Tucannon River
Spring Chinook Salmon
Captive Broodstock Program

2007 Annual Report

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Abstract

This report summarizes the objectives, tasks, and accomplishments of the Tucannon River Spring Chinook Captive Broodstock Program during 2007. Results should be considered preliminary until published is a peer-reviewed journal.

The WDFW initiated a captive broodstock program in 1997. The captive broodstock program collected juvenile hatchery supplementation fish from five (1997-2001) brood years (BY) with additional fish collected from the 2002 BY. The overall goal of the Tucannon River captive broodstock program is for the short-term, and eventually long-term, rebuilding of the Tucannon River spring Chinook salmon population, with the hope that natural production will sustain itself in the future. The project goal is to rear captive salmon selected from the supplementation program to adults, spawn them, rear their progeny, and release approximately 150,000 smolts annually into the Tucannon River between 2003-2007. These smolts, in combination with the current conventional hatchery supplementation program and wild production, are expected to produce 600-700 returning adult spring Chinook to the Tucannon River each year from 2005-2010.

Seven captive brood progeny adult returns were recovered during 2007. The number of captive brood returns was expanded to 19 for the total run. Survival to adult returns has been poor for this program to date.

Microsatellite DNA analysis to date provides evidence that the captive broodstock program has been an effective method of preserving overall genetic variation in Tucannon River spring Chinook while providing additional smolts for release.

During April 2008, WDFW volitionally released 78,176 BY 2006 captive broodstock progeny smolts from Curl Lake Acclimation Pond into the Tucannon River. These fish were marked only with a CWT in order to differentiate them from the supplementation fish (CWT/Left Blue VIE/No Finclip and CWT/Left Purple VIE/No Finclip). One thousand captive brood progeny smolts were PIT tagged to compare their outmigration with smolts from the supplementation program. Monitoring their survival and adult returns, along with future natural production levels, will be used to determine the success or failure of this captive broodstock program. A final report, including complete results of the genetics analysis, will be submitted by September 2009.

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Introduction

Reporting Period

This report summarizes the accomplishments of the Tucannon River spring Chinook salmon (*Oncorhynchus tshawytscha*) captive brood program for 2007. This report, while originally intended to cover activities accomplished exclusively under the Fiscal Year (FY) 2007 contract, includes some events during FY2008 as well. This was done to provide readers with complete results from the tagging, rearing, and acclimation and release activities that have occurred. Results should be considered preliminary until published in a peer-reviewed journal.

Tucannon River Spring Chinook Program Overview

Prior to 1985, artificial production of spring Chinook in the Tucannon River was nearly nonexistent, with only two fry releases in the 1960s (WDFW et al. 1999). In August 1962 and June 1964, 16,000 Klickitat (2.3 g fish or 197 fish/lb) and 10,500 Willamette (2.6 g fish or 175 fish/lb) stock spring Chinook, respectively, were released by the Washington Department of Fisheries into the Tucannon River. The out-planting program was discontinued after a major flood destroyed the rearing ponds in 1965. Neither of these releases is believed to have returned any significant number of adults. After completion of the four lower Snake River dams, the Lower Snake River Compensation Plan (LSRCP) program was created to provide hatchery compensation for the loss of spring and fall Chinook salmon, and summer steelhead in the Snake River resulting from construction and operation of the four lower Snake River power dams (USACE 1975). In 1985, Washington Department of Fish and Wildlife (WDFW) began the hatchery spring Chinook production program in the Tucannon River by trapping wild (unmarked) adults for the hatchery broodstock. Hatchery-origin fish have been returning to the Tucannon River since 1988. The hatchery broodstock since 1989 has consisted of natural and hatchery-origin fish.

In 1992, the National Marine Fisheries Service (NMFS) listed Snake River spring/summer Chinook as "endangered" (April 22, 1992 Federal Register, Vol. 57, No. 78, p 14653), which included the Tucannon River stock. The listing status was changed to "threatened" in 1995 (April 17, 1995 Federal Register, Vol 60, No 73, p 19342). Between 1993-1998, WDFW operated the supplementation program under Section 10 direct take permit #848 for artificial propagation and research. From 1998-2003, WDFW operated both the supplementation and captive broodstock program under Section 10 direct take permits #1126 (artificial propagation), and #1129 (research), and since 2003 has operated under the Tucannon River Spring Chinook Hatchery and Genetic Management Plan.

The Endangered Species Act (ESA) allows for "the use of all methods and procedures which are necessary to bring any endangered species or threatened species to the point at which the measures pursuant to the Act are no longer necessary" (ESA 1973). Consistent with that provision, WDFW and the co-managers [The Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and the Nez Perce Tribe (NPT)] decided in 1997 to implement the Tucannon River captive broodstock program to sustain and potentially recover this listed

population. Both of the hatchery programs (supplementation and captive brood) are being conducted with the recognition that artificial propagation may have potentially deleterious direct and indirect effects on the listed fish (Hard et al. 1992; Cuenco et al. 1993; Busack and Currens 1995; Campton 1995). These effects may include genetic and ecological hazards that cause maladaptive genetic, physiological, or behavioral changes in donor or target populations, with attendant losses in natural productivity (Hard et al. 1992). However, WDFW and the co-managers believed the risk of extinction in the Tucannon River was high enough to warrant intervention beyond the supplementation program. Araki et al. (2007) found that even a few generations of domestication may have negative effects on natural reproduction of fish in the wild. This program was defined to last for only one-generation cycle (five brood years), and any potential negative effects should hopefully be reduced due to the short-term nature of the program.

Annual adult returns between 1985-1993 were estimated to be 400-750 wild and hatchery fish combined (Figure 1). In 1994, the adult escapement declined severely to less than 150 fish, and the run in 1995 was estimated at 54 fish. In 1995, WDFW started the Captive Broodstock Program but discontinued it based upon higher predicted 1996-97 returns. Unfortunately, the 1996 and 1997 returns were not strong. In addition, major floods in 1996 and 1997 on the Tucannon River destroyed most of the natural production for both brood years. Moreover, an 80% loss of the hatchery egg take occurred in 1997 due to a malfunction of a water chiller that cold shocked the eggs. Because of the lower returns, and losses to both natural and hatchery production, the Tucannon River spring Chinook captive broodstock program was re-initiated with the 1997 brood year.

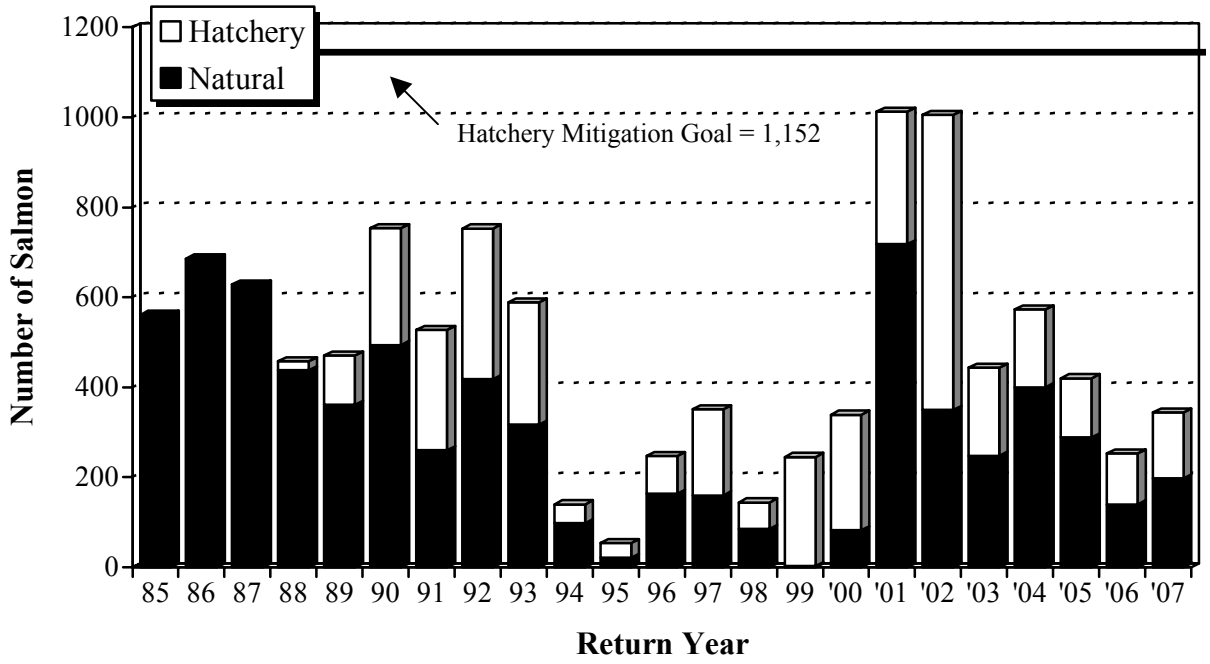

Figure 1. Total estimated escapement of Tucannon River spring Chinook salmon from 1985-2007.

Key to the Tucannon River spring Chinook restoration effort will be whether or not the natural population can consistently return above the replacement level. Since 1985, WDFW has monitored and estimated the performance of the natural population for comparison to the hatchery program as part of the LSRCP program (USFWS 1998). Monitoring efforts to date have shown the natural population below replacement almost every year (Figure 2). Unless the natural population returns to a point above replacement, the overall goal of the Tucannon River spring Chinook restoration program will not be met.

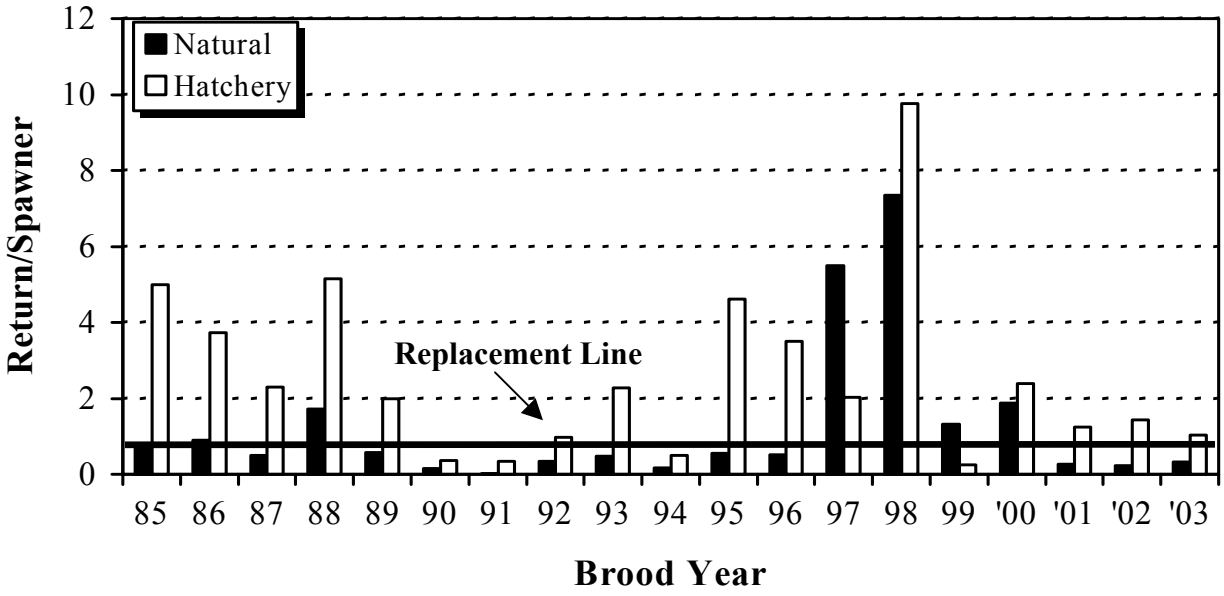

Figure 2. Return per spawner (with replacement line) for Tucannon River spring Chinook salmon for the 1985-2003 brood years (2003 brood year incomplete).

Tucannon River Watershed Characteristics

The Tucannon River empties into the Snake River between Little Goose and Lower Monumental dams approximately 622 river kilometers (rkm) from the mouth of the Columbia River (Figure 3). Stream elevation rises from 150 m at the mouth to 1,640 m at the headwater (Bugert et al. 1990). Total watershed area is about 1,295 km^2. Mean discharge is 4.9-m^3/sec with a mean low of 1.7-m^3/sec (August) and a mean high flow of 8.8-m^3/sec (April/May). Local habitat problems related to logging, road building, recreation, and agriculture/livestock grazing has limited the production potential of spring Chinook in the Tucannon River. Spring Chinook typically spawn and rear above rkm 40. WDFW and the co-managers believe producing smolts will maximize recovery efforts from the captive brood and supplementation programs, and releases in the upper watershed have the best chance for high survival.

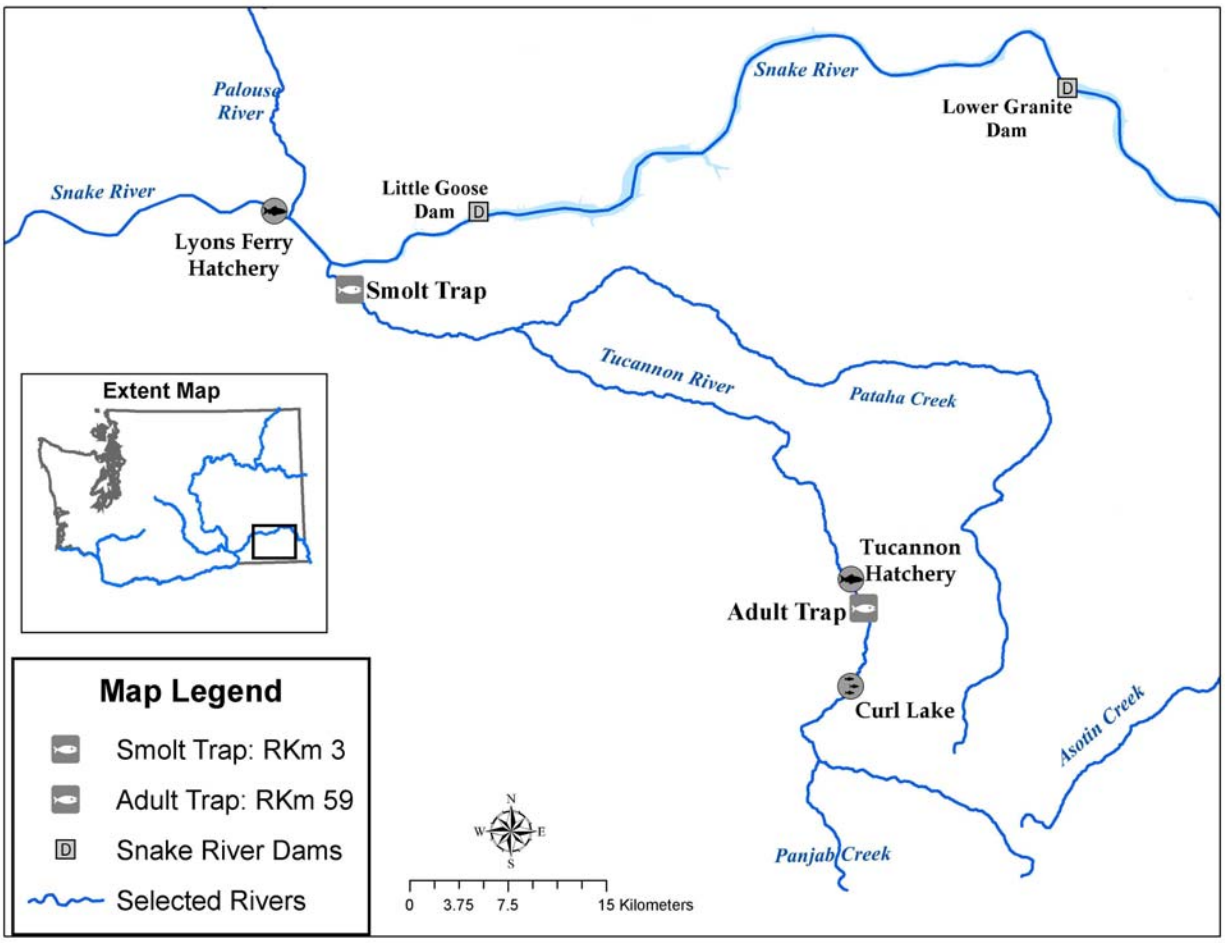

Figure 3. Location of the Tucannon River within the Snake River Basin, and locations of Lyons Ferry Hatchery, Tucannon Hatchery, and Curl Lake Acclimation Pond within the Tucannon River Basin.

It is hoped that initiatives for habitat improvement within the Tucannon Basin (BPA funded Tucannon River Model Watershed Program and Subbasin Plan, and the State of Washington Governor's Salmon Recovery Plan) that are aimed at increasing in-river survival, improved ocean conditions, and continued adult and juvenile passage improvements at Federal Columbia River Power System (FCRPS) dams, will be enough to return the natural population productivity to above the replacement level. For example, broad based goals of the Tucannon Model Watershed Program are to: 1) restore and maintain natural stream stability, 2) reduce water temperatures, 3) reduce upland erosion and sediment delivery rates, 4) improve and re-establish riparian vegetation, and 5) increase amounts of large woody debris. Managers hope that these habitat recovery efforts will ultimately increase survival of naturally reared spring Chinook in the river. While this will only provide an increase to juvenile population numbers (parr or smolts), greater numbers of juveniles should return more adult fish to the Tucannon River even if passage problems and ocean conditions remain unchanged. The captive brood program was intended to provide a quick increase in the number of adults that will produce progeny to take advantage of improved habitat.

Facility Descriptions

The spring Chinook supplementation program currently utilizes three different WDFW facilities: Lyons Ferry Hatchery (LFH), Tucannon Fish Hatchery (TFH), and Curl Lake Acclimation Pond (AP). Lyons Ferry Hatchery is located on the Snake River (rkm 90) at its confluence with the Palouse River (Figure 3). LFH was constructed with funds provided by the Army Corps of Engineers, and has subsequently been funded through the LSRCP program of the U.S. Fish and Wildlife Service. Ultimately, the FCRPS through BPA bears the cost of the LSRCP program. Lyons Ferry is used for adult broodstock holding and spawning, and incubation and early life stage rearing until production marking. Fifteen 1.2-m diameter circular starter tanks were purchased when the captive broodstock program was started in 1995. In 1999, LSRCP purchased and supplied the funding for installation of eight 6.1-m diameter circular rearing tanks for the adults, and for relocation of the small circular tanks. The tanks were installed during August and September of 1999 in the captive broodstock rearing area at LFH. During 2000, BPA supplied funding for security fencing around the broodstock rearing area.

Tucannon Hatchery, located at rkm 59 on the Tucannon River (Figure 3), has an adult collection trap on-site. Following marking at LFH, juveniles are transferred to TFH to rear through winter. In mid-February, the fish are transferred to Curl Lake AP for a minimum of three weeks acclimation. Curl Lake AP is a 0.85 ha natural bottom lake with a mean depth of 2.8 meters (pond volume estimated at 22,203 m^3). Sometime between the middle of March and the first of April, the pond exit is opened and the fish are allowed to volitionally emigrate from the lake until the third week of April when they are forced out.

Monitoring and Evaluation

As previously mentioned, the LSRCP Tucannon River spring Chinook supplementation program has ongoing evaluations. Some of the monitoring and evaluation activities include or have included: smolt release sampling, smolt trapping, spawning ground surveys, genetic monitoring, snorkel surveys for juvenile population estimates, spawning, fecundity monitoring, and experimental release strategies for smolts. Through these and other activities, survival rates of the natural and hatchery fish have been documented for the span of the supplementation program. These and other activities will continue to play a major role in evaluating the success of the captive broodstock program in the future (for both parents and progeny).

As part of the monitoring plan, survival and rate of maturation were documented by family groups within each brood year. Fecundity and egg size have been documented for all spawned captive broodstock females. Maturation timing, as well as overall growth rates, were monitored for each brood year. Smolt migration will be monitored through the use of Passive Integrated Transponder (PIT) tags, and adult return rates will be monitored through coded-wire tag (CWT) recovery during adult trapping, and carcass recoveries during spawning ground surveys.

Goal

The captive broodstock goal was to collect 290,000 eggs/year from captive brood females when three complete age classes (Age 3-Age 5) were spawned concurrently. Under the original program design, these eggs were expected to produce about 150,000 smolts for release from the Curl Lake AP. Depending on smolts produced each year this should provide a return of about 300 adult fish of captive broodstock origin per year between 2005-2010. These fish combined with fish from the hatchery supplementation program and natural production from the river should return 600-700 fish annually between 2005-2010. While this return is still well below the LSRCP mitigation goal, it would increase the in-river population level to a pre-1994 level. As described in the Tucannon Master Plan, measures have been taken to minimize and mitigate potential genetic and/or ecological hazards of this program to the listed population (WDFW et al. 1999).

Source of Captive Population

The captive population originated from the hatchery supplementation program during the 1997-2001 BYs (WDFW et al. 1999). Additional eggs were collected from the 2002 BY, initially to have extra males available at the end of the program. Supplementation broodstock consist of both natural and hatchery returns (generally 1:1 ratio). Returning hatchery fish used in the supplementation broodstock are verified to have come from the Tucannon River stock through CWT verification. Collection of eggs/fry from the supplementation program was done to lessen the effects of removing more fish from the natural population. Also, disease history and origin of parents would be known, and the overall effect to the supplementation program would be minimal.

During the spawning process in the supplementation program, the eggs of two females were split in half with each lot fertilized by a different primary male (each male also acts as a secondary male). Due to the relatively small population size, a 2 x 2 mating (Figure 4) strategy has been incorporated into the supplementation program to increase genetic variation. Milt from a secondary male was added as a backup after 30 seconds. Actual fertilization takes place in a few seconds, so the backup male is not likely to contribute substantially to each individual egg lot unless semen from the primary male is non-viable.

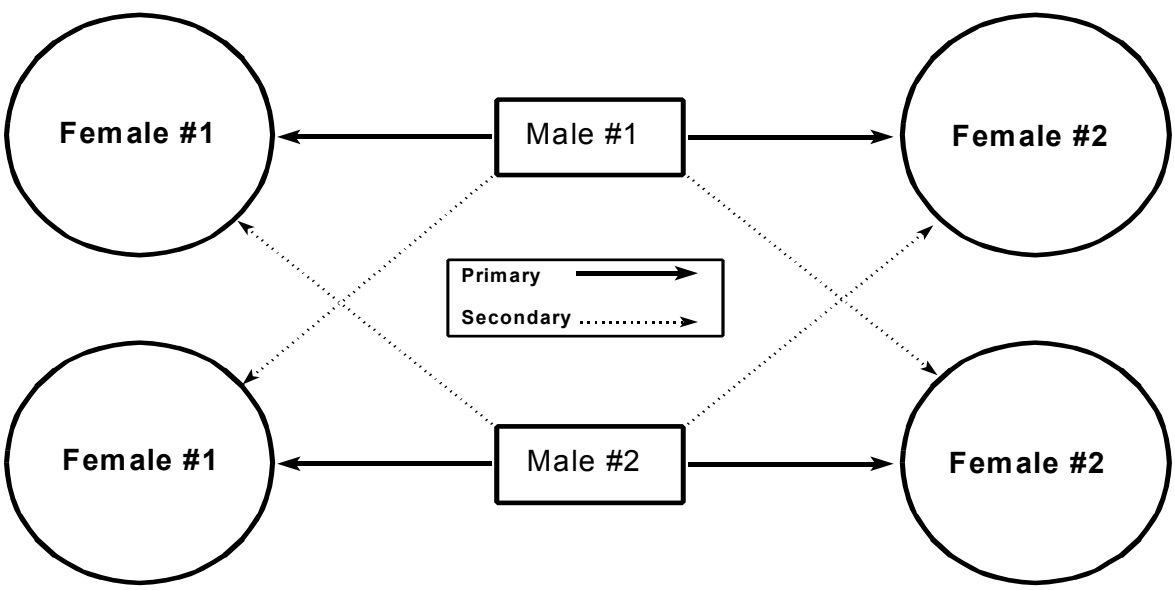

Figure 4. Diagram of the 2 x 2 mating scheme used by WDFW in the conventional supplementation and captive broodstock program.

Because of the mating strategy, some progeny from the two females are likely related as a family unit. Therefore, we consider all crosses with identical males (whether as primary or secondary to the mating) as one family unit to avoid within-family matings in the future. So while only 15 "family" units were chosen for the program, actual contribution of male and female parents (population size) to the captive broodstock program on a yearly basis has been higher. The actual number of parents that comprise the 1997-2002 BYs are given in Appendix A. Effective population size (N_e) for each brood year was calculated by the formula:

$$N_e = 4\,(N_M)(N_F)/(N_M + N_F)$$

Where: N_M = number of males
N_F = number of females

The effective population sizes of the 1997-2002 BYs were 53, 58, 42, 56, 58, and 59, respectively. Allendorf and Ryman (1987) and Verspoor (1988) have suggested that little (<1%) genetic variability will be lost in most salmonid species if the N_e of the founding population is greater than 50.

Selection of eggs/fry for the captive brood program was based on Bacterial Kidney Disease (BKD) and virology screening of females, parent origin, and matings (Appendix A). Spawned females were examined for BKD using the Enzyme Linked Immunosorbent Assay (ELISA) technique. Only females that were given a "Low" (0.11 - 0.19 Optical Density (OD)) or "Below Low" (< 0.11 OD) ELISA result were selected, with priority given to "Below Low" females. Priority for selection (in the following order) of eggs/fry was given to Wild x Wild, Wild x Hatchery (Mixed), and Hatchery x Hatchery crosses. All BYs identified for the program followed the same criteria.

Screening for BKD was a major factor in WDFW's decision to collect eggs/fry from the supplementation program. By having the test results prior to selection, and by having rearing criteria that called for minimal sampling/handling, we felt that BKD outbreaks would be minimized. To date, we know of no mortalities that can be attributed to BKD in the captive brood population.

Eighty fish from each of the 15 "family units" were selected (1,200 total fish) from each BY and moved to the 1.2-m circular fiberglass tanks. After rearing for one year, each of the "family" groups was reduced to 30 fish/family (450 fish/BY) by random selection just prior to marking. Excess fish were returned to the supplementation production group. Fish destined for the captive broodstock program were marked by "family" group with a CWT in the snout and adipose fin (backup). This was to verify "family" groups during future spawning activities so that full or half-siblings were not mated together. In addition to the CWT, an alphanumeric visual implant (VI) tag was placed behind the left or right eye to identify each fish. The VI tag, should it be retained, would provide a quicker "family" identification method than the CWT. In addition, fish that retain the VI would provide individual growth rates. After the fish were tagged, they were transferred to one of the 6.1-m circular fiberglass tanks for rearing to maturity. Once the fish were transferred to the larger rearing tanks, they were not moved again unless survival rates were greater than anticipated, or density limits were exceeded within the rearing tanks. At maturity, fish were transferred to the adult raceway located in the spawning building. Family size and marking procedures were the same for all brood years collected.

Density limits for each rearing tank were established prior to any stocking of fish. Most of the density limits prescribed were taken from the WDFW Dungeness River Captive Broodstock Program, where similar size starter and adult rearing tanks were used. Based on those density limits and expected survival and maturation rates, we were able to design the facilities needed. The current fish number maximums are as follows: 1.2-m circular tanks = no more than 200 fish/tank at Age 1; 6.1-m circular tanks = no more than 150 fish/tank at Age 3, or 100 fish/tank at Age 4.

Fry from each brood year were collected as described above, with appropriate families chosen for the program (Appendix A). Data on average length (mm), weight (g), and condition factor (K) for each "family" group were compiled during tagging (Appendix B).

Rearing, Spawning, and Release

Captive brood fish are reared at LFH using standard fish culture practices and approved theraputants in pathogen free well water that is a constant 11ºC. Each 6.1-m circular captive tank is supplied with about 581 L/min water flow, while the 1.2-m tanks receive about 23 L/min. To reduce the risk of catastrophic fish loss due to hatchery facility or operational failure, a number of safeguards are in place. LFH is staffed full time by personnel living on-station, providing for the protection of fish from vandalism and predation. The hatchery is also equipped with back-up generators in the event of power outages. All staff are trained in proper fish handling, transport, rearing, biological sampling, and WDFW fish health maintenance procedures to minimize the risk of fish loss due to human error. All fish are handled, transported, and propagated in accordance with the WDFW Fish Health Manual (WDFW 1996) and Pacific Northwest Fish Health Protection Committee (PNFHPC 1989) disease prevention

and control standards to minimize loss due to disease. Sanitation procedures are employed to reduce the transfer and incidence of fish diseases, and to promote quality fish in accordance with PNFHPC (1989) and Integrated Hatcheries Operations Team (1995) guidelines.

A variety of high quality commercial feed is provided through a state contract, and feed size varies with the estimated fish size of the different BYs. To date, we have used Moore-Clark Nutra™, Moore-Clark Fry™, Bio-Products Salmon Brood Feed™, and Moore-Clark Pedigree Trout Brood Feed™ on the captive brood. Estimated size only is generally used to prescribe feeding rates, as WDFW decided initially that too much handling of the fish to determine growth and size would jeopardize fish health. This decision resulted from problems that Oregon Department of Fish and Wildlife (ODFW) and Idaho Department of Fish and Game (IDFG) captive programs experienced during their first years of operation with monthly fish sampling (Bumgarner and Gallinat 2001). Due to the degree of early maturation of females in the 1997 and 1998 brood years, size-at-age recommendations were revised to produce more mature Age 4 and 5 fish. Size-at-age goals are: Age 1, 20-25 g; Age 2, 150-200 g; Age 3, 900 g; and Age 4, 4,000 g. All captive brood fish are reared outside under natural photoperiod conditions. However, each of the 6.1-m circular tanks are covered with camouflage netting which shades the pond. The netting also prevents fish from jumping out of the tank.

During the summer (late June to early July), captive brood fish that are Age 2 or greater are examined for signs of sexual maturation. Maturation is determined by change in body coloration, as other morphological sexual characteristics are not as obvious. Mature female captive broodstock were injected with Erythromycin (0.5 cc/4.5 kg of body weight) at sorting to prevent Bacterial Kidney Disease. The broodstock are also treated with a formalin flush (167 ppm) every other day to control fungus. Mature fish (primarily Age 2 jacks) not used for spawning are sacrificed at the end of the spawning season.

All captive brood progeny smolts are marked differently from supplementation progeny for identification upon adult return. Smolts are unclipped and marked with an agency-only wire tag (2000-2002 BYs) or CWT in the snout (production fish have an elastomer tag and CWT). When supplementation or captive brood fish return as adults at the TFH adult trap, each unmarked (no adipose clip) adult spring Chinook will be scanned for wire in the snout and examined for a VI tag. If the fish is not adipose fin clipped, and wire is present in the snout and no VI is present, the fish is likely from the captive broodstock program and will be passed upstream to spawn in the river.

2006 Progeny

The 2006 BY captive brood juveniles (78,705 fish) were marked with a CWT in the snout on 11-13 September, 2007. Marked fish were transported to the Tucannon Fish Hatchery on 3 October. Fish were tagged with Passive Integrated Transponders (PIT) for outmigration comparisons on 31 January (1,000 conventional supplementation fish and 1,000 captive brood progeny) before transfer to Curl Lake AP. The captive brood progeny were moved to Curl Lake for final rearing 11-12 February 2008. Pre-release length and weight samples were collected on 8 April. Mean length of a subsample of 250 released captive brood progeny was 158.5 mm (S.D. 29.8 mm) with a coefficient of variation of 18.8. Mean weight was 57.8 g (S.D. 29.8 g) with an average condition factor (K) of 1.28. There was one precocial fish in the subsample.

Volitional release began 8 April and continued until 22 April when the remaining fish were forced out. Mortalities were low in Curl Lake and 78,176 BY 2006 captive broodstock progeny were released into the Tucannon River (Table 1). These fish were marked with a CWT and no fin clips in order to differentiate them from the supplementation fish (CWT/Left Blue VIE/No Finclip and CWT/Left Purple VIE/No Finclip). Monitoring their survival and future releases to adult returns, along with future natural production levels, will determine the success or failure of the captive broodstock program. A summary of fish releases from the program to date can be found in Appendix C.

Table 1. Spring Chinook captive brood progeny smolt releases in the Tucannon River, 2006 brood year.

Release		Release		Total	CWT	Number	Ad-only	
Year	(BY)	Location	Date	Released	Code	Tagged	Marked	Kg
2008	2006	Curl Lake	4/08-4/22	78,176	63/41/94	75,283	N.A.	4,488.8

N.A. = Not Applicable.

PIT Tagging

In 2007, we used passive integrated transponder (PIT) tags to compare emigration travel timing and relative success of the 2005 BY captive brood progeny with our conventional hatchery supplementation fish. We tagged 1,000 captive brood progeny and 1,002 conventional supplementation fish during early February before transferring them to Curl Lake AP for acclimation and volitional release (Table 2). No fish were killed during PIT tagging, though some minor delayed mortality may have occurred after transfer. Dam detections were 47% for conventional supplementation fish (compared to 33% in 2006) and 41% for captive brood origin fish (compared to 28% in 2006). The smolts were released at a larger size in 2007 (57 g vs. 35 g).

Table 2. Cumulative detection (one unique detection per tag code) and travel time (TD) summaries of PIT tagged hatchery spring Chinook salmon released from Curl Lake Acclimation Pond (rkm 65.6) on the Tucannon River at downstream Snake and Columbia River dams during 2007. (Fish were volitionally released from 4/02/07-4/23/07).

	Release Data			Recapture Data										
Hatchery		Mean		Mean	LMJ		MCJ		JDJ		BONN		Total[a]	
Origin	N	Length	S.D.	Length	N	TD	N	TD	N	TD	N	TD	N	%
Supp.	1,002	134.3	15.8	134.5	138	20.8	131	24.2	126	28.5	26	30.3	467	46.6
C.B.	1,000	135.1	19.6	135.4	88	22.0	135	25.0	109	28.7	34	30.4	413	41.3

[a] Total includes detections at Ice Harbor Dam and from trawl surveys.
Note: Mean travel times listed are from total number of fish detected at each dam, not unique recoveries for a tag code. Abbreviations are as follows: LMJ-Lower Monumental Dam, MCJ-McNary Dam, JDJ-John Day Dam, Bonn-Bonneville Dam, S.D.-standard deviation, TD – Mean Travel Days.

Survival probabilities were estimated by the Cormack Jolly-Seber methodology using the Survival Under Proportional Hazards (SURPH) computer model. The data files were created using the PitPro version 4.8 computer program to translate raw PIT Tag Information System (PTAGIS) data of the Pacific States Marine Fisheries Commission (PSMFC) into usable capture histories for the SURPH program. Survival estimates from Curl Lake to Lower Monumental Dam were 0.68 (± 0.05) and 0.61 (± 0.06) for supplementation and captive brood progeny, respectively. While estimated survival was slightly lower for captive brood progeny fish the difference was not significant ($P > 0.05$).

Adult Returns

Seven captive brood progeny adult returns (6 females, 1 male) were recovered during 2007 (Table 3). Four of the returns were recovered during spawning ground surveys and three were collected for broodstock at the adult trap. Only one captive brood progeny was recovered below the adult trap (rkm 49.6). The number of captive brood returns was expanded to 19 for the total run.

Table 3. Captive brood progeny adult returns collected from hatchery spawning and carcass recoveries from the Tucannon River during 2007.

Date	Spawned or Rkm	Sex	Fork Length (cm)	POH Length (cm)	Age	Brood Year	DNA Sample #
9/11/07	SP	F	68.5	60.0	4	2003	07AB10
9/18/07	SP	F	65.0	56.0	4	2003	07AB21
9/21/07	49.6	F	66.0	60.0	4	2003	07AB138
9/25/07	SP	M	70.0	58.0	4	2003	07AB29
9/26/07	65.3	F	---	59.5	4	2003	07AB133
9/26/07	64.7	F	64.0	54.0	4	2003	07AB135
9/26/07	64.1	F	---	55.0	4	2003	07AB136

Survival Rates

Point estimates of population sizes have been calculated for various life stages (Table 4) of the captive brood fish based on fecundity estimates, hatchery records, smolt trapping and redd surveys. From these data, survivals between life stages have been calculated to assist in evaluation of the captive brood program (Table 5).

Table 4. Estimates of Tucannon River spring Chinook salmon captive brood abundance by life stage for the 2000-2006 brood years.

Brood Year	Females Spawned	Mean Fecundity[a]	Number of Eggs	Number of Parr	Number of Smolts	Progeny (returning adults)
2000	12	1,298	14,577	4,323	3,055	0
2001	166	1,765	281,303	195,264	140,396	17
2002	121	1,561	176,544	50,462	44,784	2
2003	223	1,389	309,416	164,800	130,064	21[b]
2004	205	1,549	310,819	140,874	132,312	0[b]
2005	167	1,595	261,845	93,971	90,056	
2006	86	1,892	162,736	79,432	78,177	

[a] Based on fully spawned females.
[b] Incomplete – brood year still returning.

Table 5. Survival rates (%) by brood year for various life stages for Tucannon River spring Chinook captive brood progeny.

Brood Year	Egg-to-Parr	Parr-to-Smolt	Egg-to-Smolt	Smolt-to-Adult
2000	29.7	70.7	21.0	0.00
2001	69.4	71.9	49.9	0.01
2002	28.6	88.7	25.4	0.00
2003	53.3	78.9	42.0	0.02[a]
2004	45.3	93.9	42.6	0.00[a]
2005	35.9	95.8	34.4	
2006	48.8	98.4	48.0	
Mean	44.4	85.5	37.6	0.01
Geometric Mean	42.5	84.8	36.0	0.00

[a] Incomplete – brood year still returning.

Egg-to-parr survival for captive brood progeny averaged 44.4% over seven years (Table 5). This is higher than the 10.1% egg-to-parr survival estimated for in-river natural-origin Tucannon River spring Chinook, but less than the 83.5% survival from the conventional hatchery supplementation program fish (Gallinat and Ross 2007). Parr-to-smolt survival averaged 85.5% for the captive brood progeny. This is in comparison to 54.4% for in-river natural-origin and 87.0% for conventional hatchery supplementation fish. Egg-to-smolt survival was 37.6% for the

captive brood fish compared to 5.8% for natural-origin fish and 72.0% for conventional hatchery-origin fish. Smolt-to-adult survival for captive brood progeny has effectively been 0.0% for the first few years of the program (Table 5) compared to SARs of 0.11% and 0.75% for conventional hatchery and natural-origin fish, respectively, for the same time period.

DNA Genetic Samples

2007 Brood Year

Since the beginning of the program in 1997, we have collected DNA samples from all spring Chinook parents that eventually contributed gametes to the captive broodstock population. Additional samples are also collected during spawning ground surveys to provide a large genetic data set that will be used to describe the population. During 2007 we collected 147 DNA samples (operculum punches) from adult salmon (95 natural origin, 36 conventional supplementation, 7 captive brood progeny and 9 hatchery-origin strays). The 2007 DNA samples were sent to the WDFW genetics lab in Olympia for baseline microsatellite DNA analysis.

2006 Brood Year

A total of 228 Tucannon River spring Chinook samples collected in 2006 were genotyped at 14 microsatellite loci (Ogo-2, Ogo-4, Ots-3M, Ssa-197, Oki-100, Ots-201b, Ots-208b, Ssa-408, Omm-1080, Ots-213, Ots-G474, Ots-9, Ots-211, and Ots-212) using an Applied Biosystems 3730 DNA analyzer (Appendix D). Analysis to date provides evidence that the captive broodstock program has been an effective method of preserving overall genetic variation in Tucannon River spring Chinook while providing additional smolts for release (Kassler and Hawkins 2008, Appendix D). Genotypes, allele frequencies, and tissue samples are stored at WDFW's Genetics Laboratory in Olympia, Washington.

Coordination and Reporting

Since BPA funding was acquired, WDFW has joined other researchers in a group known as the Captive Broodstock Technical Oversight Committee (CBTOC). The CBTOC is a forum for all BPA funded projects working with captive broodstock or captive rearing programs. The CBTOC goal is to ensure that all groups are coordinated, and communication is occurring between projects. The CBTOC also gives each of the researchers a chance to ask questions about other program's successes and failures, so each respective program can be adapted for better results.

WDFW also provides the co-managers with a monthly update on the captive broodstock and supplementation program activities. This monthly program update informs them about fish on hand, mortalities incurred, and any up-coming actions that may warrant their attention.

This annual progress report is produced by WDFW to disseminate the information gathered from this project to other researchers in the Columbia and Snake River basins. The final report, including complete results of the genetics analysis, will be submitted by the end of September 2009. Additional reports and papers will also be published following complete returns of all captive brood origin fish back to the Tucannon River.

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APPENDIX A

Table 1. Selection of progeny for the Tucannon River spring Chinook captive broodstock program based on origin, crosses, and BKD ELISA results, 1997 and 1998 BYs.

Brood Year	Eggtake Date	Female Numbers	Male Numbers	Crosses	BKD ELISA[1]	Tank/Family Number
97	09/16	H885 + H886	W108 + W110	Mixed	LOW, BL	TANK 1
97	09/16	H889	W116 + W120	Mixed	BL	TANK 2
97	09/23	W958 + W957	H122 + H123	Mixed	BL	TANK 3
97	09/16	W897 + W898	H156 + H199	Mixed	BL	TANK 4
97	09/09	H872 + H871	W159 + W161	Mixed	BL	TANK 5
97	09/09	H873	W163 + W165	Mixed	LOW	TANK 6
97	09/09	W881 + W882	H167 + H175	Mixed	BL	TANK 7
97	09/16	W951 + W952	H149 + H157	Mixed	BL	TANK 8
97	09/09	W874 + W875	H171 + H173	Mixed	BL	TANK 9
97	09/09	W878 + W876	H179 + H181	Mixed	LOW, BL	TANK 10
97	09/02	W869 + W867	H191 + H193	Mixed	BL	TANK 11
97	09/09	H879	W169 + W177	Mixed	BL	TANK 12
97	09/16	W899	H153 + H154	Mixed	BL	TANK 13
97	09/02	W870	H183 + H185	Mixed	BL	TANK 14
97	09/02	H868	W187 + W189	Mixed	BL	TANK 15
98	08/25	W1003 + W1004	H754 + H753	Mixed	BL	TANK 1
98	08/25	W1005 + W1006	H751 + W131	Mixed	LOW, BL	TANK 2
98	09/08	W3001 + W3002	H758 + H759	Mixed	LOW, BL	TANK 3
98	09/08	W3003 + W3004	H755 + H756	Mixed	BL	TANK 4
98	09/08	W3005 + W3006	H757 + H760	Mixed	BL	TANK 5
98	09/08	W3007 + W3008	W128 + W129	Wild	BL	TANK 6
98	09/08	H3009 + H3010	W130 + W133	Mixed	LOW, BL	TANK 7
98	09/11	H4001 + H4002	W135 + W134	Mixed	LOW, BL	TANK 8
98	09/11	W4003 + W4004	H762 + H761	Mixed	LOW, BL	TANK 9
98	09/11	W4007 + W4008	H767 + H765	Mixed	LOW, BL	TANK 10
98	09/11	W4009 + W4010	H769 + H768	Mixed	BL	TANK 11
98	09/15	W5002	H777 + H773	Mixed	LOW	TANK 12
98	09/15	W5003	H772 + H771	Mixed	LOW	TANK 13
98	09/22	W6005 + W6006	H781 + H780	Mixed	BL	TANK 14
98	09/22	W6007 + W6008	H783 + H782	Mixed	BL	TANK 15

[1] Low = 0.11-0.19 Optical Density; Below Low = < 0.11 Optical Density.

Table 2. Selection of progeny for the Tucannon River spring Chinook captive broodstock program based on origin, crosses, and BKD ELISA results, 1999 and 2000 BYs.

Brood Year	Eggtake Date	Female Numbers	Male Numbers	Crosses	BKD ELISA[1]	Tank/Family Number
99	08/31	H101	H1+H2+H526	Hatchery	LOW	TANK 1
99	09/07	H203	H12+H13+H536	Hatchery	BL	TANK 2
99	09/07	H204	H15+H530+H531	Hatchery	LOW	TANK 3
99	09/07	W205	H18+H532+H533	Mixed	LOW	TANK 4
99	09/07	H206	H528+H529+H534	Hatchery	BL	TANK 5
99	09/07	H212	H19+H20	Hatchery	BL	TANK 6
99	09/14	H305	W31+H571	Mixed	LOW	TANK 7
99	09/14	H306	W21+H576	Mixed	LOW	TANK 8
99	09/14	H307	H40+H550	Hatchery	LOW	TANK 9
99	09/14	H309	H23+H549	Hatchery	BL	TANK 10
99	09/14	H310	H39+H572	Hatchery	LOW	TANK 11
99	09/14	H311	H36+H568	Hatchery	LOW	TANK 12
99	09/14	H312	H24+H544	Hatchery	LOW	TANK 13
99	09/21	H403	H45+H580	Hatchery	LOW	TANK 14
99	09/21	H404	H581+H582+H583	Hatchery	LOW	TANK 15
00	8/29	H102	H1 + H2	Hatchery	BL	TANK 1
00	8/29	H103 + H104	H3 + H4	Hatchery	BL	TANK 2
00	8/29	H105 + W106	H5 + H6	Mixed	BL	TANK 3
00	9/05	H202	W1 + H19	Mixed	BL	TANK 4
00	9/05	H203 + H204	W2 + H7	Mixed	BL	TANK 5
00	9/05	H205 + H206	H8 + H9	Hatchery	BL	TANK 6
00	9/05	H209 + H210	H12 + H13	Hatchery	BL	TANK 7
00	9/05	H211	H14 + H15	Hatchery	BL	TANK 8
00	9/05	H213 + H214	H16 + H17	Hatchery	BL	TANK 9
00	9/05	W215	H10 + H11	Mixed	BL	TANK 10
00	9/12	H301 + H302	H20 + H24	Hatchery	BL	TANK 11
00	9/12	H303 + H304	W3 + H23	Mixed	BL	TANK 12
00	9/12	H308 + H311	W5 + H22	Mixed	BL	TANK 13
00	9/19	W401 + H402	H30 + H31	Mixed	BL	TANK 14
00	9/19	H403 + H404	W6 + H32	Mixed	BL	TANK 15

[1] Low = 0.11-0.19 Optical Density; Below Low = < 0.11 Optical Density.

Table 3. Selection of progeny for the Tucannon River spring Chinook captive broodstock program based on origin, crosses, and BKD ELISA results, 2001 and 2002 (for extra males) BYs.

Brood Year	Eggtake Date	Female Numbers	Male Numbers	Crosses	BKD ELISA[1]	Tank/Family Number
01	8/28	H101 + H103	28A2 + BCCC	Mixed	BL	TANK 1
01	9/04	W201 + W203	HM8 + HM9	Mixed	BL	TANK 2
01	9/04	W205 + W207	HM4 + HM5	Mixed	BL	TANK 3
01	9/04	H206 + H208	B2F4 + AAE7	Mixed	BL	TANK 4
01	9/04	W211 + W212	HM3 + HM6	Mixed	BL	TANK 5
01	9/04	H210 + H213	AOFB + DB6E	Mixed	BL	TANK 6
01	9/04	W214 + W220	HM2 + HM7	Mixed	BL	TANK 7
01	9/11	W301 + W303	HM10 + HM11	Mixed	BL	TANK 8
01	9/11	W314	HM16 + HM23	Mixed	BL	TANK 9
01	9/11	W304 + W305	HM12 + HM14	Mixed	BL	TANK 10
01	9/11	W307 + W308	HM13 + HM17	Mixed	BL	TANK 11
01	9/11	H309 + H311	9890 + 2912	Mixed	BL	TANK 12
01	9/11	H312	FEAC + 5F6F	Mixed	BL	TANK 13
01	9/18	W401 + W409	HM25 + HM26	Mixed	BL	TANK 14
01	9/18	W410 + W411	2626 + AF96	Wild	BL	TANK 15
02	8/27	W103 + W104	HM1 + HM2	Mixed	BL	TANK 1
02	8/27	H110	D0AA + AB01	Mixed	BL	TANK 2
02	9/03	W203 + W204	HM5 + HM6	Mixed	BL/LOW	TANK 3
02	9/03	W211 + W215	HM7 + HM8	Mixed	BL	TANK 4
02	9/03	W217 + W219	HM9 + HM10	Mixed	BL	TANK 5
02	9/03	H209 + H210	B5BD + 8D07	Mixed	BL	TANK 6
02	9/03	H212 + H213	A6CE + BC25	Mixed	BL	TANK 7
02	9/03	H214 + H216	A0CD + 29BC	Mixed	BL	TANK 8
02	9/10	W301 + W303	HM11 + HM12	Mixed	BL	TANK 9
02	9/10	W307 + W309	HM15 + HM16	Mixed	BL/LOW	TANK 10
02	9/17	H401 + H402	1515 + 98BA	Mixed	BL	TANK 11
02	9/17	H403 + H404	C045 + BF27	Mixed	BL	TANK 12
02	9/17	H405 + H408	A58C + BEB0	Mixed	BL	TANK 13
02	9/17	W406 + W407	HM24 + HM25	Mixed	BL	TANK 14
02	9/17	W409 + W410	HM19 + HM20	Mixed	LOW/BL	TANK 15

[1] Low = 0.11-0.19 Optical Density; Below Low = < 0.11 Optical Density.

APPENDIX B

Average length (mm), weight (g), and condition factor (K) with standard deviations for each family unit from the 1997, 1998, 1999, 2000 and 2001 BYs of captive broodstock at the time of tagging.

Brood Year	Family Unit	Number of Fish	Mean Length	S.D.	Mean Weight	S.D.	K
1997	1	29	113	7.8	19.4	4.4	1.31
1997	2	14	110	5.2	17.3	2.7	1.29
1997	3	31	125	9.1	28.4	6.0	1.44
1997	4	29	118	9.3	22.7	6.0	1.37
1997	5	31	119	9.3	22.7	5.8	1.30
1997	6	30	119	8.6	22.6	5.2	1.33
1997	7	30	117	7.2	21.3	4.3	1.32
1997	8	29	121	10.2	24.8	6.8	1.36
1997	9	30	117	8.1	21.8	5.0	1.32
1997	10	30	115	11.0	19.7	6.1	1.27
1997	11	30	101	6.4	13.1	2.6	1.25
1997	12	30	120	12.5	24.5	8.0	1.38
1997	13	30	121	9.3	24.4	6.6	1.34
1997	14	30	112	6.2	18.8	3.2	1.33
1997	15	30	109	9.6	18.7	4.8	1.41
Totals / Means		**433**	**116**	**10.5**	**21.5**	**6.4**	**1.34**
1998	1	30	120	15.6	22.3	8.6	1.23
1998	2	29	108	10.0	15.9	5.0	1.25
1998	3	30	112	13.1	18.6	7.8	1.26
1998	4	30	112	11.5	17.7	6.4	1.24
1998	5	30	117	16.0	20.5	9.9	1.20
1998	6	28	117	15.0	21.6	11.0	1.26
1998	7	32	120	18.0	23.2	11.6	1.26
1998	8	30	129	12.0	26.5	7.8	1.21
1998	9	30	121	16.9	23.0	9.9	1.24
1998	10	28	130	9.0	26.0	4.9	1.18
1998	11	25	120	13.6	22.3	7.7	1.26
1998	12	31	127	10.1	24.0	4.9	1.16
1998	13	29	122	11.4	22.0	6.7	1.19
1998	14	27	120	13.2	21.6	7.7	1.20
1998	15	29	138	11.0	30.3	6.7	1.14
Totals / Means		**438**	**121**	**15.2**	**22.4**	**8.7**	**1.22**
1999	1	27	147	14.6	41.1	11.3	1.25
1999	2	28	138	13.1	35.7	8.9	1.34
1999	3	28	133	11.6	33.9	11.3	1.42
1999	4	30	145	8.9	39.2	6.7	1.27
1999	5	25	136	15.8	35.4	11.8	1.34
1999	6	30	136	10.7	33.8	8.9	1.32
1999	7	27	129	20.9	30.0	14.8	1.29
1999	8	29	129	12.0	29.9	9.0	1.35
1999	9	25	128	16.3	29.3	11.6	1.33
1999	10	23	130	18.9	31.0	14.4	1.32
1999	11	23	137	13.1	36.0	10.7	1.37
1999	12	28	141	13.5	38.4	10.2	1.33
1999	13	30	133	13.9	31.9	9.1	1.34
1999	14	30	133	10.7	31.6	7.6	1.32
1999	15	26	132	16.6	34.1	14.1	1.39
Totals / Means		**409**	**135**	**15.1**	**34.1**	**11.2**	**1.33**

Appendix B (cont.). Average length (mm), weight (g), and condition factor (K) with standard deviations for each family unit from the 1997, 1998, 1999, 2000 and 2001 BYs of captive broodstock at the time of tagging.

Brood Year	Family Unit	Number of Fish	Mean Length	S.D.	Mean Weight	S.D.	K
2000	1	30	164	11.8	52.3	8.4	1.19
2000	2	30	157	11.1	45.5	8.1	1.16
2000	3	30	152	10.1	37.9	5.9	1.08
2000	4	30	152	11.0	43.0	8.0	1.20
2000	5	30	152	8.4	38.6	5.9	1.09
2000	6	30	138	11.3	31.2	6.1	1.18
2000	7	30	140	10.1	31.4	5.4	1.14
2000	8	30	147	8.4	35.0	5.4	1.10
2000	9	30	151	9.5	37.3	6.3	1.07
2000	10	30	151	7.7	37.4	5.7	1.08
2000	11	30	143	13.9	34.9	8.3	1.18
2000	12	30	147	9.1	35.4	5.2	1.12
2000	13	30	144	13.5	34.1	8.7	1.13
2000	14	30	136	9.4	27.1	4.5	1.08
2000	15	30	132	10.8	25.1	5.1	1.10
Totals / Means		**450**	**147**	**13.4**	**36.4**	**9.4**	**1.13**
2001	1	30	95	6.7	10.4	2.1	1.22
2001	2	30	101	8.7	12.6	3.0	1.22
2001	3	30	100	5.0	12.8	1.9	1.27
2001	4	30	107	6.9	14.8	3.9	1.21
2001	5	30	110	8.3	17.5	3.2	1.30
2001	6	30	104	7.7	14.7	3.6	1.29
2001	7	30	101	6.9	13.1	2.4	1.27
2001	8	30	105	8.2	14.6	2.6	1.25
2001	9	30	106	9.2	13.8	3.1	1.17
2001	10	30	97	6.5	11.4	2.4	1.24
2001	11	30	101	7.5	12.7	2.7	1.21
2001	12	30	101	5.0	12.5	1.8	1.21
2001	13	30	100	7.5	12.2	2.9	1.20
2001	14	30	100	8.8	12.2	2.9	1.22
2001	15	30	99	7.6	12.2	2.7	1.25
Totals / Means		**450**	**102**	**8.3**	**13.2**	**3.2**	**1.24**

APPENDIX C

Summary of captive brood progeny releases from the Tucannon River Spring Chinook Captive Broodstock Program.

Release Year	BY[1]	Release Date	CWT	No Wire	Wire	Total Released	Lbs	Fish/Lb
2002	2000 (S)	3/15-4/23	63	24	3,031	3,055	343	8.9
2002	2001 (P)	5/06	63/14/30	157	20,435	20,592	124.8	165.0
2003	2001 (S)	4/01-4/21	63	5,995	134,401	140,396	10,100	13.9
2004	2002 (S)	4/01-4/20	63	1,909	42,875	44,784	3,393	13.2
2005	2003 (S)	3/28-4/15	63/27/78	4,760	125,304	130,064	9,706	13.4
2006	2004 (S)	4/03-4/26	63/28/65	5,150	127,162	132,312	8,648	15.3
2007	2005 (S)	4/02-4/23	63/34/77	1,171	88,885	90,056	12,170	7.4
2008	2006 (S)	4/08-4/22	63/41/94	2,893	75,283	78,176	9,896	7.9

[1] S = Smolt release; P = Parr release.

APPENDIX D

Genetic Assessment of Spring Chinook in the Tucannon River (2006) Using a Microsatellite DNA Analysis

by

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Abstract

A total of 228 spring Chinook samples from the Tucannon River were analyzed from collections made in 2006 using 14 microsatellite loci. Analyses were performed on captive brood samples, supplementation spawners, and in-river spawners. The supplementation and in-river spawners were of natural or hatchery-origin (based on coded-wire tags) and were divided into those two groups for further analysis. The observed heterozygosity for all collections was relatively high for each group. Genotypic tests of differentiation indicated significant differences between the captive brood spawners and both the supplementation spawners and the in-river spawners. The supplementation and in-river spawners were also significantly different from each other. The composition of hatchery and natural-origin samples in the supplementation and in-river samples was not equal and may have influenced this result. Analysis of the collections re-grouped into hatchery and natural-origin indicated significant differences among these two groups and the captive brood. The significant difference between the hatchery and natural-origin fish versus the lower level of differentiation between the supplementation and in-river spawners provides genetic evidence that the supplementation program has been effective in mixing the two-spawner groups (supplementation and in-river). The pairwise F_{ST} values identify the variation between any two groups is less than 1.0% indicating the differences among the groups is small, but there are still significant differences as detected by the genotypic tests of differentiation. These results provide evidence that the supplementation and captive brood programs have not decreased the overall genetic diversity in spring-run Chinook in the Tucannon River while providing additional smolts for release.

Introduction

Prior to 1985, only two fry releases of spring Chinook salmon (*O. tshawytscha*) occurred in the Tucannon River. In August 1962, 16,000 Klickitat River spring Chinook fry were released and in June 1964, 10,500 Willamette, Oregon spring Chinook fry were released by the Washington Department of Fisheries into the Tucannon River. Neither of these releases is believed to have returned any significant number of adults (Gallinat 2004). In 1985, the hatchery spring Chinook production program was started by the Washington Department of Fisheries in the Tucannon River by capturing wild (unmarked) adults from the Tucannon River. Since 1988, hatchery-origin spring Chinook have been returning to the Tucannon River and beginning in 1989 the hatchery broodstock has consisted of both natural and hatchery-origin fish. This supplementation program is part of the Lower Snake River Compensation Plan (LSRCP) mitigation program, and will continue as long as mitigation is required under the LSRCP.

In 1994, the adult escapement declined severely to less than 150 fish, and the run in 1995 was estimated at 54 fish. In 1995, the Tucannon River spring Chinook population was listed as threatened under the ESA because of declining numbers of returning spring Chinook despite the supplementation program. As a result, WDFW and the co-managers believed intervention beyond the supplementation program was warranted in the form of a captive broodstock program.

The plans for the captive broodstock program were determined and spring Chinook from the Tucannon River supplementation program were collected from 1997-2001 brood years (BY) to be raised to adults and spawned. Males were also collected from the 2002 BY in order to have enough to spawn with the captive brood females towards the end of the program. Each year, fish that mature from the initial group of captive broodstock are spawned. The captive brood program is scheduled to produce smolts for release through 2008. A description of the captive brood program development and the number of families used for each brood year is described in Gallinat (2006).

Both the supplementation and captive brood programs are being conducted with the understanding that artificial propagation may have potentially deleterious direct and indirect effects on spring Chinook in the Tucannon River. These effects could include genetic and ecological changes that result in maladaptive genetic, physiological, or behavioral changes in the donor or target populations, thereby causing losses in natural productivity. A report by Gallinat (2004) describes the restoration program for spring Chinook in the Tucannon River.

The goal of this report is to analyze spring Chinook collected in 2006 to assess the genetic differences in the captive brood program, the supplementation program, and fish that are spawning naturally in-river. Additional analyses will assess the genetic differentiation of hatchery-origin and natural-origin spawners to determine if the artificial production programs are having any genetic effects on the natural-origin Chinook.

Materials and Methods

Collections

A total of 228 spring-run Chinook samples were analyzed at 14 microsatellite loci (13 coastwide GAPS loci plus *Ssa-197*) from three sources in 2006: the Tucannon River supplementation program, in-river (naturally produced Chinook in the Tucannon River), and samples from the captive brood program (Table 1). Collections were grouped in two ways for analysis. The first comparisons (spawner) involved groups comprised of fish that actually spawned in the various environments (i.e., supplementation hatchery, in-river, or part of the captive brood program). Both the supplementation spawner and in-river spawner groups are comprised of natural and hatchery-origin fish. Marking and tagging operations in the hatchery made it possible to positively identify each hatchery-origin Chinook. Chinook that were unmarked were considered to be natural-origin, however they could have been from a hatchery and lost identifying tags or they could be strays from out of basin. Based on the identity of each fish they were re-distributed into groups based on their genetic-origin. The second comparison involved Chinook from the hatchery versus natural-origin (genetic-origin). The captive brood group was the same in both sets of comparisons.

Tissue samples were collected for all fish spawned in both the supplementation and captive broodstock programs in 2006. However, not all of the fish that spawned in-river were genetically sampled, therefore, the entire Tucannon River spring Chinook escapement was not represented. Collection codes, number of samples analyzed per collection, sample types and collection sources are given in Table 1.

Laboratory Analyses

Genomic DNA was extracted by digesting a small piece of fin tissue using the nucleospin tissue kits obtained from Macherey-Nagel following the recommended conditions in the user manual. Extracted DNA was eluted with a final volume of 100 µL.

Descriptions of the loci assessed in this study and polymerase chain reaction (PCR) conditions are given in Table 2. PCR reactions were run with a simple thermal profile consisting of: denaturation at 95^{o}C for 3 min, denaturation at 95^{o}C for 15 sec, anneal for 30 sec at the appropriate temperature for each locus (Table 2), extension at 72^{o}C for 1 min, repeat cycle (steps 2-4), final extension at 72^{o}C for 30 minutes. PCR products were then processed with an ABI-3730 DNA Analyzer. Genotypes were visualized with a known size standard (GS500LIZ 3730) using GeneMapper 3.7 software. Alleles were binned in GENEMAPPER using the standardized allele sizes established for the Chinook coastwide standardization efforts (Seeb et al. 2007).

Statistical Analyses

Allele frequencies, the overall number of alleles (per locus and collection), and the number of private alleles (per collection and locus) were calculated with CONVERT (version 1.3, Glaubitz 2003).

Tests for Hardy-Weinberg proportions between all pairs of loci within each group were performed using GENEPOP (version 3.4, Raymond and Rousset 1995). Heterozygosity (observed and expected) was computed for each collection group using GDA (Lewis

and Zaykin 2001) and evaluated using a Bonferroni correction of p-values to account for multiple, simultaneous tests (Rice 1989). Allelic richness and Weir and Cockerham's (1984) inbreeding coefficient (F_{IS}) were calculated using FSTAT (version 2.9.3.2, Goudet 2001). Linkage disequilibrium was compared for each collection using GENEPOP v 3.4 (10,000 dememorizations, 100 batches, and 5,000 iterations per batch). Statistical significance for the linkage disequilibrium analysis was evaluated using a Bonferroni correction of p-values to account for multiple, simultaneous tests (Rice 1989).

Pairwise estimates of genetic differentiation between collection groups were calculated to examine population structure. Estimates of genotypic population differentiation and F_{ST} pairwise estimates were calculated using GENEPOP (version 3.4, Raymond and Rousset 1995). Statistical significance for the tests of genotypic differentiation was evaluated using a Bonferroni correction of p-values to account for multiple, simultaneous tests (Rice 1989).

Results and Discussion

Four individual fish samples were excluded before analysis because they were identified as strays or as unknowns. Two other samples identified as DIPs (dead in pond) were included in the analysis of hatchery and natural-origin fish because although their origin was known, they could not be included in the analysis of in-river and supplementation spawners because they did not spawn. Good quality DNA was obtained and analyzed for all other samples and genotypes were collected for those samples. All samples with genotypes for eight or more loci were included in the analysis, and over all three collections only 20 samples were excluded because of missing data. The number of samples that were analyzed and then excluded because of missing data for each collection is shown in Table 1. The hatchery-origin and in-river spawner groups had the lowest number of individuals that were scored at all loci and included in the analysis (Table 1). Samples collected from fish carcasses in-river were of lower quality given the state of tissue decomposition when collected. All other

samples were handled in the hatchery facility while the fish were still alive providing higher quality tissue. These differences in tissue quality are reflected in the higher number of samples with missing data in the carcass collections.

Global tests for Hardy-Weinberg Equilibrium (HWE) did not reveal any significant deviations from expected values for any locus or collection after implementation of Bonferroni correction for multiple tests (Rice 1989). All collections analyzed were also within the expected HWE proportions suggesting random mating within each group (Table 3).

A large positive value of the inbreeding coefficient (F_{IS}) that is significant is an indication of an excess of homozygotes in a collection and can result from small population size and inbreeding (Table 3). The F_{IS} values for each of the collections were small and not significant indicating they were not inbred or from a small population. Allelic richness is an additional measure of population diversity and therefore an indication of the health and stability of the population; high values indicate increased genetic diversity (Table 3). Analysis of allelic richness for the natural-origin, hatchery-origin, and captive brood samples, requires complete data for all loci that are included and was based on a total of 48 individuals per collection while the analysis of supplementation, in-river spawners and captive brood was conducted on a total of 29 individuals per collection. As a result, the range for allelic richness for the evaluation of the hatchery-origin, natural-origin, and captive brood was 12.6 – 14.3 while the range of the evaluation of the supplementation, in-river, and captive brood was 11.3 – 12.9. In both analyses, the collection with the larger number of natural-origin samples (natural-origin and in-river spawners) had the highest calculated allelic richness (14.3 and 12.9). Allelic richness for the supplementation and natural-origin collections in the Tucannon River were comparable to two collections of fall Chinook broodstock from Lyons Ferry Hatchery (12.85) and a collection of fall Chinook from the Umatilla River Hatchery (13.70, unpublished WDFW data) while allelic richness values for two spring Chinook collections in the Yakima River Basin were higher (upper Yakima River – 16.3, Naches River – 17.2) than detected in the Tucannon River (unpublished WDFW data). The F_{IS} values were not significant and the observed heterozygosities were not significantly different from the expected Hardy-

Weinberg expected values indicating that there was not an excess of homozygotes (which would be an indication of inbreeding).

Tests for linkage for the 2006 sample groups was consistent with those reported by Hawkins and Frye (2005), Kassler and Hawkins (2006), and Kassler and Hawkins (2007). The largest number of significant linkage disequilibrium tests occurred in the captive brood spawners (Table 3). Linkage disequilibria can be the result of genetic drift, sampling a relatively small number of families of related individuals, or assortative mating and/or analysis of an admixed collection. In the captive brood collection, the linkage disequilibria are likely the result of sampling a small number of families.

The combined results for the tests of genotypic differentiation (Table 4a) and tests of pairwise F_{ST} (Table 4b) suggest that the collections are genetically differentiated. The tests for genotypic differentiation among either genetic-origin or spawner groups revealed that all three groups are highly significantly different from each other (Table 4a). The pairwise F_{ST} values also indicate all the comparisons are significantly different from zero with p-values between 0.0003 - 0.0119 (adjusted p-value 0.0167). The p-value for the supplementation and in-river spawners is significantly different from zero; however the p-value is closer to the adjusted p-value than for the observed for the other comparisons. This suggests that there is less differentiation between these two groups than the other groups. The F_{ST} values are highly affected by the level of heterozygosity at each locus and may limit the usefulness of these comparisons.

Evaluation of private alleles provides an understanding of the genetic differentiation and similarities among a group of collections. If there are numerous private alleles in a collection, then it may indicate that the collections compared are not random samples of the populations, or are not large enough to capture all of the genetic diversity in the populations. More explicitly, the samples analyzed may not represent all of the alleles present in the population and some alleles would appear to be private but were simply not represented. There may also be more private alleles in a collection if samples from multiple brood years are compared to a collection from a single brood year. For example, samples from the captive brood program would have the same alleles as

samples from the supplementation program when it began, however, the number and identity of alleles found in the individuals from the supplementation group can change each year dependent on the broodstock used to produce them. Alleles that were present in the supplementation group may be lost while samples from the captive brood are maintained simply by chance. If multiple temporal collections are analyzed and compared it is likely that there would be fewer private alleles detected because there would be more complete allelic representation of the diversity.

Assessment of the private alleles (Table 3 and Appendix 1a) detected in the analysis among natural-origin, hatchery-origin, and captive brood samples revealed the largest number in the natural-origin samples (N = 28). The lowest number of private alleles was detected in the hatchery-origin samples (N = 7). The analysis of the supplementation spawners, in-river spawners, and captive brood samples (Table 3 and Appendix 1b) revealed the fewest private alleles in the captive brood group (N = 12) while the supplementation and in-river spawners had 19 and 20 alleles respectively.

The number and distribution of the alleles observed in each group can give insights into the relationships among the different collection types. A side-by-side comparison of the private alleles (Appendix 1a and 1b) provides an understanding of how the results differ depending on how the fish are grouped. Because there are natural-origin fish in both spawner groups, alleles that are unique to the natural-origin fish (N = 28) can be present in either the supplementation fish (N = 9), in-river spawners (N = 16), or they can be present in both groups (N = 3). Because this hatchery program is an integrated supplementation hatchery designed to augment the natural production, the presence of alleles unique to the natural-origin fish in both spawner groups identifies that the natural genetic diversity was spread among groups.

The overall number of alleles per locus ranged from 4 – 34 (*Ots-9** – *Omm-1080** respectively; Table 5). In theory, it would be expected that a healthy natural population would exhibit higher genetic diversity and thus contain more alleles than captive broodstock or hatchery-origin samples derived from a limited number of founders. Comparison of the genetic diversity in the captive brood program to the diversity of the

supplementation program would presumably be equal because the captive brood program was initiated with samples from the supplementation program. However if there were a larger number of fish from more brood years represented in the captive brood program samples than in the hatchery-origin samples, or the collection from the captive brood captured the genetic diversity more completely than the hatchery-origin collection, there would be higher diversity detected in the captive brood program. For comparisons among genetic-origin groups, the natural-origin collection has the most alleles and highest allelic richness. In general, the hatchery-origin collection has the fewest number of alleles and lowest allelic richness. In spawner-group comparisons, the supplementation spawners have more total alleles, but a lower allelic richness due to the disparity in sample size between the supplementation and in-river collections and the greater number of hatchery-origin fish in the supplementation collection. Although the diversity (allelic richness and total number of alleles) of the captive brood is lower in comparison to the natural-origin collection, it is higher than what is observed in the hatchery-origin indicating that the captive brood program has maintained genetic diversity.

Conclusions

The overall genetic diversity of the natural-origin, hatchery-origin, and captive brood samples suggests that there has not been a severe loss of genetic diversity. Likewise the in-river and supplementation samples (a combined group of hatchery and natural-origin samples) also do not show any serious loss of genetic diversity. The values of the genetic diversity in this report have changed slightly from the values reported by Hawkins and Frye (2005), Kassler and Hawkins (2006), and Kassler and Hawkins (2007); however the differences do not support any conclusion that there has been a significant loss of diversity. The natural-origin samples revealed the highest level of diversity while the supplementation spawners and the captive brood spawners have had lower values. This result is possibly a sampling effect as fewer of the hatchery-origin fish were sampled than the natural-origin population. The lower diversity in the supplementation group and captive brood spawners likely reflects a smaller population size compared to the natural-origin population (causing genetic drift to have a strong

effect), and the relatively small number of families (varying in the number of individuals per family) for the captive brood spawners. Changes in sampling or variation in the run from year to year can also affect the quantity and distribution of alleles. The results and comparisons of the different collection types provides evidence that the captive broodstock program and supplementation program have been successful in preserving genetic variation, and that the supplementation program has been effective in minimizing the genetic differences between the hatchery and natural-origin fish.

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Table 1. Collection code, collection description, and number of samples collected and used in the analysis of the 2006 samples. Collection description includes the following: hatchery-origin, natural-origin, and captive broodstock (hatchery-origin fish originated in the hatchery in their respective brood year and all natural-origin fish originated in the river in their respective broodyear). The hatchery-origin and natural-origin samples were divided into supplementation hatchery and in-river spawners and re-analyzed.

Collection Description	Collection Code	# collected	# excluded a,b,c	# used in analysis
natural-origin	06AG	72	0, 0, 6	66
hatchery-origin	06AH	67	4, 0, 10	53
supplementation - natural-origin	06AG	36	0, 0, 1	35
supplementation - hatchery-origin	06AH	56	2, 2, 4	48
supplementation spawners - total	06AG and 06AH	92	2, 2, 5	83
in-river - natural-origin	06AG	36	0, 0, 5	31
in-river - hatchery-origin	06AH	11	2, 0, 6	3
in-river spawners - total	06AG and 06AH	47	2, 0, 11	34
captive broodstock	06AI	89	0, 0, 4	85

a - Samples identified as Umatilla River strays or uknowns were dropped from analysis

b - Samples identified as a DIP (dead in pond) or morts were dropped from analysis of supplementation and in-river samples

c - Individual samples were excluded if data was not available for eight or more loci.

Table 2. PCR conditions and microsatellite locus information (number alleles/locus and allele size range) for multiplexed loci. Also included are the observed and expected heterozygosity (H_o and H_e) for each locus and p-values for deviations from Hardy-Weinberg equilibrium (HWE). P-values for deviations from Hardy Weinberg Equilibrium (HWE) were defined as significant after implementation of Bonferroni correction for multiple tests (Rice 1989). Adjusted alpha p-value was 0.05/42 = 0.0012. Because HWE is dependent on the fish combined in a group, values are given for both the spawner group collections (supplementation and In-river spawners) and the genetic-origin collections (hatchery and natural-origin).

PCR Conditions						Locus statistics		Heterozygosity		HWE	
Poolplex	Locus	Dye Label	Annealing temp (^{O}C)	Primer conc. (mM)	Cycles	# Alleles/ Locus	Allele Size Range (bp)	H_o	H_e	Spawner group	Genetic origin
Ots-M	*Oki-100**	vic	50	0.36	40	21	220-313	0.9072	0.9164	0.1666	0.2939
	*Ots-201b**	6fam	50	0.32	40	25	153-278	0.9010	0.9278	0.1656	0.2564
	*Ots-208b**	ned	50	0.18	40	26	162-286	0.9234	0.9208	0.8586	0.6836
	*Ssa-408**	pet	50	0.20	40	20	184-300	0.8724	0.8963	0.7609	0.6563
Ots-N	*Ogo-2**	pet	63	0.07	40	10	202-232	0.6891	0.7260	0.4089	0.3683
	*Ssa-197**	ned	63	0.25	40	19	189-305	0.9054	0.8937	0.6580	0.6766
Ots-O	*Ogo-4**	6fam	56	0.18	40	12	132-166	0.8181	0.8135	0.7049	0.7381
	*Ots-213**	ned	56	0.18	40	20	222-314	0.9146	0.9113	0.8757	0.8833
	*Ots-G474**	pet	56	0.14	40	6	156-200	0.5126	0.5081	0.3932	0.1609
Ots-R	*Omm-1080**	vic	56	0.22	40	34	190-354	0.9686	0.9294	0.9732	0.9839
	*Ots-3M**	6fam	63	0.12	40	6	138-150	0.4900	0.4888	0.5548	0.6333
Ots-S	*Ots-9**	pet	63	0.04	40	4	103-109	0.5330	0.5993	0.0723	0.0859
	*Ots-211**	ned	63	0.07	40	21	208-312	0.9010	0.8813	0.8150	0.9500
	*Ots-212**	6fam	63	0.30	40	15	131-203	0.8650	0.8602	0.5425	0.5149

Table 3. Descriptive statistics for the collections analyzed, including the number of significant pairwise linkage disequilibria detected (Linkage), observed and expected heterozygosities (H_o and H_e), allelic richness (number of alleles corrected for sample size, averaged over all loci), inbreeding coefficient (F_{IS}), and the number of alleles that were only found in an individual collection (private alleles). P-values were defined as significant after implementation of Bonferroni correction for multiple tests (Rice 1989). Adjusted alpha p-values are shown for each test.

		Linkage	Heterozygosity					
Collection	Collection Code	(# locus pairs significant before/after Bonferroni correction) [a]	H_o	H_e	HWE P-value [b]	Allelic Richness [c]	F_{IS} (p-value) [d]	Number of private alleles
Natural-origin	06AG	15 / 3	0.796	0.808	0.293	14.3	0.015 (0.144)	28
Hatchery origin	06AH	23 / 10	0.808	0.803	0.939	13.0	-0.006 (0.669)	7
Captive brood spawners	06AI	40 / 16 - 39 / 13 [e]	0.798	0.797	0.900	12.6 – 11.3 [e]	-0.001 (0.539)	12
Supplementation spawners	06AG and 06H	25 / 7	0.798	0.804	0.764	12.1	0.007 (0.287)	20
In-river spawners	06AG and 06AH	3 / 0	0.813	0.814	0.599	12.9	0.001 (0.460)	19

a: 91 Pairwise comparisons and Bonferroni corrected alpha p-value = 0.0005 (0.05/91)
b: 42 Pairwise comparisons and Bonferroni corrected alpha p-value = 0.0012 (0.05/42)
c: Allelic richness based on 14 loci, and 48 individuals (natural or hatchery-origin) or 29 individuals (supplementation or in-river).
d: 42 Pairwise comparisons and Bonferroni corrected alpha p-value = 0.0012 (0.05/42)
e: Value when analyzed with the natural and hatchery-origin fish - value when analyzed with the supplementation and in-river fish

Table 4a. P-values for tests of genotypic differentiation. The first comparison is of hatchery-origin, natural-origin, and captive brood samples and the second comparison is of in-river spawners, supplementation spawners, and captive brood samples. All values were significantly different from each other after implementation of Bonferroni correction for multiple tests (Rice 1989; adjusted alpha p-value = 0.0167 (0.05/3).

	06 Hatchery	06 Natural	06 Captive Brood
06 Hatchery	**X**		
06 Natural	**0.0000**	**X**	
06 Captive Brood	**0.0000**	**0.0000**	**X**

	06 Supplementation	06 In-river	06 Captive Brood
06 Supplementation	**X**		
06 In-river	**0.0002**	**X**	
06 Captive Brood	**0.0000**	**0.0000**	**X**

Table 4b. Pairwise F_{ST} values across all loci are shown below the diagonal. The comparisons are the same as listed above. Pairwise F_{ST} values can range between 0.0000 – 1.0000. The F_{ST} value represents the amount of genetic differentiation that exists between the pairwise groups being tested and the larger the F_{ST} value identifies that the populations are more genetically differentiated. P-value for each comparison are shown above the diagonal. All values were significantly different from zero after implementation of Bonferroni correction for multiple tests (Rice 1989; adjusted alpha p-value = 0.0167 (0.05/3).

	06 Hatchery	06 Natural	06 Captive Brood
06 Hatchery	**X**	**0.0010**	**0.0085**
06 Natural	**0.0063**	**X**	**0.0003**
06 Captive Brood	**0.0043**	**0.0060**	**X**

	06 Supplementation	06 In-River	06 Captive Brood
06 Supplementation	**X**	**0.0119**	**0.0028**
06 In-River	**0.0048**	**X**	**0.0053**
06 Captive Brood	**0.0038**	**0.0061**	**X**

Table 5. Number of alleles observed at each of 14 loci for five collections and the total number of alleles in all collections. Number of alleles in bold type identify the highest number of alleles observed for each locus and number that is underlined is for the fewest number of alleles observed at each locus.

Collection	Collection Code	Number of samples	Oki-100	Ots-201b	Ots-208b	Ssa-408	Ogo-2	Ssa-197	Ogo-4	Omm-1080	Ots-213	Ots-G474	Ots-3M	Ots-9	Ots-211	Ots-212
Natural-origin	06AG	66	19	**23**	**23**	**17**	**9**	15	**12**	**29**	18	**6**	**6**	4	18	13
Hatchery-origin	06AH	53	18	17	18	15	8	15	11	22	18	4	5	4	17	12
Captive brood spawners	06AI	85	16	20	18	**17**	8	**16**	10	28	16	4	5	4	18	10
Supplementation spawners	06AG / 06AH	35 / 48*	18	21	22	**17**	**9**	15	**12**	27	**20**	5	**6**	4	**19**	11
In-river spawners	06AG / 06AH	31 / 3*	17	20	21	14	7	**16**	11	23	15	5	5	4	15	**14**
Number of alleles in all collections			21	25	26	20	10	19	12	34	20	6	6	4	21	15

* Number of samples that were of natural-origin / hatchery-origin

Appendix 1a. Allele frequencies for the hatchery-origin, natural-origin, and captive broodstock spring Chinook in the Tucannon River in 2006. The column labeled "private" identifies specific alleles that were only scored in the collection that is identified.

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Oki-100	220	0.023	0.010	0.013	
Oki-100	224	0.008			Natural-origin
Oki-100	228	0.008			Natural-origin
Oki-100	232	0.031	0.059	0.019	
Oki-100	236	0.016	0.039	0.019	
Oki-100	240	0.117	0.167	0.076	
Oki-100	244	0.023	0.029	0.076	
Oki-100	248	0.086	0.069	0.114	
Oki-100	252	0.039	0.010	0.057	
Oki-100	256	0.094	0.039	0.019	
Oki-100	260	0.133	0.098	0.165	
Oki-100	264	0.008	0.010	0.044	
Oki-100	268	0.078	0.049	0.044	
Oki-100	270	0.039	0.157	0.038	
Oki-100	272	0.156	0.147	0.139	
Oki-100	275	0.063	0.029	0.070	
Oki-100	279		0.029	0.025	
Oki-100	283	0.063	0.039	0.082	
Oki-100	287	0.008	0.010		
Oki-100	290		0.010		Hatchery-origin
Oki-100	313	0.008			Natural-origin
# of samples		64	51	79	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-201b	153	0.058	0.077	0.050	
Ots-201b	165	0.008			Natural-origin
Ots-201b	169	0.025	0.010	0.031	
Ots-201b	173	0.017	0.039	0.006	
Ots-201b	178	0.058	0.106	0.069	
Ots-201b	182	0.092	0.115	0.088	
Ots-201b	186	0.092	0.125	0.144	
Ots-201b	190	0.108	0.135	0.156	
Ots-201b	194	0.075	0.077	0.063	
Ots-201b	198	0.008		0.019	
Ots-201b	202	0.033	0.019	0.031	
Ots-201b	206	0.067	0.010	0.038	
Ots-201b	210		0.029	0.038	
Ots-201b	214	0.025	0.029	0.019	
Ots-201b	218	0.100	0.029	0.100	
Ots-201b	222	0.067	0.039	0.069	
Ots-201b	226	0.033		0.025	
Ots-201b	230	0.017	0.067	0.013	
Ots-201b	234	0.025	0.019		

Appendix 1a continued.

Ots-201b	238	0.050	0.077	0.006	
Ots-201b	246	0.008			Natural-origin
Ots-201b	262	0.008			Natural-origin
Ots-201b	266	0.008			Natural-origin
Ots-201b	274			0.013	Captive Brood
Ots-201b	278	0.017		0.025	
# of samples		60	52	80	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-208b	162	0.070	0.042	0.036	
Ots-208b	170	0.008			Natural-origin
Ots-208b	174	0.008	0.021		
Ots-208b	178		0.010		Hatchery-origin
Ots-208b	182	0.039	0.031	0.060	
Ots-208b	186	0.008	0.063	0.030	
Ots-208b	190	0.008			Natural-origin
Ots-208b	194	0.016			Natural-origin
Ots-208b	198	0.102	0.094	0.089	
Ots-208b	202	0.070	0.125	0.101	
Ots-208b	206	0.023			Natural-origin
Ots-208b	210	0.047	0.021	0.036	
Ots-208b	214	0.031	0.115	0.113	
Ots-208b	218	0.031	0.021		
Ots-208b	222	0.031	0.021	0.036	
Ots-208b	226	0.031		0.012	
Ots-208b	230	0.016	0.010	0.042	
Ots-208b	234	0.070	0.073	0.095	
Ots-208b	238	0.117	0.073	0.036	
Ots-208b	242	0.164	0.135	0.202	
Ots-208b	246	0.031	0.115	0.042	
Ots-208b	250	0.063	0.021	0.036	
Ots-208b	254		0.010	0.012	
Ots-208b	262	0.008		0.018	
Ots-208b	274	0.008			Natural-origin
Ots-208b	286			0.006	Captive Brood
# of samples		64	48	84	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ssa-408	184	0.008	0.038	0.018	
Ssa-408	188	0.172	0.198	0.213	
Ssa-408	192	0.066	0.104	0.079	
Ssa-408	196	0.303	0.085	0.079	
Ssa-408	200	0.016		0.061	
Ssa-408	204	0.066	0.066	0.061	
Ssa-408	208	0.156	0.123	0.110	
Ssa-408	212	0.008	0.104	0.116	
Ssa-408	216	0.016	0.009	0.024	

Appendix 1a continued.

Ssa-408	220	0.016	0.028	0.012	
Ssa-408	224	0.041	0.066	0.085	
Ssa-408	228		0.019	0.006	
Ssa-408	240	0.025	0.057	0.012	
Ssa-408	244			0.012	Captive Brood
Ssa-408	252	0.008			Natural-origin
Ssa-408	280	0.016	0.009		
Ssa-408	288	0.033	0.047	0.018	
Ssa-408	292	0.008			Natural-origin
Ssa-408	296	0.041	0.047	0.073	
Ssa-408	300			0.018	Captive Brood
# of samples		61	53	82	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ogo-2	202		0.031		Hatchery-origin
Ogo-2	214	0.133	0.135	0.147	
Ogo-2	216	0.458	0.396	0.477	
Ogo-2	218	0.033		0.024	
Ogo-2	220	0.183	0.260	0.147	
Ogo-2	222	0.142	0.104	0.124	
Ogo-2	226	0.008	0.052	0.071	
Ogo-2	228	0.008	0.010	0.006	
Ogo-2	230	0.008			Natural-origin
Ogo-2	232	0.025	0.010	0.006	
# of samples		60	48	85	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ssa-197	189		0.010		Hatchery-origin
Ssa-197	201	0.047	0.087	0.077	
Ssa-197	209	0.078	0.010	0.006	
Ssa-197	213		0.010	0.006	
Ssa-197	221			0.006	Captive Brood
Ssa-197	249	0.008			Natural-origin
Ssa-197	252	0.047	0.010	0.012	
Ssa-197	256	0.047	0.144	0.082	
Ssa-197	261	0.070	0.106	0.053	
Ssa-197	265	0.133	0.135	0.200	
Ssa-197	269	0.070	0.115	0.141	
Ssa-197	273	0.219	0.164	0.194	
Ssa-197	277	0.070	0.077	0.071	
Ssa-197	281	0.023	0.058	0.035	
Ssa-197	285	0.047	0.010	0.018	
Ssa-197	289	0.031		0.006	
Ssa-197	293	0.086	0.058	0.077	
Ssa-197	297			0.018	Captive Brood
Ssa-197	305	0.023	0.010		
# of samples		64	52	85	

Appendix 1a continued.

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ogo-4	132	0.008	0.019	0.006	
Ogo-4	136	0.064	0.028	0.055	
Ogo-4	138	0.024	0.019	0.012	
Ogo-4	148	0.119	0.208	0.207	
Ogo-4	152	0.008			Natural-origin
Ogo-4	154	0.048	0.085	0.110	
Ogo-4	156	0.349	0.255	0.311	
Ogo-4	158	0.206	0.264	0.189	
Ogo-4	160	0.064	0.028	0.037	
Ogo-4	162	0.048	0.028	0.012	
Ogo-4	164	0.048	0.047	0.061	
Ogo-4	166	0.016	0.019		
# of samples		63	53	82	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-213	222	0.015	0.010		
Ots-213	226	0.015		0.043	
Ots-213	230		0.039		Hatchery-origin
Ots-213	234	0.008	0.019		
Ots-213	238	0.023		0.006	
Ots-213	258	0.015	0.019	0.068	
Ots-213	262	0.197	0.164	0.161	
Ots-213	266	0.038	0.019	0.012	
Ots-213	270	0.129	0.164	0.124	
Ots-213	274	0.046	0.029	0.049	
Ots-213	278	0.091	0.058	0.099	
Ots-213	282	0.038	0.048	0.043	
Ots-213	286	0.015	0.010		
Ots-213	290	0.030	0.048	0.043	
Ots-213	294	0.068	0.019	0.086	
Ots-213	298	0.008	0.058	0.043	
Ots-213	302	0.136	0.125	0.080	
Ots-213	306	0.023	0.067	0.043	
Ots-213	310		0.019	0.025	
Ots-213	314	0.106	0.087	0.074	
# of samples		66	52	81	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-G474	156	0.692	0.628	0.645	
Ots-G474	164	0.008			Natural-origin
Ots-G474	168	0.169	0.294	0.265	
Ots-G474	184	0.008			Natural-origin
Ots-G474	192	0.046	0.049	0.072	
Ots-G474	200	0.077	0.029	0.018	
# of samples		65	51	83	

Appendix 1a continued.

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Omm-1080	190	0.008	0.020	0.013	
Omm-1080	194	0.032	0.040	0.019	
Omm-1080	206			0.026	Captive Brood
Omm-1080	214	0.016			Natural-origin
Omm-1080	218	0.016		0.019	
Omm-1080	222	0.008			Natural-origin
Omm-1080	226			0.006	Captive Brood
Omm-1080	230	0.198	0.180	0.205	
Omm-1080	234	0.032	0.010	0.032	
Omm-1080	238			0.032	Captive Brood
Omm-1080	242	0.024	0.010	0.019	
Omm-1080	250	0.008	0.010		
Omm-1080	254			0.006	Captive Brood
Omm-1080	258	0.016	0.050	0.045	
Omm-1080	262	0.040	0.060	0.026	
Omm-1080	266	0.008			Natural-origin
Omm-1080	270	0.032		0.026	
Omm-1080	274	0.008			Natural-origin
Omm-1080	282	0.008		0.006	
Omm-1080	286		0.030	0.045	
Omm-1080	290	0.016	0.050		
Omm-1080	294	0.024	0.040	0.039	
Omm-1080	298	0.048	0.100	0.039	
Omm-1080	302	0.079	0.040	0.064	
Omm-1080	306	0.048	0.020	0.019	
Omm-1080	310	0.016	0.010	0.013	
Omm-1080	314	0.056	0.060	0.019	
Omm-1080	318	0.016	0.060	0.058	
Omm-1080	322	0.071	0.020	0.045	
Omm-1080	326	0.040	0.020	0.051	
Omm-1080	334	0.016	0.040	0.006	
Omm-1080	338	0.103	0.080	0.109	
Omm-1080	342	0.008	0.050	0.006	
Omm-1080	354	0.008		0.006	
# of samples		63	50	78	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-3M	138	0.023	0.028	0.019	
Ots-3M	142	0.008	0.028	0.019	
Ots-3M	144	0.015			Natural-origin
Ots-3M	146	0.242	0.189	0.290	
Ots-3M	148	0.644	0.726	0.654	
Ots-3M	150	0.068	0.028	0.019	
# of samples		66	53	81	

Appendix 1a continued.

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-211	208	0.008	0.019	0.012	
Ots-211	236			0.006	Captive Brood
Ots-211	240	0.108	0.115	0.094	
Ots-211	244	0.015	0.039	0.047	
Ots-211	248	0.015	0.010	0.018	
Ots-211	252	0.023	0.010		
Ots-211	256	0.039		0.012	
Ots-211	260	0.008	0.010		
Ots-211	264	0.015	0.029	0.012	
Ots-211	268	0.115	0.192	0.082	
Ots-211	272	0.046	0.058	0.124	
Ots-211	276	0.231	0.269	0.206	
Ots-211	280	0.031	0.048	0.012	
Ots-211	284	0.169	0.048	0.224	
Ots-211	288	0.031	0.029	0.041	
Ots-211	292	0.031	0.019	0.012	
Ots-211	296			0.006	Captive Brood
Ots-211	300	0.008			Natural-origin
Ots-211	304	0.062	0.067	0.071	
Ots-211	308		0.019	0.018	
Ots-211	312	0.046	0.019	0.006	
# of samples		65	52	85	

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-212	131	0.038	0.019	0.025	
Ots-212	135	0.046	0.057	0.056	
Ots-212	139	0.068	0.038	0.074	
Ots-212	143	0.212	0.189	0.154	
Ots-212	147	0.068	0.085	0.117	
Ots-212	151	0.152	0.293	0.253	
Ots-212	155	0.152	0.113	0.167	
Ots-212	159	0.136	0.151	0.105	
Ots-212	163	0.068	0.009	0.025	
Ots-212	167	0.023			Natural-origin
Ots-212	171	0.023	0.009	0.025	
Ots-212	175	0.008			Natural-origin
Ots-212	179		0.009		Hatchery-origin
Ots-212	199	0.008			Natural-origin
Ots-212	203		0.028		Hatchery-origin
# of samples		66	53	81	

Appendix 1a continued.

Locus	Size	06 Natural	06 Hatchery	06 Captive	Private
Ots-9	103	0.025	0.028	0.036	
Ots-9	105	0.393	0.340	0.295	
Ots-9	107	0.434	0.509	0.602	
Ots-9	109	0.148	0.123	0.066	
# of samples		61	53	83	

Appendix 1b. Allele frequencies for the supplementation spawners (includes both natural- and hatchery-origin), in-river spawners (includes both natural- and hatchery-origin), and captive broodstock spring Chinook in the Tucannon River in 2006. The column labeled "private" identifies specific alleles that were only scored in the collection that is identified.

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Oki-100	220	0.006	0.047	0.013	
Oki-100	224	0.006			Supplementation
Oki-100	228		0.016		In-River
Oki-100	232	0.049	0.031	0.019	
Oki-100	236	0.031	0.016	0.019	
Oki-100	240	0.146	0.125	0.076	
Oki-100	244	0.031	0.016	0.076	
Oki-100	248	0.092	0.047	0.114	
Oki-100	252	0.031	0.016	0.057	
Oki-100	256	0.049	0.109	0.019	
Oki-100	260	0.110	0.141	0.165	
Oki-100	264	0.012		0.044	
Oki-100	268	0.055	0.094	0.044	
Oki-100	270	0.110	0.047	0.038	
Oki-100	272	0.171	0.094	0.139	
Oki-100	275	0.043	0.063	0.070	
Oki-100	279	0.018		0.025	
Oki-100	283	0.037	0.094	0.082	
Oki-100	287		0.031		In-River
Oki-100	290	0.006			Supplementation
Oki-100	313		0.016		In-River
# of samples		82	32	79	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-201b	153	0.082	0.032	0.050	
Ots-201b	165		0.016		In-River
Ots-201b	169	0.019	0.016	0.031	
Ots-201b	173	0.032	0.016	0.006	
Ots-201b	178	0.095	0.048	0.069	
Ots-201b	182	0.120	0.065	0.088	
Ots-201b	186	0.101	0.129	0.144	
Ots-201b	190	0.108	0.145	0.156	
Ots-201b	194	0.057	0.129	0.063	
Ots-201b	198		0.016	0.019	
Ots-201b	202	0.032	0.016	0.031	
Ots-201b	206	0.032	0.048	0.038	
Ots-201b	210	0.019		0.038	
Ots-201b	214	0.032		0.019	
Ots-201b	218	0.076	0.048	0.100	
Ots-201b	222	0.044	0.081	0.069	
Ots-201b	226	0.019	0.016	0.025	
Ots-201b	230	0.044	0.016	0.013	
Ots-201b	234	0.013	0.048		

Appendix 1b continued.

Ots-201b	238	0.057	0.081	0.006	
Ots-201b	246	0.006			Supplementation
Ots-201b	262	0.006			Supplementation
Ots-201b	266		0.016		In-River
Ots-201b	274			0.013	Captive Brood
Ots-201b	278	0.006	0.016	0.025	
# of samples		79	31	80	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-208b	162	0.038	0.113	0.036	
Ots-208b	170		0.016		In-River
Ots-208b	174	0.013	0.016		
Ots-208b	178	0.006			Supplementation
Ots-208b	182	0.038	0.032	0.060	
Ots-208b	186	0.038	0.016	0.030	
Ots-208b	190		0.016		In-River
Ots-208b	194	0.013			Supplementation
Ots-208b	198	0.095	0.113	0.089	
Ots-208b	202	0.095	0.081	0.101	
Ots-208b	206	0.013	0.016		
Ots-208b	210	0.038	0.032	0.036	
Ots-208b	214	0.082	0.032	0.113	
Ots-208b	218	0.025	0.016		
Ots-208b	222	0.025	0.016	0.036	
Ots-208b	226	0.013	0.032	0.012	
Ots-208b	230	0.013	0.016	0.042	
Ots-208b	234	0.089	0.032	0.095	
Ots-208b	238	0.108	0.081	0.036	
Ots-208b	242	0.146	0.177	0.202	
Ots-208b	246	0.076	0.032	0.042	
Ots-208b	250	0.025	0.097	0.036	
Ots-208b	254	0.006		0.012	
Ots-208b	262		0.016	0.018	
Ots-208b	274	0.006			Supplementation
Ots-208b	286			0.006	Captive Brood
# of samples		79	31	84	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ssa-408	184	0.019	0.032	0.018	
Ssa-408	188	0.173	0.210	0.213	
Ssa-408	192	0.086	0.081	0.079	
Ssa-408	196	0.198	0.194	0.079	
Ssa-408	200	0.006	0.016	0.061	
Ssa-408	204	0.068	0.065	0.061	
Ssa-408	208	0.124	0.194	0.110	
Ssa-408	212	0.056	0.032	0.116	
Ssa-408	216	0.019		0.024	
Ssa-408	220	0.025	0.016	0.012	
Ssa-408	224	0.056	0.048	0.085	

Appendix 1b continued.

Ssa-408	228	0.012		0.006	
Ssa-408	240	0.049	0.016	0.012	
Ssa-408	244			0.012	Captive Brood
Ssa-408	252		0.016		In-River
Ssa-408	280	0.019			Supplementation
Ssa-408	288	0.037	0.048	0.018	
Ssa-408	292	0.006			Supplementation
Ssa-408	296	0.049	0.032	0.073	
Ssa-408	300			0.018	Captive Brood
# of samples		81	31	82	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ogo-2	202	0.020			Supplementation
Ogo-2	214	0.169	0.035	0.147	
Ogo-2	216	0.416	0.448	0.477	
Ogo-2	218	0.007	0.052	0.024	
Ogo-2	220	0.214	0.241	0.147	
Ogo-2	222	0.104	0.190	0.124	
Ogo-2	226	0.039		0.071	
Ogo-2	228	0.013		0.006	
Ogo-2	230		0.017		In-River
Ogo-2	232	0.020	0.017	0.006	
# of samples		77	29	85	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ssa-197	189		0.015		In-River
Ssa-197	201	0.063	0.074	0.077	
Ssa-197	209	0.006	0.147	0.006	
Ssa-197	213	0.006		0.006	
Ssa-197	221			0.006	Captive Brood
Ssa-197	249		0.015		In-River
Ssa-197	252	0.025	0.029	0.012	
Ssa-197	256	0.094	0.074	0.082	
Ssa-197	261	0.094	0.074	0.053	
Ssa-197	265	0.144	0.118	0.200	
Ssa-197	269	0.088	0.088	0.141	
Ssa-197	273	0.213	0.162	0.194	
Ssa-197	277	0.088	0.044	0.071	
Ssa-197	281	0.050	0.015	0.035	
Ssa-197	285	0.031	0.029	0.018	
Ssa-197	289	0.019	0.015	0.006	
Ssa-197	293	0.069	0.088	0.077	
Ssa-197	297			0.018	Captive Brood
Ssa-197	305	0.013	0.015		
# of samples		80	34	85	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ogo-4	132	0.012	0.015	0.006	
Ogo-4	136	0.043	0.061	0.055	

Appendix 1b continued.

Ogo-4	138	0.019	0.030	0.012	
Ogo-4	148	0.179	0.106	0.207	
Ogo-4	152	0.006			Supplementation
Ogo-4	154	0.080	0.030	0.110	
Ogo-4	156	0.265	0.409	0.311	
Ogo-4	158	0.272	0.152	0.189	
Ogo-4	160	0.037	0.076	0.037	
Ogo-4	162	0.031	0.046	0.012	
Ogo-4	164	0.037	0.061	0.061	
Ogo-4	166	0.019	0.015		
# of samples		81	33	82	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-213	222	0.012			Supplementation
Ots-213	226	0.012		0.043	
Ots-213	230	0.024			Supplementation
Ots-213	234	0.012	0.015		
Ots-213	238	0.006	0.029	0.006	
Ots-213	258	0.018	0.015	0.068	
Ots-213	262	0.189	0.177	0.161	
Ots-213	266	0.024	0.044	0.012	
Ots-213	270	0.146	0.118	0.124	
Ots-213	274	0.037	0.044	0.049	
Ots-213	278	0.055	0.132	0.099	
Ots-213	282	0.043	0.044	0.043	
Ots-213	286	0.018			Supplementation
Ots-213	290	0.043	0.015	0.043	
Ots-213	294	0.043	0.059	0.086	
Ots-213	298	0.037	0.015	0.043	
Ots-213	302	0.140	0.118	0.080	
Ots-213	306	0.049	0.029	0.043	
Ots-213	310	0.012		0.025	
Ots-213	314	0.079	0.147	0.074	
# of samples		82	34	81	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-G474	156	0.698	0.591	0.645	
Ots-G474	164	0.006			Supplementation
Ots-G474	168	0.241	0.182	0.265	
Ots-G474	184		0.015		In-River
Ots-G474	192	0.025	0.091	0.072	
Ots-G474	200	0.031	0.121	0.018	
# of samples		81	33	83	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Omm-1080	190	0.019		0.013	
Omm-1080	194	0.051		0.019	
Omm-1080	206			0.026	Captive Brood
Omm-1080	214	0.006	0.015		

Appendix 1b continued.

Omm-1080	218	0.006	0.015	0.019	
Omm-1080	222		0.015		In-River
Omm-1080	226			0.006	Captive Brood
Omm-1080	230	0.184	0.212	0.205	
Omm-1080	234	0.013	0.046	0.032	
Omm-1080	238			0.032	Captive Brood
Omm-1080	242	0.019	0.015	0.019	
Omm-1080	250	0.013			Supplementation
Omm-1080	254			0.006	Captive Brood
Omm-1080	258	0.038	0.015	0.045	
Omm-1080	262	0.063	0.015	0.026	
Omm-1080	266		0.015		In-River
Omm-1080	270	0.006	0.046	0.026	
Omm-1080	274		0.015		In-River
Omm-1080	282	0.006		0.006	
Omm-1080	286	0.019		0.045	
Omm-1080	290	0.038	0.015		
Omm-1080	294	0.038	0.015	0.039	
Omm-1080	298	0.076	0.061	0.039	
Omm-1080	302	0.038	0.106	0.064	
Omm-1080	306	0.013	0.091	0.019	
Omm-1080	310	0.006	0.030	0.013	
Omm-1080	314	0.076	0.015	0.019	
Omm-1080	318	0.044	0.015	0.058	
Omm-1080	322	0.044	0.046	0.045	
Omm-1080	326	0.019	0.061	0.051	
Omm-1080	334	0.025	0.030	0.006	
Omm-1080	338	0.095	0.091	0.109	
Omm-1080	342	0.038		0.006	
Omm-1080	354	0.006		0.006	
# of samples		79	33	78	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-3M	138	0.036		0.019	
Ots-3M	142	0.018	0.015	0.019	
Ots-3M	144	0.006	0.015		
Ots-3M	146	0.211	0.250	0.290	
Ots-3M	148	0.693	0.632	0.654	
Ots-3M	150	0.036	0.088	0.019	
# of samples		83	34	81	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-211	208	0.012	0.015	0.012	
Ots-211	236			0.006	Captive Brood
Ots-211	240	0.124	0.074	0.094	
Ots-211	244	0.031	0.015	0.047	
Ots-211	248	0.012	0.015	0.018	
Ots-211	252	0.019			Supplementation
Ots-211	256	0.019	0.029	0.012	

Appendix 1b continued.

Ots-211	260	0.012			Supplementation
Ots-211	264	0.025	0.015	0.012	
Ots-211	268	0.173	0.088	0.082	
Ots-211	272	0.056	0.044	0.124	
Ots-211	276	0.210	0.338	0.206	
Ots-211	280	0.043	0.029	0.012	
Ots-211	284	0.105	0.147	0.224	
Ots-211	288	0.025	0.044	0.041	
Ots-211	292	0.025	0.029	0.012	
Ots-211	296			0.006	Captive Brood
Ots-211	300	0.006			Supplementation
Ots-211	304	0.062	0.074	0.071	
Ots-211	308	0.012		0.018	
Ots-211	312	0.031	0.044	0.006	
# of samples		81	34	85	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-212	131	0.036	0.015	0.025	
Ots-212	135	0.054	0.044	0.056	
Ots-212	139	0.048	0.074	0.074	
Ots-212	143	0.181	0.250	0.154	
Ots-212	147	0.078	0.074	0.117	
Ots-212	151	0.259	0.074	0.253	
Ots-212	155	0.133	0.147	0.167	
Ots-212	159	0.151	0.132	0.105	
Ots-212	163	0.024	0.088	0.025	
Ots-212	167		0.044		In-River
Ots-212	171	0.018	0.015	0.025	
Ots-212	175		0.015		In-River
Ots-212	179		0.015		In-River
Ots-212	199		0.015		In-River
Ots-212	203	0.018			Supplementation
# of samples		83	34	81	

Locus	Size	06 Supplementation	06 In-River	06 Captive	Private
Ots-9	103	0.024	0.017	0.036	
Ots-9	105	0.378	0.350	0.295	
Ots-9	107	0.463	0.500	0.602	
Ots-9	109	0.134	0.133	0.066	
# of samples		82	30	83	

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