Tucannon River
Spring Chinook Salmon
Captive Broodstock Program

2006 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 2006. 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 hatchery supplementation program (132,000 smolts) and wild production, are expected to produce 600-700 returning adult spring Chinook to the Tucannon River each year from 2005-2010.

The 2006 eggtake from the 2001 brood year (Age 5) was 17,042 eggs from 8 ripe females. Egg survival was 54%. Mean fecundity based on the 8 fully spawned females was 2,130 eggs/female. The 2006 eggtake from the 2002 brood year (Age 4) was 145,694 eggs from 78 ripe females. Egg survival was 62%. Mean fecundity based on the 78 fully spawned fish was 1,868 eggs/female. The total 2006 eggtake from the captive brood program was 162,736 eggs. A total of 63,316 dead eggs (38.9%) were removed with 99,420 live eggs remaining for the program. An additional 19,988 dead eggs/fry (20.1%) were picked at ponding leaving 79,432 fish for rearing.

Only two captive brood progeny adult returns were recovered during 2006. 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 2007, WDFW volitionally released 90,056 BY 2005 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/Right Red VIE/No Finclip). One thousand captive brood progeny smolts were PIT tagged to compare their outmigration with smolts from the supplementation program (1,002 tagged). 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.

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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 2006. This report, while originally intended to cover activities accomplished exclusively under the Fiscal Year (FY) 2006 contract, includes some events during FY2007 as well. This was done to provide readers with complete results from the tagging, rearing, and spawning activities that have occurred.

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 (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. Further, this program was defined to last for only one-generation cycle (five brood years), and any potential negative effects should 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.
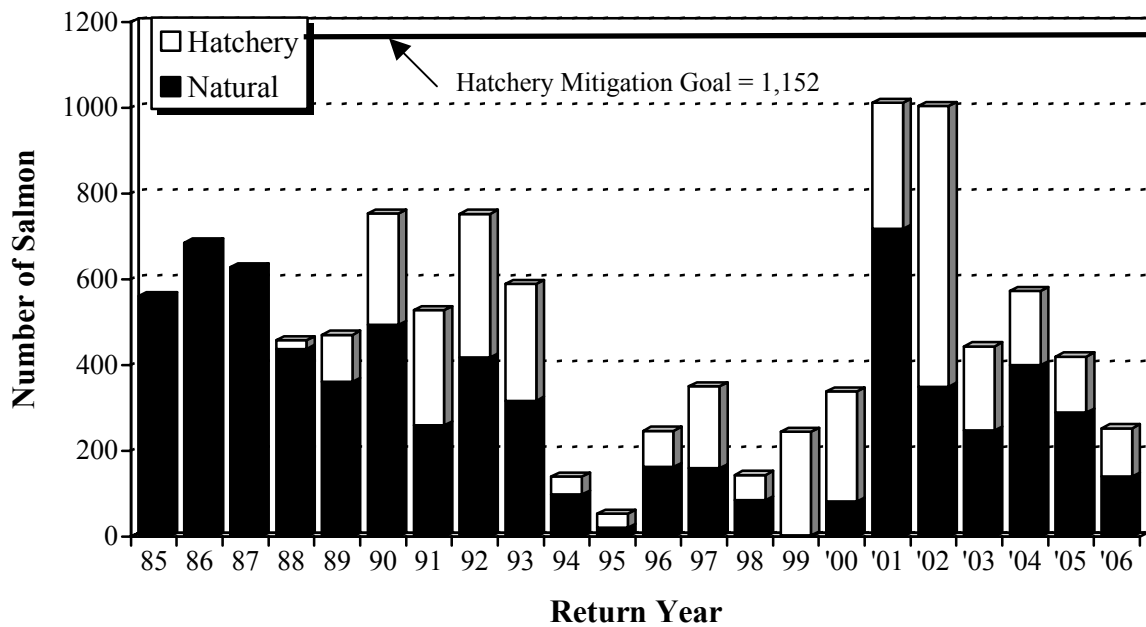
Figure 1. Total estimated escapement of Tucannon River spring Chinook salmon from 1985-2006.

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.
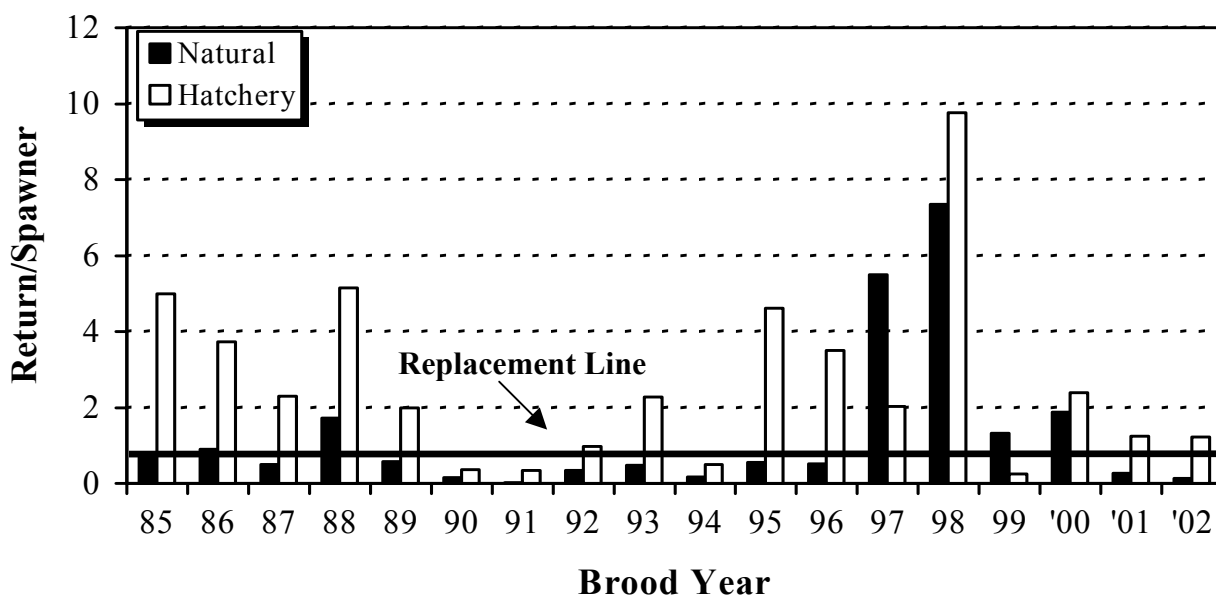
Figure 2. Return per spawner (with replacement line) for Tucannon River spring Chinook salmon for the 1985-2002 brood years.

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 smolt releases in the upper watershed have the best chance for high survival, and recovery effects from the captive brood and supplementation programs will be maximized by producing smolts.
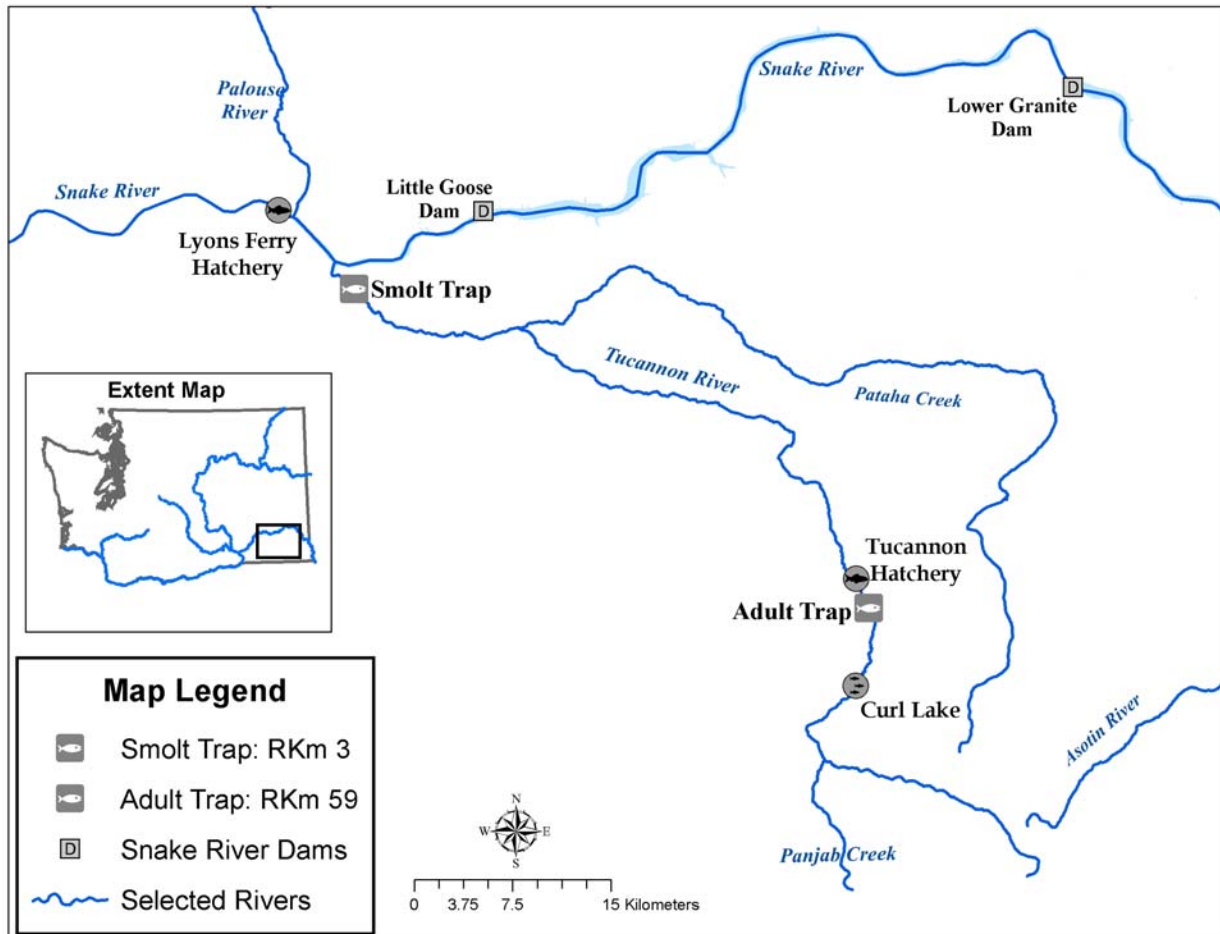
Figure 3. Location of the Tucannon River within the Snake River Basin, and locations of Lyons Ferry Hatchery, Tucannon Hatchery, and Curl Lake Acclimation Pond within the Tucannon River Basin.

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, and 4) improve and re-establish riparian vegetation. 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). Each of these facilities will also be used in some manner for the captive broodstock program for rearing, release and subsequent adult capture upon return. 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 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. A diagram of the captive broodstock facility is shown in Appendix A.

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 m³). 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: 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 same 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 are being documented by family groups within each brood year. Fecundity and egg size in relation to spawning success will be documented for all spawned captive broodstock females. Maturation timing will be monitored as well as overall growth rates 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 adult trapping and carcass recoveries during spawning ground surveys.

Captive Broodstock Program

The overall goal of the Tucannon River spring Chinook salmon captive broodstock program is for the short-term, and eventually long-term, rebuilding of the natural run, with the intent that the natural population will sustain itself. The current hatchery mitigation goal under the LSRCP is to return 1,152 adult spring Chinook of Tucannon River stock to the river annually. Attempts to reach the LSRCP mitigation goal through an annual release of 132,000 smolts have failed largely because of poor smolt-to-adult survival rates. Currently, there is not an escapement goal for naturally produced spring Chinook in the Tucannon River. It is hoped that through re-negotiation of the Columbia River Fish Management Plan (CRFMP), and as part of the development of a Snake River Chinook recovery plan, an agreed upon natural production goal will be established.

The captive broodstock program is not intended to replace the hatchery supplementation program. Rather, it is to provide a quick "boost" to the population in the short term because of poor runs initially predicted through 2000. A quick "boost" would not be possible under the existing supplementation program, as it would require about 200 adults for hatchery broodstock each year. This was not believed possible by WDFW biologists, as returns from 1998-2000 were expected to be less than 200 total fish annually. Further, such an increase would have required taking more fish from the river, nearly eliminating all natural production. WDFW believed that the low runs between 1997-2000 would limit both natural and hatchery production, possibly to a point where the run would not be able to recover. Based on this conclusion, the captive broodstock program was initiated. The program is scheduled to terminate with the final release of smolts in 2008. Successes and failures during and after the program ends will be evaluated by WDFW concurrently with the LSRCP hatchery evaluation program.

The captive broodstock goal is to collect 290,000 eggs/year from captive brood females when three complete age classes (Age 3-Age 5) are spawned concurrently. Under the original program design, these eggs are 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 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).

Captive brood program production (adults, eggs, or juveniles) in excess of the smolt goal may be released by other methods as discussed in the Master Plan (WDFW et al. 1999). Options include adult outplants, remote site egg incubation, fry outplants, or smolt releases into other systems deemed suitable for Tucannon River spring Chinook stock introductions.

Source of Captive Population

As described in the Tucannon Master Plan (WDFW et al. 1999), the captive population originated from the hatchery supplementation program during the 1997-2001 BYs. 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 Coded-Wire Tag (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.

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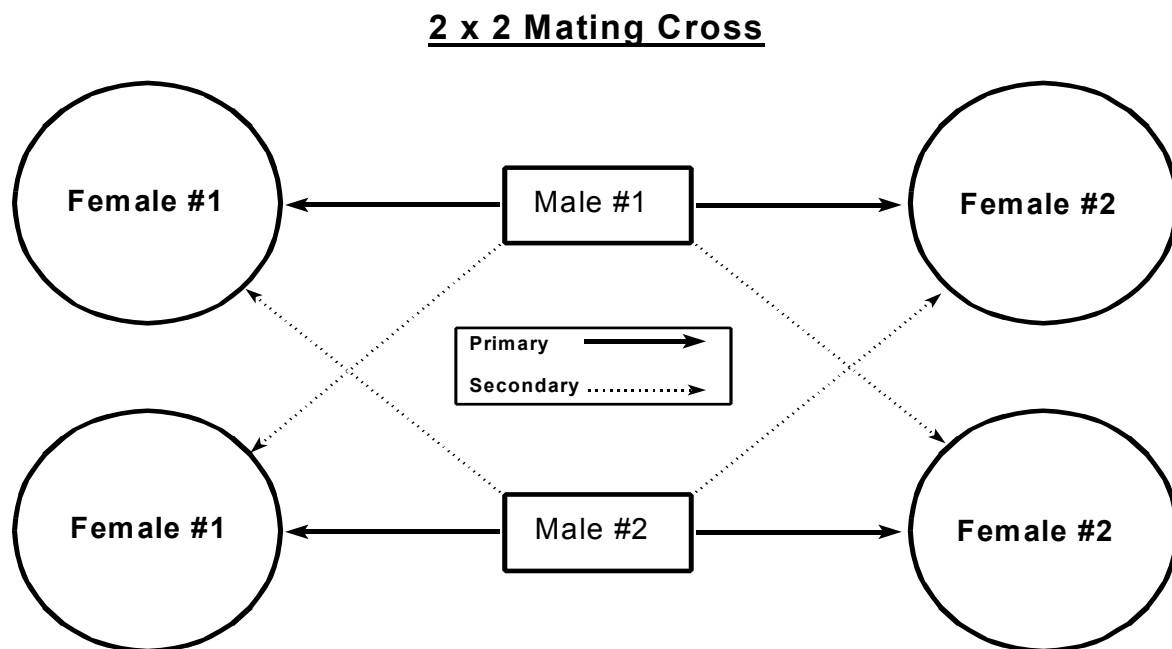
Figure 4. Diagram of the 2 x 2 mating scheme used by WDFW in the conventional supplementation and captive broodstock program.

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 B. Effective population size 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 B). 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 B). Data on average length (mm), weight (g), and condition factor (K) for each "family" group were compiled during tagging (Appendix C).

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[TM], Moore-Clark Fry[TM], Bio-Products Salmon Brood Feed[TM], and Moore-Clark Pedigree Trout Brood Feed[TM] 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. Only if the run completely collapses would any of the captive broodstock fish be collected for hatchery broodstock.

We started the year (Jan. 1, 2006) with 15 01BY and 90 02BY fish on hand. The paragraphs below detail the rearing, sorting, spawning activities, and mortalities for each BY during 2006 as well as the inventory and release information for the 2005 and 2006 progeny groups.

2001 Brood Rearing

We began 2006 with 15 BY 2001 fish on hand. Fish from this brood remained healthy throughout their rearing at LFH. There were three mortalities during the year not related to spawning (Appendix D). Since Age 1, there have only been 31 (7.0%) mortalities not related to maturation. The captive broodstock were sorted for maturity on June 21, 2006. Since we are only keeping each broodstock to the age of 5, all 12 fish from the 01 BY were transported to the spawning raceway for holding. All captive brood fish at the spawning building were held downstream of the supplementation broodstock captured at the adult trap on the Tucannon River to aid in maturation timing. Mature captive broodstock were held upstream of broodstock collected from the river in 2003 to address possible disease concerns, however spawn timing appeared to be adversely affected (Gallinat 2004). Length and weight samples were not collected from the 01 BY before transport.

Mortalities by age for each stage of maturity have been followed since program inception (Figure 5). Fish from the captive brood program have matured earlier than fish from the supplementation program (Figure 5, Appendix D). Captive brood males began to mature at Age 2 and captive brood females began to mature at Age 3 (Figure 5). Mature fish not used for spawning are fish that were in excess of the number required for spawning or mature fish that did not become ripe in time for spawning (Figure 5).
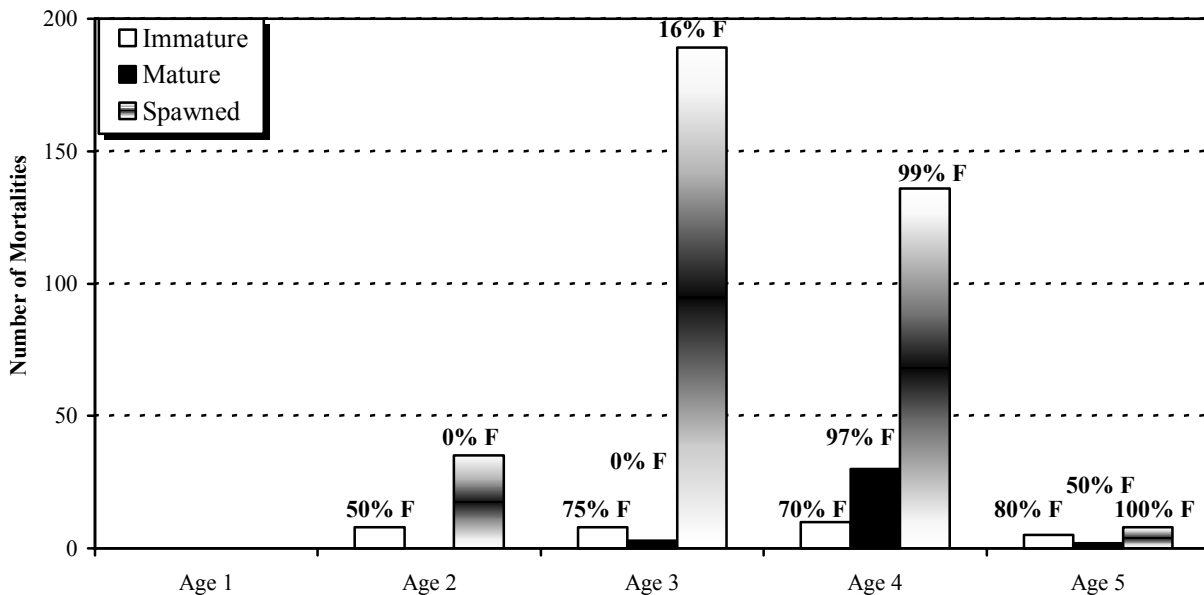
Figure 5. Number of mortalities by age and percent composition of females (F) for each stage of maturity for the 2001 brood year.

2002 Brood Rearing

We determined that there would be insufficient captive brood males to spawn with females at the end of the captive broodstock program. To prevent this from occurring, 20 fish from 15 families (300 fish total) were selected from the 2002 supplementation fish in order to have extra males available in the future. It was later agreed that females from this brood year would also be spawned so their eggs could contribute to the program.

WDFW began 2006 with 90 BY 2002 fish on hand. Fish from this brood have remained healthy throughout their rearing at LFH, with one mortality during the year prior to sorting. As 2006 was the last spawn for the Tucannon Captive Broodstock Program, all 89 remaining fish were transported to the spawning building during sorting on June 21.

2006 Spawning, with Comparisons to the Supplementation Broodstock

One of the 12 fish from the 2001 brood year (Age 5) was a mature male but was not needed for spawning. Length and weight of the male was 47.5 cm and 953.4 g (Appendix E, Table 1). The remaining 11 fish were females. Of those, eight were spawned, one had non-viable eggs, and two were immature and were killed. Mean length and weight of the Age 5 mature females was 53.8 cm and 2,088.4 g, respectively (Appendix E, Table 1). The two immature females averaged 44 cm and 908 g. Length-weight relationships by sex are found in Appendix E, Table 2.

Eggs were initially disinfected and water hardened for one hour in iodophor (100 ppm). During incubation, formalin (1,000 ppm) was added every other day for a 30 min treatment period to control fungus on the eggs. Eggtake from the 2001 brood year was 17,042 eggs and egg survival

was 54%. Mean fecundity of the eight fully spawned females was 2,130 eggs/female. Fecundity by size relationship for Age 4 females was expressed by the formula:

$$\text{Fecundity} = -4,776.59 + 125.29 \times \text{Fork Length (cm)} \qquad (r^2 = 0.76; P < 0.01)$$

Peak spawning was one week later than observed for the supplementation fish (Figure 6). Six of the 2001 BY females were crossed with wild (unmarked) males, one with hatchery-origin males, and one with a combination of hatchery and wild-origin males.
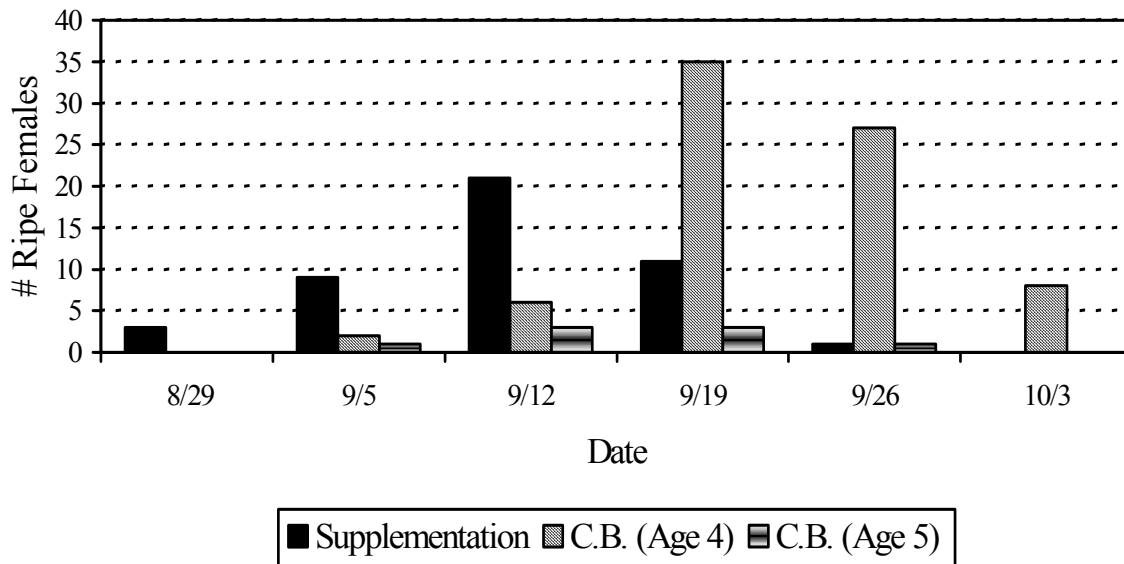
Figure 6. Spawn timing comparison by origin for the 2006 spawning season.

Four of the 89 2002 BY (Age 4) fish were males, of which two were immature and the remaining two fish were mature but not used for spawning. Mean length and weight for mature Age 4 males was 50.3 cm and 1,452.8 g (Appendix E, Table 1). The two immature males averaged 44.5 cm and 1,271.2 g. The remaining 85 fish were females. Seventy-eight of the females were spawned, three were pre-spawn mortalities, and four were immature. Mean length and weight of mature Age 4 females was 53.9 cm and 2,364.8 g (Appendix E, Table 1). Length-weight relationships by sex are found in Appendix E, Table 2. Eggtake was 145,694 eggs and egg survival was 62%. Mean fecundity based on the 78 fully spawned fish was 1,868 eggs/female. Fecundity by size relationship for Age 4 females was expressed by the formula:

$$\text{Fecundity} = -2,917.62 + 88.45 \times \text{Fork Length (cm)} \qquad (r^2 = 0.70; P < 0.01)$$

Peak spawning was one week later than observed for the supplementation fish (Figure 6). Thirty 2002 BY females were crossed with wild (unmarked) males, 38 with hatchery-origin males, and 10 with a combination of hatchery and wild-origin males.

The 2006 eggtake for the captive brood program was 162,736 eggs. A total of 63,316 dead eggs (38.9%) were removed leaving 99,420 live eggs in the incubators. An additional 19,988 dead eggs/fry (20.1%) were picked at ponding leaving 79,432 fish for rearing.

Analysis of variance was performed to determine if there were significant differences (at the 95% confidence interval) in mean fecundities between captive brood (Age 4) and wild and hatchery origin females (Age 4) trapped from the Tucannon River for the supplementation program. Age 4 fish trapped for the supplementation program (both hatchery and wild origin) had significantly higher fecundities than Age 4 captive brood females (P < 0.01) (Figure 7).
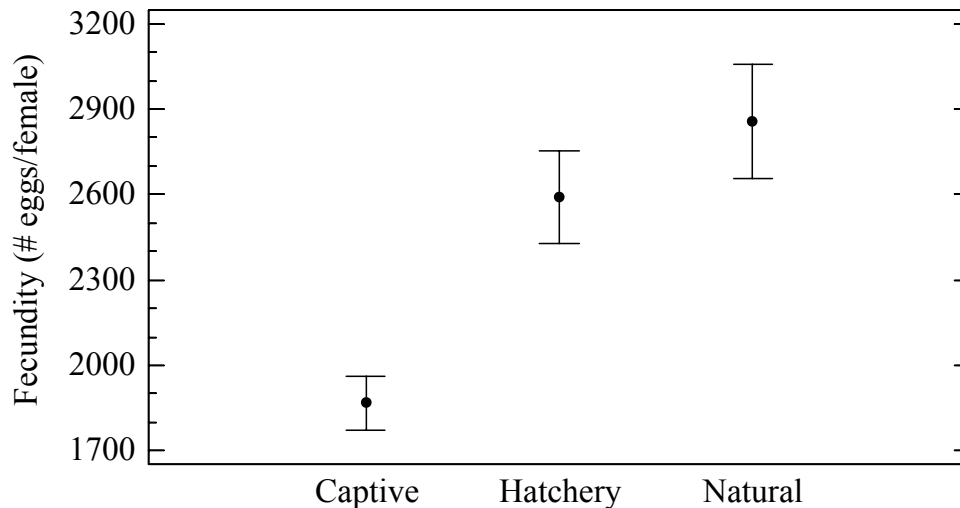
Figure 7. Mean fecundity (with 95% confidence intervals) of Age 4 captive, hatchery and natural origin spawned females, 2006.

Egg size (g/egg) has been tracked in the supplementation program since 1988. Mean egg size for 4-year-old females was significantly different at the 95% confidence level between hatchery-origin, natural-origin, and captive brood fish (P < 0.05) (Figure 8). Heath et al. (2003) found that Chinook salmon raised in a commercial hatchery in Canada developed significantly smaller eggs within four generations in captivity. We have found the opposite, with hatchery and captive brood eggs significantly larger than eggs from wild origin fish, at least for Age 4 fish (Figure 8).
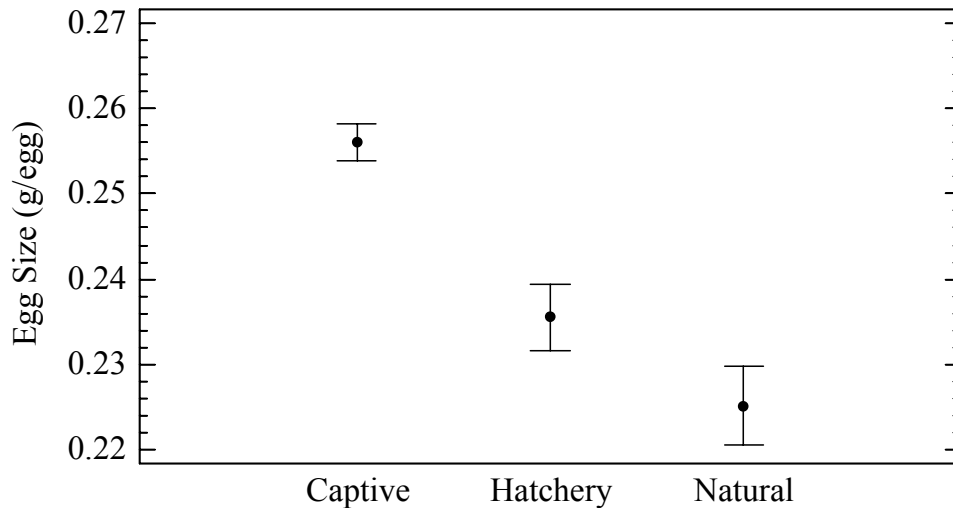
Figure 8. Mean egg size (g/egg) with 95% confidence intervals for Age 4 captive brood females (2001-2006) compared to Age 4 natural and hatchery origin females from the supplementation program, 1988-2006.

Surprisingly, captive brood eggs are significantly larger than eggs from broodstock trapped from the Tucannon River even though captive broodstock females are significantly smaller (P < 0.05) (Table 1). Captive brood females may be able to allocate more energy into producing larger eggs because of their protection in the hatchery environment. These large eggs in small fish results in lower captive broodstock fecundities than fish trapped from the wild.

Table 1. Comparison of mean fork length (cm) and mean egg size (g/egg) from female captive broodstock (2000-2006) and female supplementation broodstock (1988-2006).

Female Origin (Age)	N	Mean Fork Length (cm)	S.D.	Mean Egg Size (g/egg)	S.D.	Range
Captive Brood (Age 3)	191	47.4	3.4	0.22	0.04	0.13-0.31
Captive Brood (Age 4)	753	52.8	5.1	0.26	0.05	0.15-0.45
Captive Brood (Age 5)	29	53.8	5.0	0.25	0.06	0.15-0.38
Wild Origin (Age 4)	167	70.8	4.2	0.23	0.03	0.15-0.33
Hatchery Origin (Age 4)	225	70.4	4.1	0.24	0.03	0.10-0.32
Wild Origin (Age 5)	81	84.0	4.0	0.27	0.04	0.13-0.35
Hatchery Origin (Age 5)	40	80.4	5.0	0.28	0.04	0.20-0.36

Using analysis of variance, mortality to the eyed egg stage was significantly higher for captive brood origin eggs than eggs from the supplementation program ($P < 0.01$) (Figure 9). The cause of such high egg mortality for the captive brood fish is unknown. It may be nutritionally or hatchery environment related.
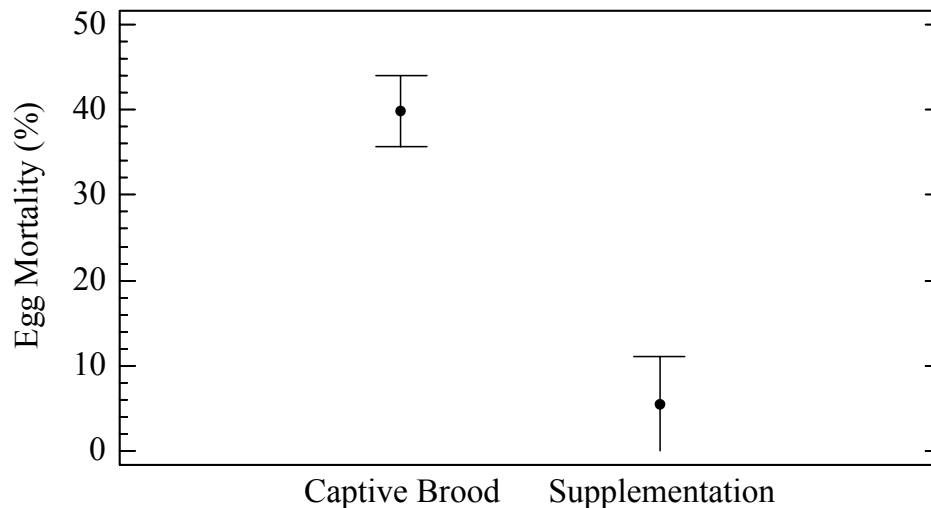
Figure 9. Mean percent egg mortality (with 95% confidence intervals) of captive brood and supplementation origin eggs from the 2006 spawning season.

2005 Progeny

The 2005 BY captive brood juveniles (90,260 fish) were marked with a CWT in the snout on September 14-18, 2006. Marked fish were transported to the Tucannon Fish Hatchery during September 28-29. Fish were sampled for length, weight, hatchery mark quality, and Passive Integrated Transponder (PIT) tagged for outmigration comparisons (1,002 supplementation fish and 1,000 captive brood progeny) before transfer to Curl Lake Acclimation Pond (Table 2). Length and weight samples were collected twice from the 2005 BY fish during the rearing cycle (Table 3). The captive brood progeny were moved to Curl Lake for final rearing February 8-9, 2007. Volitional release began April 2 and continued until April 23 when the remaining fish were forced out. Mortalities were low in Curl Lake and 90,056 BY 2005 captive broodstock progeny were released into the Tucannon River (Table 4). These fish were marked with a CWT and no fin clips in order to differentiate them from the supplementation fish (CWT/Right Red 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. Fish releases from the program to date can be found in Appendix F.

Table 2. Length and weight statistics of the 2005 brood year supplementation (Supp.) and captive brood (C.B.) progeny PIT tagged in February 2007.

Origin	N	Mean Length (mm)	Coefficient of Variation	Mean Weight (g)	Condition Factor (K)	Number PIT Tagged
Supp.	250	135	10.9	32.4	1.27	1,002
C.B.	250	136	12.9	32.4	1.23	1,000

Table 3. Summary of sample sizes (N), mean lengths (mm), coefficients of variation (CV), condition factors (K), and fish/lb (FPP) of 2005 BY juveniles sampled at TFH and Curl Lake.

Date	Progeny Type	Sample Location	N	Mean Length	CV	K	FPP
2/05/07	Captive Brood	TFH	250	136.1	12.9	1.23	14.0
4/05/07	Captive Brood	Curl Lake	250	166.3	14.3	1.25	7.4

Table 4. Summary of spring Chinook captive brood progeny smolt releases in the Tucannon River, 2005 brood year.

Release Year	(BY)	Release Location	Date	Total Released	CWT Code	Number Tagged	Ad-only Marked	Kg
2007	2005	Curl Lake	4/02-4/23	90,056	63/34/77	88,885	N.A.	5,525.2

N.A. = Not Applicable.

2006 Progeny

As of May 1, 2007 we had 83,392 BY 2006 captive brood progeny on hand after adjustments based on actual counts at Lyons Ferry Hatchery. These fish will be coded-wire tagged and volitionally released during March-April 2008.

PIT Tagging

In 2006, WDFW used passive integrated transponder (PIT) tags to compare emigration travel timing and relative success of the 2004 BY captive brood progeny with our conventional hatchery supplementation fish. We tagged 1,002 captive brood progeny and 1,001 supplementation fish during early February before transferring them to Curl Lake AP for acclimation and volitional release (Table 5). No fish were killed during PIT tagging, though it is likely some minor delayed mortality occurred after transfer. Detection rates were low (Table 5), but similar to rates from previous releases at Curl Lake (Bumgarner et al. 1998).

Table 5. 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 2006. (Fish were volitionally released from 4/03/06-4/26/06).

Hatchery Origin	Release Data			Recapture Data										
		Mean		Mean	LMJ		MCJ		JDJ		BONN		Total[a]	
	N	Length	S.D.	Length	N	TD	N	TD	N	TD	N	TD	N	(%)
Supp.	1,001	128.0	13.1	128.3	136	13.6	97	16.1	40	21.2	18	22.5	327	(32.7)
C.B.	1,002	125.3	14.6	127.0	127	12.4	87	16.7	30	22.7	14	18.6	279	(27.8)

[a] Total includes detections at Ice Harbor Dam.
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 0.84 ($\pm$ 0.08) and 0.83 ($\pm$ 0.08) 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

Only two captive brood progeny adult returns (1 female, 1 jack) were recovered during 2006 (Table 6). Both of the returns were recovered during spawning ground surveys with one recovered above the adult trap (rkm 59). The number of captive brood returns was expanded to four for the total run.

Table 6. Captive brood progeny adult returns collected from the Tucannon River during 2006.

Date	Rkm	Sex	Fork Length (cm)	POH Length (cm)	Age	Brood Year	DNA Sample #
9/14/06	67.8	F	61.5	52.0	4	2002	06AH98
9/25/06	55.9	J	43.0	35.0	3	2003	06AH90

Survival Rates

Point estimates of population sizes have been calculated for various life stages (Table 7) 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 8).

Table 7. 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[b]
2003	223	1,389	309,416	164,800	130,064	2[b]
2004	205	1,549	310,819	140,874	132,312	
2005	167	1,595	261,845	93,971	90,056	
2006	86	1,892	162,736	79,432		

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

Table 8. 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[a]
2003	53.3	78.9	42.0	0.00[a]
2004	45.3	93.9	42.6	
2005	35.9	95.8	34.4	
2006	48.8			
Geometric Mean	42.5	82.7	34.3	0.00

[a] Incomplete – brood year still returning.

Egg-to-parr survival for captive brood progeny averaged 42.5% (geometric mean) over seven years (Table 8). This is higher than the 7.7% found for in-river natural-origin Tucannon River spring Chinook, but less than the 80.1% survival from the conventional hatchery supplementation program fish (Gallinat and Ross 2006). Parr-to-smolt survival averaged 82.7% for the captive brood progeny. This is in comparison to 40.2% for in-river natural-origin and 85.0% for conventional hatchery supplementation fish. Egg-to-smolt survival was 34.3% for the captive brood fish compared to 4.8% for natural-origin fish and 67.7% 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 compared to SARs of 0.15% and 0.76% for hatchery and natural-origin fish, respectively (Gallinat and Ross 2006).

DNA Genetic Samples

2006 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 2006 we collected 140 DNA samples (operculum punches) from adult salmon (73 wild and 67 hatchery spring Chinook, including the two captive brood progeny adult returns) and 89 samples from captive broodstock spawners. The 2006 DNA samples were sent to the WDFW genetics lab in Olympia for baseline microsatellite DNA analysis.

2005 Brood Year

A total of 343 Tucannon River spring Chinook samples collected in 2005 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 G). 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 2007, Appendix G). 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 (i.e., sorting of mature fish) 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. 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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Captive Broodstock Facility at Lyons Ferry

(Security Fencing)

Existing Water Intake Line

Main Valve
(with Alarm)

Flat Plate
Screen Drain

Tank 4 Tank 3 Tank 2 Tank 1

Tank 8 Tank 7 Tank 6 Tank 5

Pond Valves
(with Pond Level Float Alarm)

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

4' Tanks

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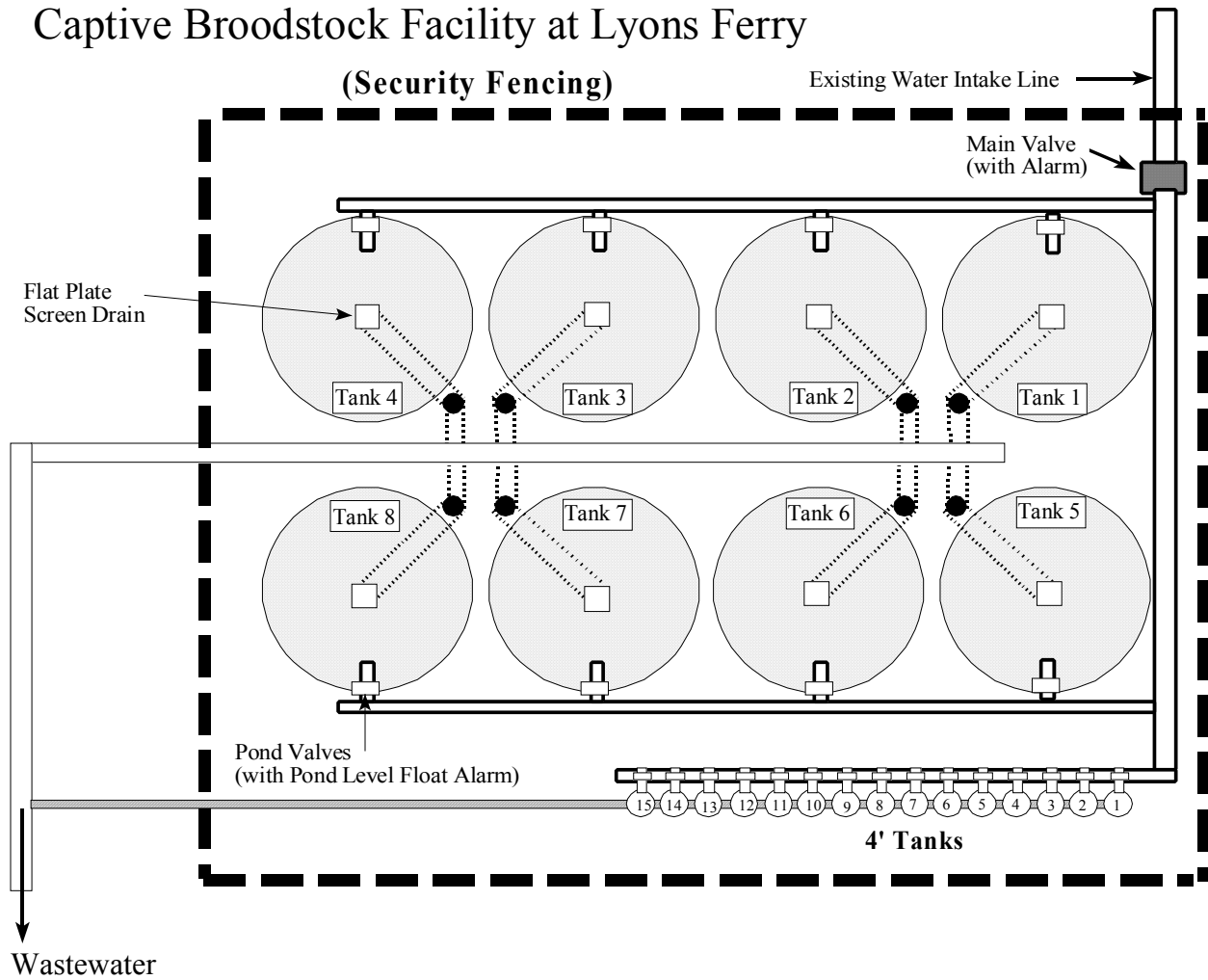
Table 1. Selection of progeny for the Tucannon River spring Chinook captive broodstock program based on origin, crosses, and BKD ELISA results, 1997 and 1998 BYs.

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	Mixed	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.

Brood Year	Family Unit	Number of Fish	Mean Length	S.D.	Mean Weight	S.D.	K
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.
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 C (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 D

Tucannon River spring Chinook captive broodstock mortalities by family unit, sex, age, and maturity for the 2001 Brood Year.

Family Unit	N	M Age1 IM	M Age2 IM	M Age2 MA	M Age2 SP	M Age3 IM	M Age3 MA	M Age3 SP	M Age4 IM	M Age4 MA	M Age5 IM	M Age5 MA	F Age1 IM	F Age2 IM	F Age2 MA	F Age3 IM	F Age3 MA	F Age3 SP	F Age4 IM	F Age4 MA	F Age4 SP	F Age5 IM	F Age5 MA	F Age5 SP	Total Mort.	% Mort.
1	30				2			13						1				1		2	7			1	27	90
2[a]	30							8	1			1						4		3	7		1		28[a]	93
3	30		1		1			13						1		2			1	2	8			1	30	100
4	30		1		3			6						2				1	2	1	13				29	97
5	30				3			11								1		1	1	2	11				30	100
6[b]	30							12								1		2		2	11				29[b]	97
7	30		1			1		9		1						1		3	1	2	10				29	97
8	30				1			14										1	1	2	10	1			30	100
9	30				8			9										5		3	4				29	97
10	30				7			4										2		2	10	2		1	28	93
11	30				3		2	11										3		1	7				27	90
12	30				4			12			1		1					1		1	8	1			29	97
13	30							12	2									1		3	5			3	26	87
14	30				1		1	11										1	1	2	12			2	31	103
15	30		1		2	1		14								1		4		1	5				29	97
Totals	450		4		35	2	3	159	3	1	1	1	1	4		6		30	7	29	135[c]	4	1	8	438[c]	97

IM = Immature, MA = Mature, SP = Spawned

[a] Total includes 3 fish of unknown sex. (Three died from family 2 during tagging).

[b] Total includes 1 fish of unknown sex (just fish head found from Age 2).

[c] Total includes 7 fish from unknown families.

APPENDIX E

Table 1. Fork length (cm) and weight (g) statistics for male, female, and both sexes combined by brood year for captive brood fish sampled during spawning, 2006.

Brood Year	Sex	N	Mean Length (cm)	Range	S.D.	Mean Wt. (g)	Range	S.D.
2001	M	1	47.5	---	---	953.4	---	---
2001	F	9	53.8	43.0-62.0	5.8	2088.4	681.0-3178.0	687.8
2001	*Both*	*10*	*53.2*	*43.0-62.0*	*5.9*	*1974.9*	*681.0-3178.0*	*741.1*
2002	M	2	50.3	48.5-52.0	2.5	1452.8	1362.0-1543.6	128.4
2002	F	81	53.9	35.5-64.5	6.1	2364.8	862.6-4313.0	836.3
2002	*Both*	*83*	*53.8*	*35.5-64.5*	*6.0*	*2342.3*	*862.6-4313.0*	*838.1*

Table 2. Length-weight relationship for male, female, and both sexes combined by brood year for the captive brood during spawning, 2006.

Brood Year	Sex	Length-Weight Relationship	r^2	Probability
2001	Female	Fork Length (cm) = 36.929 + 0.0081 x Wt (g)	0.90	< 0.01
2001	Male[a]	N/A	---	---
2001	*Combined*	*Fork Length (cm) = 38.392 + 0.0075 x Wt (g)*	*0.89*	*< 0.01*
2002	Female	Fork Length (cm) = 38.904 + 0.0064 x Wt (g)	0.88	< 0.01
2002	Male[a]	N/A	---	---
2002	*Combined*	*Fork Length (cm) = 39.097 + 0.0063 x Wt (g)*	*0.88*	*< 0.01*

[a] Small sample size.

APPENDIX F

Release Year	BY[1]	Release Date	CWT	No Wire	Wire	Total Released	Lbs	Fish/Lb
Summary of captive brood progeny releases from the Tucannon River Spring Chinook Captive Broodstock Program.
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

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

APPENDIX G

Genetic Assessment of Spring Chinook in the Tucannon River (2005) Using a Microsatellite DNA Analysis

by

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Abstract

A total of 343 spring Chinook samples from the Tucannon River were analyzed from collections made in 2005 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. All collections were found to have relatively high and similar levels of genetic diversity. Genotypic tests of differentiation indicated highly 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, however these samples were more similar to each other than they were to the captive brood samples. 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 highly significant differences among these two groups and the captive brood. The highly significant difference between the hatchery and natural-origin fish versus the low 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 captive broodstock program has also been effective at maintaining overall genetic variation 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 2005 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

<u>Collections</u>

A total of 343 spring-run Chinook samples were analyzed at 14 microsatellite loci (13 coastwide GAPS loci plus *Ssa-197*) from three sources in 2005: 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 Chinook as

hatchery-origin. 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 2005. 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 separately for each microsatellite locus with a simple thermal profile consisting of: denaturation at 95°C for 3 min, denaturation at 95°C for 15 sec, anneal for 30 sec at the appropriate temperature for each locus (Table 2), extension at 72°C for 1 min, repeat cycle (steps 2-4), final extension at 72°C for 30 minutes. PCR products for each locus were subsequently combined into multiplexes to be processed with an ABI-3730 DNA Analyzer. Genotypes were visualized with a known size standard (GS500LIZ 3730) using GeneMapper 3.7 software. Allele binning and naming were accomplished using MicrosatelliteBinner-v.1.h (Young, WDFW

available from the author). MicrosatelliteBinner creates groups (bins) of alleles with similar mobilities (presumably alleles with the same number of repeat units). The upper and lower bounds of the bins are determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins are then named as the mean allele size for the cluster rounded to an integer.

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: three samples were identified as strays and one as an unknown. One other sample identified as a DIP (dead in pond) was included in the analysis of hatchery and natural-origin fish because although its origin was known, it could not be included in the analysis of in-river and supplementation spawners because it 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 16 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.

Tests for Hardy-Weinberg Equilibrium (HWE) revealed no significant deviations from expected values after implementation of Bonferroni correction for multiple tests (Rice 1989) at any locus and therefore no loci were dropped from analysis (Table 2). 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 52 individuals per collection while the analysis of supplementation, in-river spawners and captive brood was conducted on a total of 13 individuals per collection. As a result the mean for allelic richness for these two different analyses differed and ranged from 8.81 - 12.45. 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 (9.13 and 13.58). 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 2005 sample groups was consistent with those reported by Hawkins and Frye (2005) and Kassler and Hawkins (2006). 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 of the pairwise F_{ST} tests and tests of genotypic differentiation (Table 4a and 4b) suggest that the collections are genetically differentiated (Table 4a). The supplementation and in-river spawners are significantly different in this analysis; however the p-value for this test is higher than observed for the other comparisons suggesting less differentiation between these two groups than the other groups. The pairwise F_{ST} values are between 0.0013 - 0.0052 indicating a relatively low level of genetic difference among all of the collections regardless of how they are sorted (Table 4b). The F_{ST} values are highly affected by the level of heterozygosity at each locus and may limit the usefulness of these comparisons (Table 2). 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).

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 there was not a random sample among all the collections being analyzed. 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. The presence of a large number of private alleles could also indicate that the sample size for a collection was not large enough to observe all alleles that exist in that collection area. 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 = 30). The lowest number of private alleles was detected in the hatchery-origin samples (N = 2). The analysis of the supplementation spawners, in-river spawners, and captive brood samples (Table 3 and Appendix 1b) revealed a more equal number of private alleles within groups. The captive brood samples had N = 17 private alleles and the supplementation spawners had N = 16. The in-river collection only had 33 samples which likely contributed to the low number of private alleles detected in that collection.

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 = 30) can be present in either the supplementation fish (N = 15), in-river spawners (N = 9), or they can be present in both groups (N = 6). 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 5 – 32 (*Ots-9** and *Ots-G474** – *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) and Kassler and Hawkins (2006), 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.

Acknowledgements

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Table 1. Collection code, collection description, and number of samples collected and used in the 2005 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[c]	# used in analysis
natural-origin	05EQ	86	14	72
hatchery-origin	05ER[a]	57	2	55
supplementation - natural-origin	05EQ[b]	46	0	46
supplementation - hatchery-origin	05ER[a]	48	0	48
supplementation spawners - total	05EQ and 05ER	94	0	94
in-river - natural-origin	05EQ[b]	40	14	26
in-river - hatchery-origin	05ER[a]	9	2	7
in-river spawners - total	05EQ and 05ER[a]	49	16	33
captive broodstock	05ES	200	0	200

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

b - One sample identified as a DIP (dead in pond) was 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 (°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	19	220-294	0.8621	0.9051	0.0318	0.0276
	Ots-201b*	6fam	50	0.32	40	22	153-278	0.8824	0.9140	0.0286	0.0615
	Ots-208b*	ned	50	0.18	40	24	162-274	0.9032	0.9137	0.4153	0.3510
	Ssa-408*	pet	50	0.20	40	22	184-300	0.8700	0.9000	0.4041	0.2358
Ots-N	Ogo-2*	pet	63	0.07	40	9	202-232	0.6310	0.6343	0.5965	0.6935
	Ssa-197*	ned	63	0.25	40	19	201-297	0.8758	0.8727	0.7058	0.7819
Ots-O	Ogo-4*	6fam	56	0.18	40	11	132-166	0.7785	0.8201	0.1333	0.1518
	Ots-213*	ned	56	0.18	40	18	226-314	0.8770	0.8903	0.2395	0.2588
	Ots-G474*	pet	56	0.14	40	5	156-200	0.5569	0.5249	0.9265	0.9533
Ots-R	Omm-1080*	vic	56	0.22	40	32	190-354	0.9557	0.9244	0.8039	0.8998
	Ots-3M*	6fam	63	0.12	40	7	128-150	0.4448	0.4827	0.2112	0.2320
Ots-S	Ots-9*	pet	63	0.04	40	5	103-111	0.6235	0.5863	0.9794	0.9837
	Ots-211*	ned	63	0.07	40	22	208-312	0.9062	0.8897	0.5932	0.7426
	Ots-212*	6fam	63	0.30	40	15	131-231	0.8957	0.8577	0.8917	0.8188

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.

Collection	Collection Code	Linkage (# locus pairs significant before/after Bonferroni correction) [a]	Heterozygosity H_o	H_e	HWE P-value[b]	Allelic Richness[c]	F_{IS} (p-value)[d]	Number of private alleles
Natural-origin	05EQ	18 / 2	0.781	0.801	0.002	13.58	0.025 (0.026)	30
Hatchery origin	05ER	23 / 5	0.797	0.794	0.568	11.81	-0.003 (0.591)	2
Captive brood spawners	05ES	73 / 53 - 76 / 56d	0.792	0.788	0.873	11.95 / 8.42d	-0.005 (0.751)	17
Supplementation spawners	05EQ and 05ER	25 / 7	0.782	0.793	0.028	8.89	0.015 (0.092)	16
In-river spawners	05EQ and 05ER	3 / 0	0.815	0.819	0.236	9.13	0.006 (0.366)	10

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 13 individuals (supplementation or in-river) or 52 individuals (natural or hatchery-origin).
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 = 0.017 (0.05/3).

	05 Hatchery	05 Natural	05 Captive Brood
05 Hatchery	**X**		
05 Natural	**0.0000**	**X**	
05 Captive Brood	**0.0000**	**0.0000**	**X**

	05 Supplementation	05 In-river	05 Captive Brood
05 Supplementation	**X**		
05 In-river	**0.0020**	**X**	
05 Captive Brood	**0.0000**	**0.0000**	**X**

Table 4b. Pairwise F_{ST} values across all loci. 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.

	05 Hatchery	05 Natural	05 Captive Brood
05 Hatchery	**X**		
05 Natural	**0.0035**	**X**	
05 Captive Brood	**0.0048**	**0.0052**	**X**

	05 Supplementation	05 In-River	05 Captive Brood
05 Supplementation	**X**		
05 In-River	**0.0016**	**X**	
05 Captive Brood	**0.0025**	**0.0013**	**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	05EQ	72	**18**	**21**	19	18	**9**	16	**11**	27	14	**5**	**6**	**5**	17	**14**
Hatchery-origin	05ER	55	<u>14</u>	<u>16</u>	<u>14</u>	<u>14</u>	7	15	<u>8</u>	23	**16**	<u>4</u>	<u>5</u>	4	<u>16</u>	<u>10</u>
Captive brood spawners	05ES	200	17	19	18	**19**	8	16	10	27	**16**	<u>4</u>	**6**	4	**19**	12
Supplementation spawners	05EQ / 05ER	46 / 48*	**18**	19	**21**	16	**9**	**17**	**11**	**28**	15	**5**	**6**	**5**	18	13
In-river spawners	05EQ / 05ER	26 / 7*	16	19	<u>14</u>	16	<u>5</u>	<u>14</u>	9	<u>19</u>	<u>13</u>	**5**	**6**	<u>3</u>	18	12
Number of alleles in all collections			19	22	24	22	9	19	11	32	18	5	7	5	22	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 2005. The column labeled "private" identifies specific alleles that were only scored in the collection that is identified.

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Oki-100	220	0.060	0.019	0.015	
Oki-100	232	0.015	0.019		
Oki-100	236	0.045		0.003	
Oki-100	240	0.119	0.083	0.071	
Oki-100	244	0.030	0.074	0.088	
Oki-100	248	0.097	0.056	0.093	
Oki-100	252	0.022		0.035	
Oki-100	256	0.030	0.056	0.020	
Oki-100	260	0.060	0.120	0.131	
Oki-100	264	0.015		0.010	
Oki-100	268	0.090	0.093	0.063	
Oki-100	270	0.112	0.102	0.086	
Oki-100	272	0.134	0.148	0.205	
Oki-100	275	0.067	0.046	0.063	
Oki-100	279	0.030		0.010	
Oki-100	283	0.052	0.157	0.101	
Oki-100	287	0.015	0.009	0.003	
Oki-100	290	0.008			Natural-origin
Oki-100	294		0.019	0.003	
# of samples		39	74	331	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-201b	153	0.042	0.028	0.073	
Ots-201b	169	0.007	0.009		
Ots-201b	173	0.007			Natural-origin
Ots-201b	178	0.070	0.056	0.096	
Ots-201b	182	0.078	0.083	0.088	
Ots-201b	186	0.113	0.093	0.121	
Ots-201b	190	0.162	0.102	0.169	
Ots-201b	194	0.021	0.093	0.056	
Ots-201b	198	0.007		0.003	
Ots-201b	202	0.021	0.019	0.010	
Ots-201b	206	0.014		0.020	
Ots-201b	210	0.028	0.037	0.020	
Ots-201b	214	0.035	0.074	0.020	
Ots-201b	218	0.169	0.157	0.078	
Ots-201b	222	0.056	0.065	0.121	
Ots-201b	226	0.078	0.093	0.028	
Ots-201b	230	0.007		0.003	
Ots-201b	234	0.042	0.019	0.005	
Ots-201b	238	0.014	0.037	0.046	
Ots-201b	254	0.007			Natural-origin
Ots-201b	274			0.008	Captive Brood
Ots-201b	278	0.021	0.037	0.035	
# of samples		71	54	198	

Appendix 1a continued.

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-208b	162	0.081	0.046	0.041	
Ots-208b	174			0.010	Captive Brood
Ots-208b	178	0.016			Natural-origin
Ots-208b	182	0.089	0.056	0.072	
Ots-208b	186			0.018	Captive Brood
Ots-208b	190	0.008			Natural-origin
Ots-208b	194	0.024			Natural-origin
Ots-208b	198	0.032	0.120	0.085	
Ots-208b	202	0.089	0.065	0.121	
Ots-208b	206	0.024			Natural-origin
Ots-208b	210		0.046	0.026	
Ots-208b	214	0.113	0.074	0.080	
Ots-208b	218		0.009	0.003	
Ots-208b	222	0.040		0.016	
Ots-208b	226	0.008	0.009	0.008	
Ots-208b	230	0.024	0.028	0.028	
Ots-208b	234	0.137	0.120	0.144	
Ots-208b	238	0.089	0.120	0.093	
Ots-208b	242	0.097	0.167	0.134	
Ots-208b	246	0.040	0.056	0.052	
Ots-208b	250	0.065	0.083	0.064	
Ots-208b	254	0.008			Natural-origin
Ots-208b	262			0.005	Captive Brood
Ots-208b	274	0.016			Natural-origin
# of samples		62	54	194	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ssa-408	184	0.064	0.056	0.035	
Ssa-408	188	0.164	0.074	0.204	
Ssa-408	192	0.014	0.120	0.073	
Ssa-408	196	0.171	0.120	0.143	
Ssa-408	200	0.014	0.019	0.030	
Ssa-408	204	0.043	0.009	0.038	
Ssa-408	208	0.200	0.259	0.085	
Ssa-408	212	0.043	0.074	0.116	
Ssa-408	216	0.014	0.009	0.053	
Ssa-408	220		0.009	0.003	
Ssa-408	224	0.079	0.111	0.070	
Ssa-408	228	0.043		0.003	
Ssa-408	232	0.007			Natural-origin
Ssa-408	240	0.021		0.025	
Ssa-408	244			0.003	Captive Brood
Ssa-408	248			0.003	Captive Brood
Ssa-408	280	0.007			Natural-origin
Ssa-408	284	0.014	0.046	0.020	
Ssa-408	288	0.036	0.037	0.023	
Ssa-408	292	0.007			Natural-origin
Ssa-408	296	0.057	0.056	0.073	
Ssa-408	300			0.003	Captive Brood
# of samples		70	54	199	

Appendix 1a continued.

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ogo-2	202	0.009			Natural-origin
Ogo-2	214	0.148	0.087	0.120	
Ogo-2	216	0.611	0.625	0.552	
Ogo-2	218	0.019		0.025	
Ogo-2	220	0.083	0.048	0.128	
Ogo-2	222	0.083	0.115	0.065	
Ogo-2	226	0.009	0.048	0.052	
Ogo-2	228	0.009	0.029	0.030	
Ogo-2	232	0.028	0.048	0.030	
# of samples		54	52	184	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ssa-197	201	0.056	0.036	0.051	
Ssa-197	205	0.007			Natural-origin
Ssa-197	209	0.035	0.064	0.026	
Ssa-197	213			0.003	Captive Brood
Ssa-197	221		0.027	0.003	
Ssa-197	225	0.007			Natural-origin
Ssa-197	249	0.021	0.027	0.026	
Ssa-197	252	0.014	0.018	0.023	
Ssa-197	256	0.120	0.136	0.089	
Ssa-197	261	0.078	0.046	0.041	
Ssa-197	265	0.190	0.182	0.199	
Ssa-197	269	0.070	0.036	0.054	
Ssa-197	273	0.232	0.236	0.247	
Ssa-197	277	0.042	0.018	0.056	
Ssa-197	281	0.035	0.018	0.028	
Ssa-197	285	0.028	0.091	0.097	
Ssa-197	289	0.014			Natural-origin
Ssa-197	293	0.049	0.027	0.038	
Ssa-197	297		0.036	0.020	
# of samples		71	55	196	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ogo-4	132	0.007	0.009	0.003	
Ogo-4	136	0.014	0.109	0.056	
Ogo-4	138	0.014		0.003	
Ogo-4	148	0.194	0.164	0.258	
Ogo-4	154	0.056	0.091	0.038	
Ogo-4	156	0.319	0.182	0.222	
Ogo-4	158	0.215	0.291	0.207	
Ogo-4	160	0.083	0.091	0.131	
Ogo-4	162	0.021		0.015	
Ogo-4	164	0.069	0.064	0.068	
Ogo-4	166	0.007			Natural-origin
# of samples		72	55	198	

Appendix 1a continued.

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Omm-1080	190	0.037	0.083	0.064	
Omm-1080	194			0.003	Captive Brood
Omm-1080	206	0.088	0.120	0.054	
Omm-1080	214	0.007			Natural-origin
Omm-1080	218	0.015		0.008	
Omm-1080	230	0.184	0.157	0.201	
Omm-1080	234	0.044	0.028	0.021	
Omm-1080	242	0.007	0.009	0.018	
Omm-1080	250	0.007			Natural-origin
Omm-1080	254		0.028	0.008	
Omm-1080	258	0.059	0.028	0.070	
Omm-1080	262	0.088	0.028	0.026	
Omm-1080	266	0.022			Natural-origin
Omm-1080	270	0.015	0.037	0.026	
Omm-1080	274		0.019	0.003	
Omm-1080	282	0.007	0.009	0.005	
Omm-1080	286			0.013	Captive Brood
Omm-1080	290	0.015	0.019	0.013	
Omm-1080	294	0.015	0.028	0.036	
Omm-1080	298	0.066	0.093	0.064	
Omm-1080	302	0.044	0.046	0.077	
Omm-1080	306	0.007	0.019	0.008	
Omm-1080	310	0.007	0.009	0.003	
Omm-1080	314	0.022	0.019	0.023	
Omm-1080	318	0.052	0.046	0.072	
Omm-1080	322	0.022	0.037	0.021	
Omm-1080	326	0.052	0.074	0.054	
Omm-1080	330	0.007			Natural-origin
Omm-1080	338	0.096	0.028	0.083	
Omm-1080	342	0.007	0.037	0.023	
Omm-1080	350	0.007			Natural-origin
Omm-1080	354			0.005	Captive Brood
# of samples		68	54	194	

Appendix 1a continued.

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-213	226		0.065	0.018	
Ots-213	230		0.009		Hatchery-origin
Ots-213	234			0.013	Captive Brood
Ots-213	238	0.069	0.019	0.023	
Ots-213	258			0.008	Captive Brood
Ots-213	262	0.146	0.222	0.240	
Ots-213	266	0.046	0.028		
Ots-213	270	0.123	0.167	0.184	
Ots-213	274	0.023	0.065	0.033	
Ots-213	278	0.100	0.056	0.081	
Ots-213	282	0.023	0.046	0.020	
Ots-213	290	0.046	0.019	0.028	
Ots-213	294	0.069	0.056	0.101	
Ots-213	298	0.062	0.046	0.063	
Ots-213	302	0.131	0.037	0.068	
Ots-213	306	0.054	0.019	0.025	
Ots-213	310	0.031	0.065	0.030	
Ots-213	314	0.077	0.083	0.066	
# of samples		65	54	198	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-G474	156	0.583	0.673	0.636	
Ots-G474	168	0.278	0.200	0.288	
Ots-G474	184	0.021			Natural-origin
Ots-G474	192	0.035	0.100	0.056	
Ots-G474	200	0.083	0.027	0.020	
# of samples		72	55	198	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-3M	128	0.014			Natural-origin
Ots-3M	138	0.007	0.009	0.018	
Ots-3M	142			0.008	Captive Brood
Ots-3M	144	0.014	0.046	0.013	
Ots-3M	146	0.285	0.291	0.249	
Ots-3M	148	0.660	0.636	0.681	
Ots-3M	150	0.021	0.018	0.033	
# of samples		72	55	199	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-9	103	0.007	0.056	0.023	
Ots-9	105	0.373	0.380	0.349	
Ots-9	107	0.486	0.500	0.548	
Ots-9	109	0.127	0.065	0.080	
Ots-9	111	0.007			Natural-origin
# of samples		71	54	199	

Appendix 1a continued.

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-211	208		0.009	0.003	
Ots-211	220	0.008			Natural-origin
Ots-211	236	0.037		0.013	
Ots-211	240	0.105	0.037	0.075	
Ots-211	244	0.045	0.046	0.040	
Ots-211	248	0.015			Natural-origin
Ots-211	252	0.030	0.009	0.005	
Ots-211	256	0.022	0.009	0.013	
Ots-211	260		0.019		Hatchery-origin
Ots-211	264	0.008		0.008	
Ots-211	268	0.075	0.130	0.123	
Ots-211	272	0.037	0.093	0.035	
Ots-211	276	0.246	0.194	0.188	
Ots-211	280	0.037	0.056	0.045	
Ots-211	284	0.082	0.130	0.234	
Ots-211	288	0.075	0.028	0.038	
Ots-211	292	0.082	0.093	0.035	
Ots-211	296		0.037	0.045	
Ots-211	300			0.005	Captive Brood
Ots-211	304	0.090	0.074	0.065	
Ots-211	308			0.003	Captive Brood
Ots-211	312	0.008	0.037	0.028	
# of samples		67	54	199	

Locus	Size	05 Natural	05 Hatchery	05 Captive	Private
Ots-212	131	0.042	0.019	0.043	
Ots-212	135	0.021	0.009	0.008	
Ots-212	139	0.035	0.083	0.035	
Ots-212	143	0.194	0.222	0.228	
Ots-212	147	0.069	0.102	0.138	
Ots-212	151	0.167	0.139	0.163	
Ots-212	155	0.194	0.222	0.155	
Ots-212	159	0.104	0.120	0.158	
Ots-212	163	0.049	0.046	0.033	
Ots-212	167	0.028		0.010	
Ots-212	171	0.069	0.037	0.025	
Ots-212	183	0.007			Natural-origin
Ots-212	191	0.014			Natural-origin
Ots-212	207			0.008	Captive Brood
Ots-212	231	0.007			Natural-origin
# of samples		72	54	200	

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 2005. The column labeled "private" identifies specific alleles that were only scored in the collection that is identified.

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Oki-100	220	0.038	0.052	0.015	
Oki-100	232	0.016	0.017		
Oki-100	236	0.027	0.017	0.003	
Oki-100	240	0.103	0.103	0.071	
Oki-100	244	0.060	0.017	0.088	
Oki-100	248	0.049	0.172	0.093	
Oki-100	252	0.011	0.017	0.035	
Oki-100	256	0.038	0.052	0.020	
Oki-100	260	0.087	0.086	0.131	
Oki-100	264	0.011		0.010	
Oki-100	268	0.098	0.069	0.063	
Oki-100	270	0.109	0.103	0.086	
Oki-100	272	0.147	0.121	0.205	
Oki-100	275	0.038	0.121	0.063	
Oki-100	279	0.016	0.017	0.010	
Oki-100	283	0.130		0.101	
Oki-100	287	0.011	0.017	0.003	
Oki-100	290		0.017		In-river
Oki-100	294	0.011		0.003	
# of samples		92	29	198	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-201b	153	0.022	0.076	0.073	
Ots-201b	169	0.005	0.015		
Ots-201b	173		0.015		In-river
Ots-201b	178	0.071	0.046	0.096	
Ots-201b	182	0.098	0.030	0.088	
Ots-201b	186	0.087	0.152	0.121	
Ots-201b	190	0.147	0.106	0.169	
Ots-201b	194	0.044	0.076	0.056	
Ots-201b	198	0.005		0.003	
Ots-201b	202	0.022	0.015	0.010	
Ots-201b	206	0.005	0.015	0.020	
Ots-201b	210	0.038	0.015	0.020	
Ots-201b	214	0.044	0.076	0.020	
Ots-201b	218	0.185	0.106	0.078	
Ots-201b	222	0.065	0.046	0.121	
Ots-201b	226	0.092	0.061	0.028	
Ots-201b	230	0.005		0.003	
Ots-201b	234	0.016	0.076	0.005	
Ots-201b	238	0.027	0.015	0.046	
Ots-201b	254		0.015		In-river
Ots-201b	274			0.008	Captive Brood
Ots-201b	278	0.022	0.046	0.035	
# of samples		92	33	198	

Appendix 1b continued.

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-208b	162	0.049	0.125	0.041	
Ots-208b	174			0.010	Captive Brood
Ots-208b	178	0.011			Supplementation
Ots-208b	182	0.054	0.146	0.072	
Ots-208b	186			0.018	Captive Brood
Ots-208b	190	0.005			Supplementation
Ots-208b	194	0.016			Supplementation
Ots-208b	198	0.071	0.083	0.085	
Ots-208b	202	0.082	0.063	0.121	
Ots-208b	206	0.011	0.021		
Ots-208b	210	0.016	0.042	0.026	
Ots-208b	214	0.114	0.021	0.080	
Ots-208b	218	0.005		0.003	
Ots-208b	222	0.016	0.042	0.016	
Ots-208b	226	0.011		0.008	
Ots-208b	230	0.022	0.042	0.028	
Ots-208b	234	0.136	0.104	0.144	
Ots-208b	238	0.098	0.125	0.093	
Ots-208b	242	0.130	0.125	0.134	
Ots-208b	246	0.049	0.042	0.052	
Ots-208b	250	0.087	0.021	0.064	
Ots-208b	254	0.005			Supplementation
Ots-208b	262			0.005	Captive Brood
Ots-208b	274	0.011			Supplementation
# of samples		92	24	194	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ssa-408	184	0.066	0.046	0.035	
Ssa-408	188	0.121	0.136	0.204	
Ssa-408	192	0.039	0.121	0.073	
Ssa-408	196	0.143	0.167	0.143	
Ssa-408	200	0.006	0.046	0.030	
Ssa-408	204	0.039		0.038	
Ssa-408	208	0.247	0.167	0.085	
Ssa-408	212	0.066	0.030	0.116	
Ssa-408	216	0.011	0.015	0.053	
Ssa-408	220		0.015	0.003	
Ssa-408	224	0.099	0.076	0.070	
Ssa-408	228	0.011	0.061	0.003	
Ssa-408	232	0.006			Supplementation
Ssa-408	240	0.011	0.015	0.025	
Ssa-408	244			0.003	Captive Brood
Ssa-408	248			0.003	Captive Brood
Ssa-408	280		0.015		In-river
Ssa-408	284	0.039		0.020	
Ssa-408	288	0.044	0.015	0.023	
Ssa-408	292		0.015		In-river
Ssa-408	296	0.055	0.061	0.073	
Ssa-408	300			0.003	Captive Brood
# of samples		91	33	199	

Appendix 1b continued.

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ogo-2	202	0.005			Supplementation
Ogo-2	214	0.113	0.154	0.120	
Ogo-2	216	0.640	0.462	0.552	
Ogo-2	218	0.011		0.025	
Ogo-2	220	0.048	0.192	0.128	
Ogo-2	222	0.091	0.154	0.065	
Ogo-2	226	0.032		0.052	
Ogo-2	228	0.022		0.030	
Ogo-2	232	0.038	0.039	0.030	
# of samples		93	13	184	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ssa-197	201	0.032	0.094	0.051	
Ssa-197	205	0.005			Supplementation
Ssa-197	209	0.053	0.031	0.026	
Ssa-197	213			0.003	Captive Brood
Ssa-197	221	0.011	0.016	0.003	
Ssa-197	225	0.005			Supplementation
Ssa-197	249	0.032		0.026	
Ssa-197	252	0.016	0.016	0.023	
Ssa-197	256	0.138	0.094	0.089	
Ssa-197	261	0.059	0.078	0.041	
Ssa-197	265	0.197	0.156	0.199	
Ssa-197	269	0.043	0.094	0.054	
Ssa-197	273	0.239	0.219	0.247	
Ssa-197	277	0.016	0.078	0.056	
Ssa-197	281	0.032	0.016	0.028	
Ssa-197	285	0.069	0.016	0.097	
Ssa-197	289		0.031		In-river
Ssa-197	293	0.032	0.063	0.038	
Ssa-197	297	0.021		0.020	
# of samples		94	32	196	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ogo-4	132	0.005	0.015	0.003	
Ogo-4	136	0.069	0.015	0.056	
Ogo-4	138	0.011		0.003	
Ogo-4	148	0.192	0.152	0.258	
Ogo-4	154	0.090	0.015	0.038	
Ogo-4	156	0.266	0.242	0.222	
Ogo-4	158	0.234	0.288	0.207	
Ogo-4	160	0.064	0.152	0.131	
Ogo-4	162	0.011	0.015	0.015	
Ogo-4	164	0.053	0.106	0.068	
Ogo-4	166	0.005			Supplementation
# of samples		94	33	198	

Appendix 1b continued.

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Omm-1080	190	0.077		0.064	
Omm-1080	194			0.003	Captive Brood
Omm-1080	206	0.115	0.065	0.054	
Omm-1080	214		0.016		In-river
Omm-1080	218	0.011		0.008	
Omm-1080	230	0.165	0.194	0.201	
Omm-1080	234	0.033	0.048	0.021	
Omm-1080	242	0.006	0.016	0.018	
Omm-1080	250	0.006			Supplementation
Omm-1080	254	0.017		0.008	
Omm-1080	258	0.028	0.097	0.070	
Omm-1080	262	0.055	0.081	0.026	
Omm-1080	266	0.006	0.032		
Omm-1080	270	0.022	0.032	0.026	
Omm-1080	274	0.011		0.003	
Omm-1080	282	0.006	0.016	0.005	
Omm-1080	286			0.013	Captive Brood
Omm-1080	290	0.022		0.013	
Omm-1080	294	0.028		0.036	
Omm-1080	298	0.088	0.048	0.064	
Omm-1080	302	0.050	0.032	0.077	
Omm-1080	306	0.006	0.032	0.008	
Omm-1080	310	0.006	0.016	0.003	
Omm-1080	314	0.017	0.032	0.023	
Omm-1080	318	0.050	0.048	0.072	
Omm-1080	322	0.039		0.021	
Omm-1080	326	0.050	0.097	0.054	
Omm-1080	330	0.006			Supplementation
Omm-1080	338	0.066	0.065	0.083	
Omm-1080	342	0.017	0.032	0.023	
Omm-1080	350	0.006			Supplementation
Omm-1080	354			0.005	Captive Brood
# of samples		91	31	194	

Appendix 1b continued.

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-213	226	0.038		0.018	
Ots-213	230		0.019		In-river
Ots-213	234			0.013	Captive Brood
Ots-213	238	0.043	0.058	0.023	
Ots-213	258			0.008	Captive Brood
Ots-213	262	0.199	0.115	0.240	
Ots-213	266	0.027	0.077		
Ots-213	270	0.134	0.173	0.184	
Ots-213	274	0.038	0.058	0.033	
Ots-213	278	0.086	0.058	0.081	
Ots-213	282	0.038	0.019	0.020	
Ots-213	290	0.043		0.028	
Ots-213	294	0.054	0.096	0.101	
Ots-213	298	0.059	0.039	0.063	
Ots-213	302	0.065	0.173	0.068	
Ots-213	306	0.032	0.058	0.025	
Ots-213	310	0.059		0.030	
Ots-213	314	0.086	0.058	0.066	
# of samples		93	26	198	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-G474	156	0.633	0.591	0.636	
Ots-G474	168	0.245	0.242	0.288	
Ots-G474	184	0.005	0.030		
Ots-G474	192	0.064	0.061	0.056	
Ots-G474	200	0.053	0.076	0.020	
# of samples		94	33	198	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-3M	128	0.005	0.015		
Ots-3M	138	0.005	0.015	0.018	
Ots-3M	142			0.008	Captive Brood
Ots-3M	144	0.027	0.030	0.013	
Ots-3M	146	0.271	0.333	0.249	
Ots-3M	148	0.676	0.576	0.681	
Ots-3M	150	0.016	0.030	0.033	
# of samples		94	33	199	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-9	103	0.038		0.023	
Ots-9	105	0.366	0.406	0.349	
Ots-9	107	0.489	0.500	0.548	
Ots-9	109	0.102	0.094	0.080	
Ots-9	111	0.005			Supplementation
# of samples		93	32	199	

Appendix 1b continued.

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-211	208	0.005		0.003	
Ots-211	220		0.018		In-river
Ots-211	236	0.022	0.018	0.013	
Ots-211	240	0.065	0.107	0.075	
Ots-211	244	0.048	0.036	0.040	
Ots-211	248	0.005	0.018		
Ots-211	252	0.022	0.018	0.005	
Ots-211	256	0.016	0.018	0.013	
Ots-211	260	0.011			Supplementation
Ots-211	264		0.018	0.008	
Ots-211	268	0.113	0.054	0.123	
Ots-211	272	0.059	0.071	0.035	
Ots-211	276	0.210	0.268	0.188	
Ots-211	280	0.043	0.054	0.045	
Ots-211	284	0.118	0.054	0.234	
Ots-211	288	0.059	0.036	0.038	
Ots-211	292	0.091	0.071	0.035	
Ots-211	296	0.016	0.018	0.045	
Ots-211	300			0.005	Captive Brood
Ots-211	304	0.075	0.107	0.065	
Ots-211	308			0.003	Captive Brood
Ots-211	312	0.022	0.018	0.028	
# of samples		93	28	199	

Locus	Size	05 Supp	05 In-river	05 Captive	Private
Ots-212	131	0.032	0.030	0.043	
Ots-212	135	0.011	0.030	0.008	
Ots-212	139	0.032	0.121	0.035	
Ots-212	143	0.231	0.136	0.228	
Ots-212	147	0.081	0.091	0.138	
Ots-212	151	0.156	0.152	0.163	
Ots-212	155	0.204	0.212	0.155	
Ots-212	159	0.102	0.136	0.158	
Ots-212	163	0.065		0.033	
Ots-212	167	0.016	0.015	0.010	
Ots-212	171	0.059	0.046	0.025	
Ots-212	183		0.015		In-river
Ots-212	191	0.005	0.015		
Ots-212	207			0.008	Captive Brood
Ots-212	231	0.005			Supplementation
# of samples		93	33	200	