate Gravel			2	55.00	49.50
Tail Substrate Sand			2	0.00	0.00
Female Fork Length			2	80.00	0.00
		Nason Creek Reach N4			
Bowl Front Depth			3	0.27	0.09
Bowl Depth			3	0.06	0.05
Redd Depth			3	0.29	0.10
Tail Depth			3	0.18	0.04
Bowl Front Velocity			3	0.35	0.16
Tail Front Bottom Velocity			3	0.26	0.11
Distance to Cover			3	2.30	1.99
Distance to Nearest Redd			3	41.17	51.62
Tail Substrate Boulder			3	3.67	1.15

Tail Substrate Cobble				3	58.33	2.89
Tail Substrate Gravel				3	29.00	6.56
Tail Substrate Sand				3	9.00	6.56
Female Fork Length				3	71.67	2.89
		Rock Creek Reach R1				
Bowl Front Depth	2	0.41	0.02			
Bowl Depth	2	0.06	0.07			
Redd Depth	2	0.35	0.09			
Tail Depth	2	0.15	0.08			
Bowl Front Velocity	2	0.25	0.08			
Tail Front Bottom Velocity	2	0.11	0.00			
Distance to Cover	2	1.65	1.63			
Distance to Nearest Redd	2	110.00	127.28			
Tail Substrate Boulder	2	0.00	0.00			
Tail Substrate Cobble	2	47.50	38.89			
Tail Substrate Gravel	2	40.00	42.43			
Tail Substrate Sand	2	12.50	3.54			
Female Fork Length	2	71.00	12.73			
		Panther Creek Reach T1				
Bowl Front Depth	1	0.32				
Bowl Depth	1	0.12				
Redd Depth	1	0.22				
Tail Depth	1	0.05				
Bowl Front Velocity	1	0.02				
Tail Front Bottom Velocity	1	0.11				
Distance to Cover	1	0.91				
Distance to Nearest Redd	1	--				
Tail Substrate Boulder	1	1.00				
Tail Substrate Cobble	1	10.00				
Tail Substrate Gravel	1	89.00				
Tail Substrate Sand	1	0.00				
Female Fork Length	1	85.00				