

AN ABSTRACT OF THE THESIS OF

Kent Corey Mayer for the degree of Master of Science in Fisheries Science presented on  
April 16, 2002. Title: Spawning Chinook Salmon (*Oncorhynchus tshawytscha*) Two Years  
In A Row: Reconditioning For Repeated Gamete Collection.

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ABSTRACT: Spring chinook salmon, *Oncorhynchus tshawytscha*, in the Snake River Basin are listed as endangered under the Endangered Species Act. The typical life history of spring chinook salmon is semelparous. An experiment was performed to see if mature male spring chinook salmon parr could be reconditioned after hand-spawning and spawned in successive years. The mature male parr were part of a 1997 and 1998 cohort of captive broodstock from the Grande Ronde River Basin in Oregon. Force-feeding was combined with volitional feeding in an attempt to inhibit senescence and increase survival time after spawning. The viscerosomatic index, fat and protein contents increased with survival time after spawning for both cohorts. There was a significant relationship between percent weight change and survival time for the 1997 cohort ( $p < 0.001$ ). Force-feeding had no effect on survival time after spawning for the 1997 cohort ( $p = 0.074$ ) or the 1998 cohort ( $p = 0.178$ ). Fat content, weight gain and survival time indicated that the reconditioning process was observable in the

1997 cohort at 77 days after spawning and was cyclical, resulting in a physiological response which allowed male chinook salmon to spawn in successive years. Fertilization trials with three reconditioned males yielded a fertilization success rate of 96.9% compared to 95.7% for 4-year old, anadromous male spring chinook salmon ( $p = 0.152$ ), measured as production of eyed-embryos. This experiment provides new knowledge of plasticity in the reproductive biology of male, stream-type, spring chinook salmon. Reconditioning and spawning male chinook salmon parr in successive years could be used to help maximize genetic diversity and aid in the recovery of endangered *Oncorhynchus* populations.

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Spawning Chinook Salmon (*Oncorhynchus tshawytscha*) Two Years In A Row:  
Reconditioning For Repeated Gamete Collection

by

Kent Corey Mayer

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APPROVED:

  
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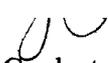
  
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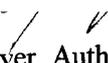
  
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Spawning Chinook Salmon (*Oncorhynchus tshawytscha*) Two Years In A Row:  
Reconditioning For Repeated Gamete Collection

ABSTRACT

Spring chinook salmon, *Oncorhynchus tshawytscha*, in the Snake River Basin are listed as endangered under the Endangered Species Act. The typical life history of spring chinook salmon is semelparous. An experiment was performed to see if mature male spring chinook salmon parr could be reconditioned after hand-spawning and spawned in successive years. The mature male parr were part of a 1997 and 1998 cohort of captive broodstock from the Grande Ronde River Basin in Oregon. Force-feeding was combined with volitional feeding in an attempt to inhibit senescence and increase survival time after spawning. The viscerosomatic index, fat and protein contents increased with survival time after spawning for both cohorts. There was a significant relationship between percent weight change and survival time for the 1997 cohort ( $p < 0.001$ ). Force-feeding had no effect on survival time after spawning for the 1997 cohort ( $p = 0.074$ ) or the 1998 cohort ( $p = 0.178$ ). Fat content, weight gain and survival time indicated that the reconditioning process was observable in the 1997 cohort at 77 days after spawning and was cyclical, resulting in a physiological response which allowed male chinook salmon to spawn in successive years. Fertilization trials with three reconditioned males yielded a fertilization success rate of 96.9% compared to 95.7% for 4-year old, anadromous male spring chinook salmon ( $p = 0.152$ ), measured as production of eyed-embryos. This experiment provides new knowledge of plasticity in the reproductive biology of male, stream-type, spring chinook salmon. Reconditioning and spawning male chinook salmon parr in successive years could be used to help maximize genetic diversity and aid in the recovery of endangered *Oncorhynchus* populations.

## INTRODUCTION

In the Snake River, a tributary of the Columbia River, spring chinook salmon, *Oncorhynchus tshawytscha*, are listed as endangered under the Endangered Species Act. Their numbers have declined over 99%, from an estimated 1.5 million in the late 1800's (Matthews and Waples 1991) to an average abundance of 3,820 natural spawners in the 1990's (National Marine Fisheries Service (NMFS) 2000). The current annual population of natural spawners in the Grande Ronde River and its six sub-basins is estimated at less than 1,000 fish (J.M. Myers, NMFS, Seattle, pers. comm.). The major factors for their decline include habitat degradation and increased mortality of juveniles during passage through mainstem reservoirs and dams, and blockage of spawning and rearing habitat (NMFS 2000).

Chinook salmon have two behavioral life-history forms (Gilbert 1913), designated "stream-type" and "ocean-type." Ocean-type fish migrate to the sea in their first year of life. Stream-type juveniles rear for a year or more in fresh water before migrating to the sea. The extended juvenile fresh water residency of stream-type salmon may be responsible for the mature male parr in the population, which is considered an alternate life history strategy (Gross 1987). As adults, male spring chinook salmon from the Snake River Basin typically enter fresh water to begin their spawning migration in the spring as 4-year olds (Myers et al. 1998), several months prior to spawning in the fall (Healey 1991).

The typical life history of spring chinook salmon is semelparous, i.e., they spawn once and die (Healey 1991). The rapidity and timing of senescence in chinook salmon after spawning may be an example of a genetically programmed death (Dickhoff 1989). However, studies of hatchery-reared fish by Robertson (1957), Bernier et al. (1993) and Unwin et al. (1999) suggest that it may be possible for some precocious male chinook salmon, known as

mature male parr, to spawn more than once. Robertson (1957) studied mature male parr in fresh water that produced milt at 14 months post-fertilization. After 5 months, autopsy revealed renewed spermatogenesis in the testes. Bernier et al. (1993) studied mature male parr that produced milt in March at 18 months post-fertilization. After transfer to seawater in June, 35% of the mature male parr died and 68% of the surviving fish produced milt again in mid-September in the same year. Unwin et al. (1999) studied fall-run mature male chinook salmon parr in fresh water that produced milt at 12 months post-fertilization. Almost 80% survived to produce milt again at age 2. However, there was degradation in the gut wall of the mature male parr, which grew slower and appeared thinner than immature parr from the same cohort. Ten fish were reared for another year and matured for a third time at age 3.

Endogenous and exogenous factors are important during significant life history changes, such as energy storage, growth and sexual maturation (Shearer 1994). Endogenous factors include size, sex and life history stage. Exogenous factors include environment (such as temperature, photoperiod and salinity) and diet (such as feed type, frequency and ration).

Johnston et al. (1987) suggest that endogenous rhythms determine the length of the refractory period following spawning in Atlantic salmon, *Salmo salar*. Johnston et al. (1987) also suggest the feeding rhythm is somewhat independent of exogenous factors, but how much or how easily environmental cues can be overridden by endogenous rhythms is unknown.

The decrease in food consumption associated with spawning (Johnston et al. 1990) results in atrophy of the gut (Unwin et al. 1999), depleted energy stores and a weakened condition (Dickhoff 1989). Force-feeding after spawning may reverse degradation of the gastro-intestinal tract in fasted and spawned salmon. McBride et al. (1963) found that force-fed sockeye salmon, *O. nerka*, resumed volitional feeding over a 4-month period after spawning. Johnston et al. (1987) also found that force-fed Atlantic salmon resumed volitional

feeding over a 4-month period after spawning. Force-feeding appeared to stimulate a feeding response in different fish at different times during the 4-month resumption periods.

Proximate analysis can be used to measure the general categories of compounds (i.e., fat, moisture, protein and ash) in animals (Hurwitz and Plavnik 1986) and is routinely used in experimental feeding trials (Shearer 1994). Proximate analysis can also be used to assess how force-feeding affects survival by determining how food is partitioned in the body during senescence and death or reconditioning after spawning.

Since mature male parr have an alternate life history to anadromous males, they may possess some physiological difference which allows them to spawn in successive years. If force-feeding mature male parr can be used as part of a reconditioning program to resume feeding and recovery after spawning (Boyer et al. 1993), then rematuration and repeated spawning may be possible (Crim et al. 1992). Reconditioning could be used as an alternative, or complimentary, method to increase the gamete pool available for breeding in any given year and aid in the recovery of selected *Oncorhynchus* populations.

There are few published studies designed to identify the variables that increase the potential for repeat sexual maturation of spring chinook salmon or the factors affecting post-spawning survival of mature male parr. This experiment was designed to determine if there is plasticity in the reproductive biology of spring chinook salmon by hand-spawning the same fish in successive years and document iteroparity in *Oncorhynchus tshawytscha*. This experiment was also designed to help define "reconditioning" after spawning.

The first objective of this experiment was to determine how force-feeding affects survival in male chinook salmon parr after spawning, while considering the river of origin, freshwater, accelerated or saltwater rearing treatments, days starved, spawning weight and tank assignment. These factors were associated with the artificial propagation of spring

chinook salmon from the Grande Ronde River Basin, under the Oregon Department of Fish and Wildlife (ODFW) Captive Broodstock program (ODFW 1995). The second objective was to investigate the relationship between viscerosomatic index, proximate composition (fat, moisture, protein and ash content) and weight change after spawning, and the explanatory variables of survival time and treatment. The final objective was to determine the efficacy of sperm from age 2+ reconditioned mature male chinook salmon spawned in successive years by analyzing the production of eyed-embryos between reconditioned male spring chinook salmon and 4 year-old, anadromous male spring chinook salmon.

## MATERIALS AND METHODS

### POST-SPAWNING SURVIVAL

The post-spawning survival experiment used mature male, stream-type, spring chinook salmon parr from the ODFW Captive Broodstock program for the Grande Ronde River Basin. As part of the captive broodstock protocols (ODFW 1995), 0-age spring chinook salmon were randomly collected in August of each year (approximately 11-months post-fertilization) using a passive seining technique, i.e., snorkeling and seining, from three rivers: the Grande Ronde River, the Lostine River and Catherine Creek in northeastern Oregon (Figure 1).

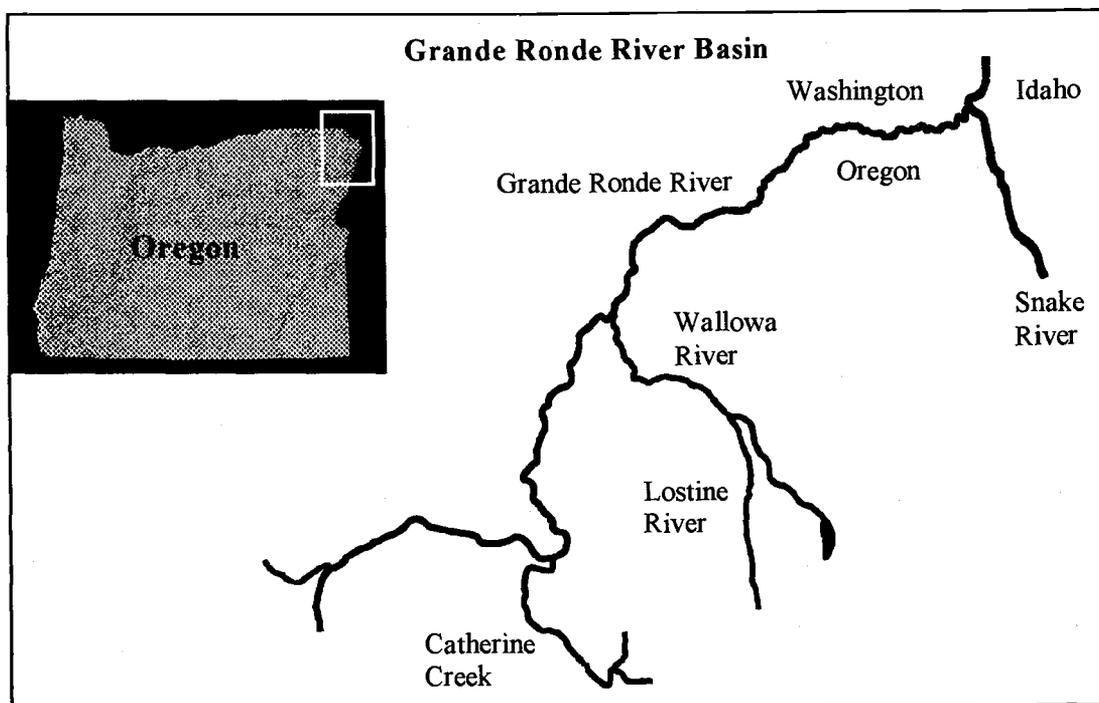


Figure 1. Map of the Grande Ronde Basin, showing the Grande Ronde River, Lostine River and Catherine Creek.

After capture, fish were reared for six months at the Lookingglass Fish Hatchery in Elgin, Oregon. Approximately 30% of the fish were placed in an "accelerated" rearing program, designed to maximize growth by controlling water temperature and feeding to satiation. Upon smolting, approximately 70% of the fish were transferred in February to the Captive Broodstock facility at the Bonneville Fish Hatchery (BFH) in Cascade Locks, Oregon. Approximately 30% of the fish were transferred to a saltwater rearing environment at the Manchester Marine Laboratory (MML) in Port Orchard, Washington. Fish that developed signs of maturation at MML were transferred in July to BFH for spawning. The 1997 cohort had all three rearing treatments (i.e., fresh water, accelerated and saltwater) and the 1998 cohort had two rearing treatments (i.e., the accelerated and saltwater).

The ODFW Captive Broodstock program performed maturity sorts (ripeness checks) by visual observation between March and September (ODFW 1995). Mature/maturing fish were placed in separate tanks from the non-maturing fish and taken off feed. Upon spawning, mature fish were anesthetized, hand-spawned (i.e., stripped by hand) and then killed.

For the 1997 cohort, the mature parr were not fed for 78 days prior to spawning. Thirty-eight mature parr were hand-spawned on October 20, 1999. After spawning, fish were alternately divided into three groups: a force-fed treatment group, a sham-fed group and a control (i.e., volitional feeding only) group, with 13, 13 and 12 fish, respectively.

For the 1998 cohort, the mature parr were not fed from between 50 to 90 days prior to spawning. One-hundred and four mature parr were hand-spawned in September and October of 2000. After spawning, fish were alternately divided into two groups: a force-fed treatment group and a sham-fed group. Ten incidents (i.e., over-anesthetization, torn esophaguses) during the 120-day experiment resulted in a final population of 94 fish.

At spawning, fish from both cohorts had an average weight of 98 grams (45 to 181 grams) and an average fork length of 190 mm (171 to 240 mm). All fish had previously been marked with passive integrated transponder (PIT) and visual implant (VI) tags.

The post-spawning survival (i.e., reconditioning) experiment consisted of three steps. The first step was to establish a baseline by weighing each fish at spawning. The second step was to force-feed the treatment group with a high-protein formula. The sham-fed group was fed a 0.65% physiological saline solution (i.e., distilled water and non-iodized salt) to minimize osmoregulatory stress. Force-feeding and sham-feeding were done twice a week for 120 days or until all fish resumed volitional feeding. Fish were volitionally fed on non-force-feeding (i.e., non-treatment) days. The third step, at the end of the 120-day treatment period or upon death, was to characterize fish health by measuring survival time, weight change, viscerosomatic index and proximate composition to determine the effects of treatment.

Fish were kept in 6,435 liter, ten-foot diameter fiberglass tanks in well water at 9-12°C, with a flow rate of 216–295 liters per minute and a dissolved oxygen concentration of 10–12 ppm. The fish were under natural photoperiod. To minimize the risk of bacterial kidney disease (BKD), both cohorts were given erythromycin via food (in a 28-day feeding regime with erythromycin-medicated feed) and by injection (at 20-40 mg/kg) during rearing. An injection of erythromycin mixed with oxytetracycline was given after spawning. Formalin, a formaldehyde solution, was administered both prophylactically and in response to the presence of *Saprolegnia* fungus, at a concentration of 1:4000 or 1:6000 (i.e., 0.045 or 0.09%) in one-hour flow-through bath treatments twice a week. Argentyne, an iodine solution, was used to decontaminate equipment prior to all experimental procedures.

All food used in this experiment was Moore-Clark's Nutra Fry-Freshwater feed, with a composition of 48% protein, 24% fat, 8% moisture, 7% nitrogen-free extracts, 7% ash and

2% fiber. Ration size was 1.5 cc, based on the volumetric gut size of spawned mature male parr from preliminary research (unpublished data). Gut evacuation was also checked by sampling 24 hours after force-feeding to verify that food had cleared the digestive system (Adams and Breck 1990). The sham-fed group was given 1.5 cc of physiological saline.

The force-feeding techniques used by McBride (1963) were modified for this experiment. Force-feeding was done by injecting food through a syringe fitted with a piece of flexible 2.0 mm diameter surgical tubing on one end. Food was powdered and mixed with physiological saline until it was capable of passing through the tubing, which was inserted by hand into the stomach via the esophagus, and the food discharged from the syringe into the foregut. To reduce irritation, the tubing was coated with glycerin prior to force-feeding.

Prior to force- and sham-feeding, fish were anesthetized with tricaine methane-sulfonate (MS-222). A 60–75 mg/l concentration of MS-222 was used to induce a stage 4 anesthesia, i.e., a total loss of equilibrium and spinal reflexes (Summerfelt and Smith 1990), necessary for force-feeding. To reduce reaction to MS-222, precautions for the use of anesthetics were followed (Bell 1987). The MS-222 was thoroughly mixed in water, aerated, and buffered with 150 mg/L sodium bicarbonate (H. Li, Oregon State University, pers. comm.). A synthetic slime coating was used to prevent mucousal and tissue damage during handling. The total time necessary for force-feeding was approximately eight minutes, including two minutes for induction of anesthesia, ten-seconds for force-feeding and five minutes for recovery.

All fish were volitionally fed pellets on non-treatment days. One concern about volitional feeding was the ability of the fish to swallow food due to generalized atrophy from starvation and spawning. Therefore, pellet size was 1.5 mm, based on esophagus diameter of previously spawned mature male parr from preliminary research (unpublished data) and was

increased by  $\frac{1}{2}$ -mm as esophagus diameter increased. Ration size for volitional feeding was 2.0% of body weight per day, dispensed by 12-hour automatic feeders. Small pieces of beef liver were used once a week as an appetite stimulant prior to volitional feeding.

At the end of the 120-day experiment, surviving fish were killed with a lethal dose of anesthetic. Upon death, carcasses were placed in air-tight bags with air and water removed. The date, VI tag number and weight were written on the outside of the bag in waterproof ink and refrigerated. Within five days of refrigeration, a viscerosomatic index was determined. A viscerosomatic index was used to measure gross nutritional state of the fish (Jensen 1980) and calculated as: gut weight/body weight x 100. The viscerosomatic index was determined by removing, cleaning and weighing the gut (i.e., gastro-intestinal tract and cecum) and then comparing the weight of the gut to the weight of the whole fish (Bowen 1996). The gut was then put back in the carcass and PIT tags removed prior to proximate analysis.

## PROXIMATE ANALYSIS

Whole fish proximate analysis was performed under the supervision of Dr. Karl Shearer of the National Marine Fisheries Service in Seattle, Washington. Moisture content was determined by oven-drying diced carcasses at 105°C until a constant weight was achieved (approximately 24 hours). The weight of the dried sample was compared to its original weight (wet basis). Dried fish carcasses were preserved under vacuum and stored for 1-6 months at -84°C to minimize changes in composition prior to proximate analysis. To analyze fat, protein and ash, the dried carcasses were ground in a coffee grinder. Fat content was determined by super-critical fluid extraction. Fat was extracted with CO<sub>2</sub> in a LECO® FA-100<sup>mm</sup> fat analyzer and compared to its original sample weight. Protein content was determined using combustion

to measure percent nitrogen by thermal conductivity in a LECO® FP-2000 Nitrogen/Protein determinator. Ash content was determined by ashing a sample in a muffle furnace at 550°C for 24 hours and comparing the weight of the ash to its original, pre-ashed weight.

Multiple linear regression (Ramsey and Schafer 1997) was used on the post-spawning survival data to examine the relationship between survival time and the river of origin, fresh water, accelerated or saltwater rearing treatments, days starved, spawning weight and tank assignment. Survival analysis (Collett 1994) was used to evaluate survival trends with respect to treatment. Multivariate regression (Johnson 1998) was used to investigate the relationship between survival time and viscerosomatic index, proximate composition and weight change.

## FERTILIZATION TRIALS

Of the 38 mature male chinook salmon parr from the 1997 cohort that were spawned in October 1999, three were fully reconditioned 355 days later when they were spawned the following year on October 9, 2000. All three fish originated from the Grande Ronde River. The gametes from these fish were used for fertilization trials, after which the fish were volitionally fed under the same holding conditions for 120 days or until death.

The sperm stripped from the reconditioned males was placed in air-tight bags. The bags were filled with air and placed in a cooler with ice and transported from BFH to the ODFW Clackamas Fish Hatchery in Clackamas, Oregon. To assure consistency in handling procedures between the two male types, the 4-year old, anadromous males followed the same procedures of stripping, bagging, icing, containerization and holding time.

Sperm from the three, age 2+, reconditioned mature males and three, 4-year old, anadromous, male spring chinook salmon were used to fertilize eggs from three, 5-year old,

anadromous female spring chinook salmon. Eggs from the same three females were crossed and fertilized by the reconditioned males and the anadromous males as shown in Table 1.

Three replicates were used for each cross, resulting in approximately 240 eggs per cross.

A total of 4,248 fertilized eggs from the three females were used in the fertilization trials.

Table 1. Setup for the fertilization trial crosses between the reconditioned males from the 1997 cohort and the 4-year old, anadromous males. Reconditioned males 1 and 2 were from the force-fed group. Reconditioned male 3 was from the control group.

Reconditioned males (rm)	Female (f) number			Anadromous males (am)	Female (f) number		
	1	2	3		1	2	3
1	f1/rm1	f2/rm1	f3/rm1	1	f1/am1	f2/am1	f3/am1
2	f1/rm2	f2/rm2	f3/rm2	2	f1/am2	f2/am2	f3/am2
3	f1/rm3	f2/rm3	f3/rm3	3	f1/am3	f2/am3	f3/am3

After mixing, the fertilized eggs were measured volumetrically using a 1-ounce measuring cup, which held an average of 80 eggs, and placed into one of 54 isolets (crosses plus replicates). However, no tray contained fertilized eggs from more than half of any one male type. Isolets were randomly distributed in six incubation trays with a continuous flow of well water at 10–12°C and a dissolved oxygen concentration of 10–12 ppm.

Observations were made at the eyed-embryo stage, i.e., an eyed-embryo with visible eyes and orange color, approximately 27 days after fertilization. The percent of eyed-embryos successfully produced was determined for the reconditioned males and the anadromous males was evaluated using analysis of variance.

## RESULTS

### POST-SPAWNING SURVIVAL

Three out of thirty-eight (7.9%) of the mature male parr from the 1997 cohort survived to spawn two years in a row. The time of the second spawning in October of 2000 (i.e., one year after 1<sup>st</sup> spawning) was normal for this population of spring chinook salmon.

There was a significant difference in survival time between males that died after first spawning and males that spawned in successive years (multiple linear regression,  $p < 0.001$ ). The mean survival time for males that died after first spawning was 15.6 days (95% CI from 4.5 to 26.7 days) and the mean survival time for males that spawned two years in a row was 441.7 days (95% CI from 323.0 to 560.1 days). A listing of potential differences between males from the 1997 cohort that died after first spawning and males from the 1997 cohort that spawned two years in a row are summarized in Table 2.

Table 3 summarizes the explanatory variables and associated p-values from the multiple linear regression used to analyze the post-spawning survival data for the 1997 cohort. The final multiple linear regression equation incorporating the treatment effect was:

$$\begin{aligned} \log(\text{Days survived}) = & 4.55 + 0.27\text{Force} - 0.77\text{Sham} - 1.77\text{Tank1} - 1.32\text{Tank3} \\ (\text{Std. error}) & \quad (\pm 0.47) \quad (\pm 0.41) \quad (\pm 0.42) \quad (\pm 0.42) \quad (\pm 0.42) \\ & - 1.76\text{Freshwater} - 1.08\text{Accelerated} \\ & \quad (\pm 0.45) \quad (\pm 0.43) \end{aligned}$$

Table 2. Comparison of the differences at the beginning of the experiment between the males that died after first spawning and males that spawned two years in a row for the 1997 cohort. Weights and lengths are averages. P-values from rank-sum test.

Spawning history	Sample size (n)	Weight (gr.)	Length (mm)	Tank (T) No.	Experimental treatment	ODFW Rearing treatment
Spawned once and died	35	99.7	199.7	13 T1	11 Force-fed	12 Natural
				10 T2	12 Sham-fed	15 Accelerated
				12 T3	12 Control	8 Saltwater
Spawned two years in a row	3	119.4	205.0	0 T1	2 Force-fed	0 Natural
				3 T2	0 Sham-fed	1 Accelerated
				0 T3	1 Control	2 Saltwater
P-value	-	0.090	0.452	-	-	-

Table 3. Explanatory variables and p-values (from multiple linear regression) for the post-spawning survival data for the 1997 cohort. The reference for the rearing variable was the saltwater treatment. The reference for the tank variable was tank No. 2. The reference for treatment variable was the control group.

1997 Variables	River of origin	Freshwater rearing	Accelerated rearing	Spawn weight	Tank 1	Tank 3	Force-fed	Sham-fed
P-value	0.468	0.001	0.022	0.114	0.249	0.005	0.756	0.087

For the 1997 cohort, there was no significant difference in survival time between treatments (multiple linear regression,  $p = 0.074$ , adjusted  $r^2 = 0.49\%$ ) (Figure 2). Survival time was influenced by the pre-spawning holding environment, e.g., the saltwater rearing treatment (Figure 3). Mean survival time for the saltwater group was 116.3 days (95% CI from 0 to 244.2 days) and was significantly greater (multiple linear regression,  $p = 0.002$ ) than the mean survival time of the fresh water group (7.7 days 95% CI from 4.1 to 11.3 days) and the accelerated group (38.5 days; 95% CI from 0 to 97.9 days). Ten male spring chinook salmon parr matured in the saltwater rearing environment.



There was evidence of a significant relationship between survival time and tank assignment for the 1997 cohort (multiple linear regression,  $p < 0.001$ ). Survival time was negatively related to tank assignment due to the presence of fungus in two of the three holding tanks prior to the beginning of the experiment. There was no evidence of significant interactions between main effects, i.e., treatment, river of origin, rearing treatment, spawning weight or tank assignment.

All three fish that spawned in consecutive years were from the Grande Ronde River (Figure 4) and had a mean survival time of 51.9 days (95% CI from 7.4 to 127.4 days), compared to the mean survival time of the fish from Catherine Creek of 26.5 days (95% CI from 0 to 81.4 days). The difference in survival time was not significant (multiple linear regression,  $p = 0.468$ ) for the 1997 cohort.

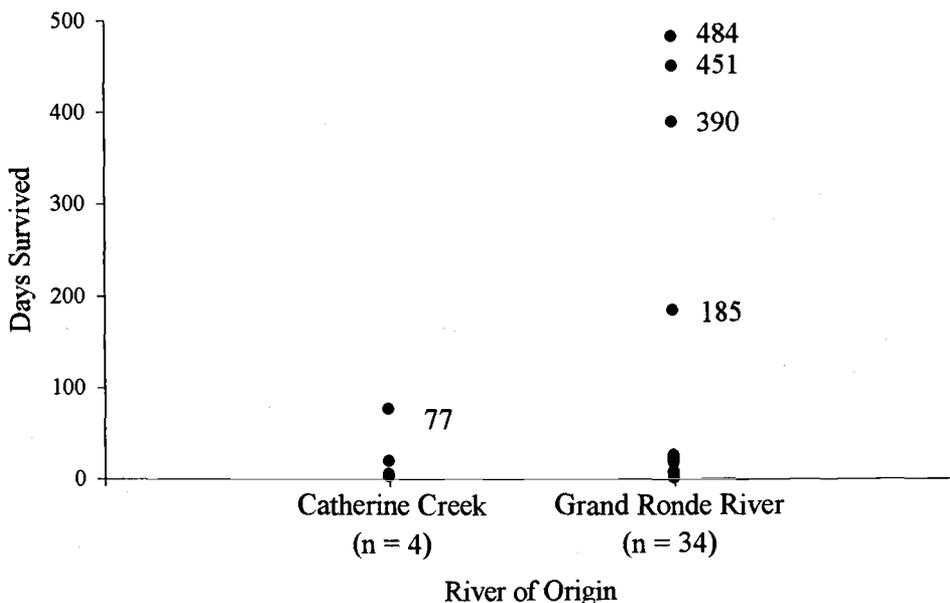


Figure 4. Number of days survived by mature male parr from the Grande Ronde River and Catherine Creek after first spawning for the 1997 cohort.

For the 1998 cohort, Table 4 summarizes the explanatory variables and associated p-values (from multiple linear regression) used to analyze the post-spawning survival data.

The final multiple linear regression equation incorporating the treatment effect was:

$$\text{Days survived} = 117.0 + 7.69\text{Force} - 0.98\text{DaysStarved} + 1.12\text{Catherine} + 35.0\text{Grande}$$

(Std. error)      ( $\pm 26.27$ )    ( $\pm 5.90$ )            ( $\pm 0.37$ )            ( $\pm 7.95$ )            ( $\pm 34.96$ )

Table 4. Explanatory variables and p-values (from multiple linear regression) for the post-spawning survival data for the 1998 cohort. The reference for the river variable was the Grande Ronde River.

1998 Variables	River of origin: Catherine Creek	River of origin: Lostine River	Rearing treatment	Days starved	Spawn weight	Treatment effect
P-value	0.004	0.001	0.930	0.035	0.098	0.162

For the 1998 cohort, there was no significant difference in survival time between treatments (multiple linear regression;  $p = 0.178$ ; adjusted  $r^2 = 0.21\%$ ) (Figure 5). The mean survival time for the force-fed treatment group was 53.7 days (95% CI from 43.8 to 63.6 days) and the mean survival time for the sham-fed group was 62.1 days (95% CI from 53.3 to 70.9 days). A Kaplan-Meier survival curve for the two groups, after accounting for the effect of river of origin, is presented in Figure 6. There was no significant difference between survival curves for the force-fed treatment and the sham-fed treatment groups (Chi square,  $p = 0.448$ ).

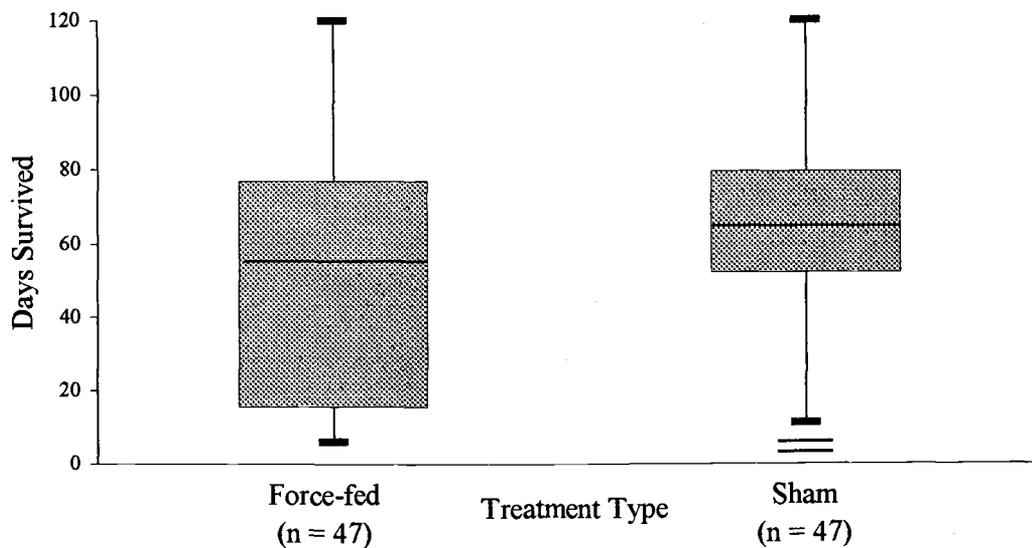


Figure 5. Treatment versus survival time for the 1998 Cohort. The shaded box represents the middle 50% of the data. The vertical lines represent the upper and lower 25% of the data. The line within the box represents the median. The separated lines (“-”) are outliers.

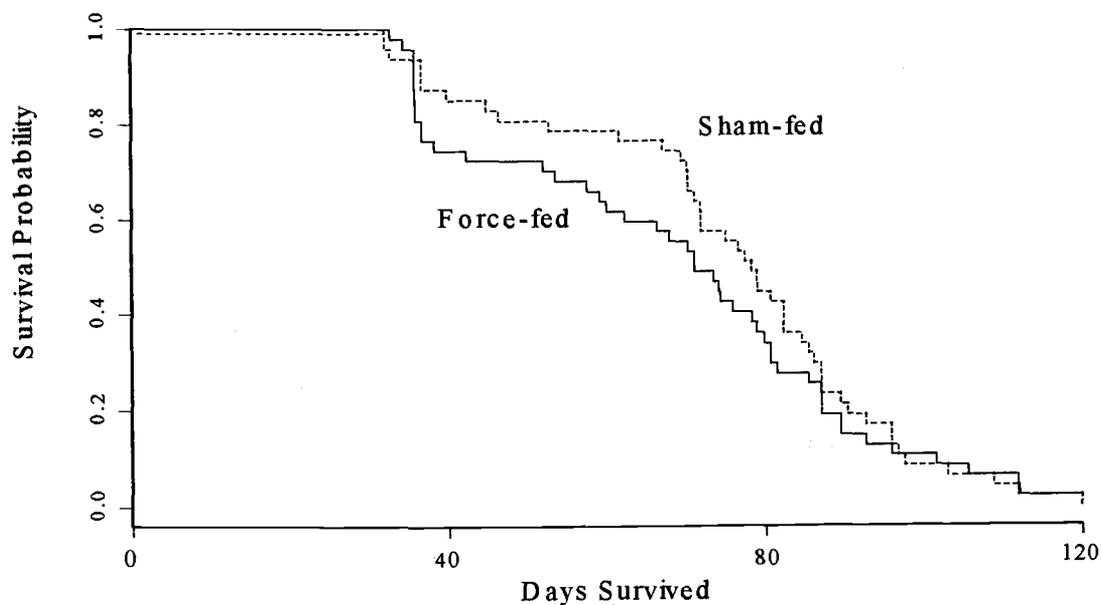


Figure 6. Kaplan-Meier survival curve for the 1998 Cohort. Two fish (one force-fed and one sham-fed) survived to the end of the 120-day experiment.  $P = 0.448$  for curve differences.

The mean survival time for fish from the Grande Ronde River was 93.7 days (95% CI from 85.9 to 108.8 days) and was significantly greater (multiple linear regression,  $p = 0.002$ ) than the mean survival time of fish from the Lostine River (52.2 days; 95% CI from 44.8 to 59.7 days) and Catherine Creek (59.2 days; 95% CI from 43.7 to 74.7 days) (Figure 7).

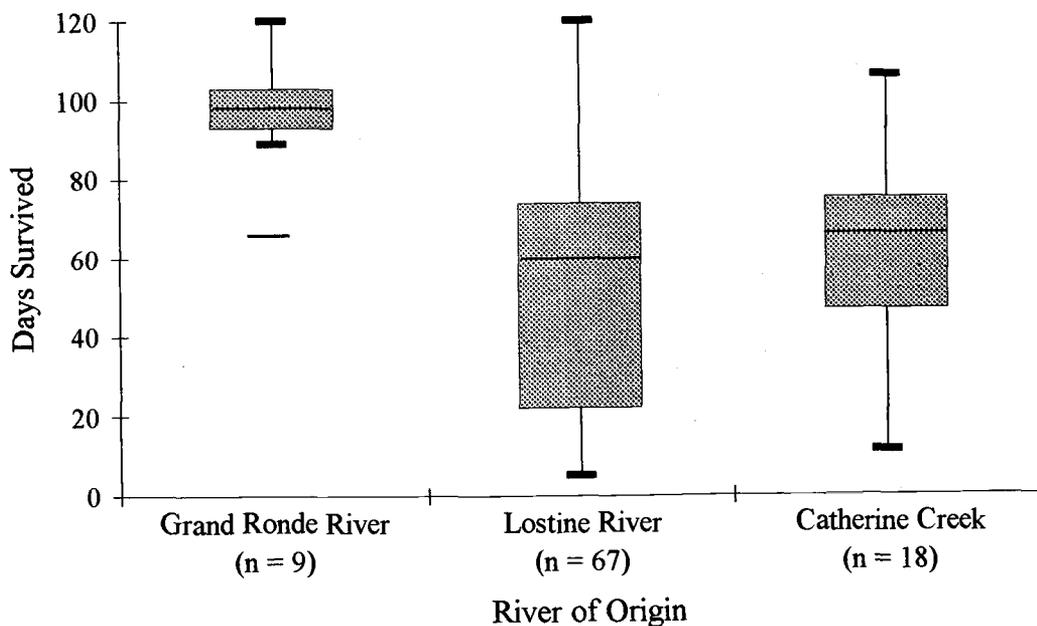


Figure 7. Number of days survived after spawning by mature male parr from the Grande Ronde River, the Lostine River and Catherine Creek for 1998 cohort. The shaded box represents the middle 50% of the data. The vertical lines represent the upper and lower 25% of the data. The line within the box represents the median. The separated line (“-”) represents an outlier.

For the 1998 cohort, there was a significant relationship between survival time after spawning and days starved prior to spawning (multiple linear regression,  $p = 0.009$ ). There was no evidence of significant interactions between main effects, i.e., treatment, river of origin, rearing treatment, days starved or spawning weight.

In the 1998 cohort, bacterial kidney disease infected all fish that lived longer than 56 days ( $n = 53$ ), i.e., the longest-lived fish. The confirmed BKD mortality rate from enzyme-linked immunosorbent assay (ELISA) tests was 71%. The first 29% died before signs of BKD were apparent upon autopsy and were not tested for BKD. There was no evidence of a relationship between ELISA levels and survival time after spawning (simple linear regression,  $p = 0.992$ ). There was evidence of a significant relationship between ELISA levels and protein content (multiple linear regression,  $p = 0.039$ ), i.e., the higher the BKD level, the lower the protein content.

#### PROXIMATE ANALYSIS

For the 1997 cohort, viscerosomatic index appeared to change with survival time in an annual, cyclical pattern after spawning. The number of fish that survived at least 77 days was small ( $n = 5$ ). Viscerosomatic index appeared to reach a maximum at 77 days after first spawning and then declined with the onset of maturation prior to second spawning (Figure 8). The mean viscerosomatic index for fish that lived at least 77 days was 2.03% (95% CI from 1.72% to 2.35%) compared to the mean viscerosomatic index of fish that lived less than 77 days of 1.06% (95% CI from 0.98% to 1.14%).

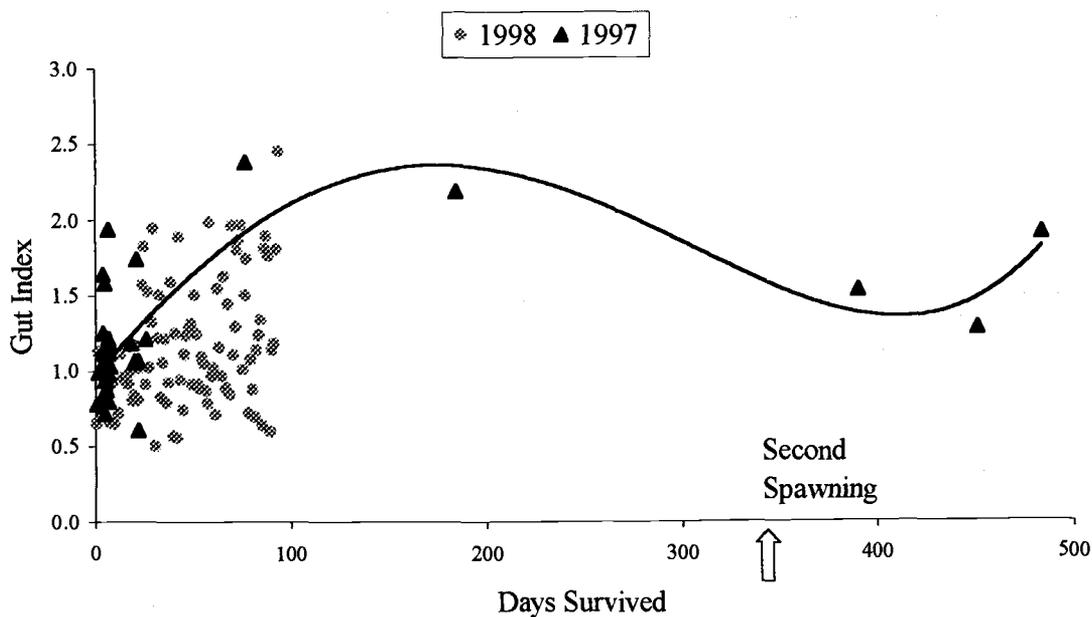


Figure 8. Relationship of viscerosomatic index versus survival time for the 1997 and 1998 cohorts ( $n = 38$  and  $94$ , respectively). The line suggests an annual cycle for the 1997 data.

Changes in proximate composition (fat, moisture, protein and ash content) were observable in the 1997 cohort at 77-days after spawning and appeared to change in an annual, cyclical pattern (Figures 9–12). Fat content increased with survival time from 1.6% at 77 days after first spawning to its highest level of 10.1% at 185 days (Figure 9). Fat content then decreased to 1.2% at 87 days after second spawning (451 days after first spawning) and increased to 8.9% at 120 days after second spawning (484 days after first spawning). Moisture content decreased from 83.1% at 77 days after first spawning to 74.0% at 185 days (Figure 10). Moisture content then increased to 85.2% at 87 days after second spawning (451 days after first spawning) and decreased to 72% at 120 days after second spawning (484 days after first spawning). Moisture content had a significant, inverse relationship with fat content (simple linear regression, 1997 cohort:  $p < 0.001$ ,  $r^2 = 0.85$ ; 1998 cohort:  $p < 0.001$ ,  $r^2 = 0.61$ ). Protein and ash contents remained relatively constant by comparison (Figures 11 and 12).

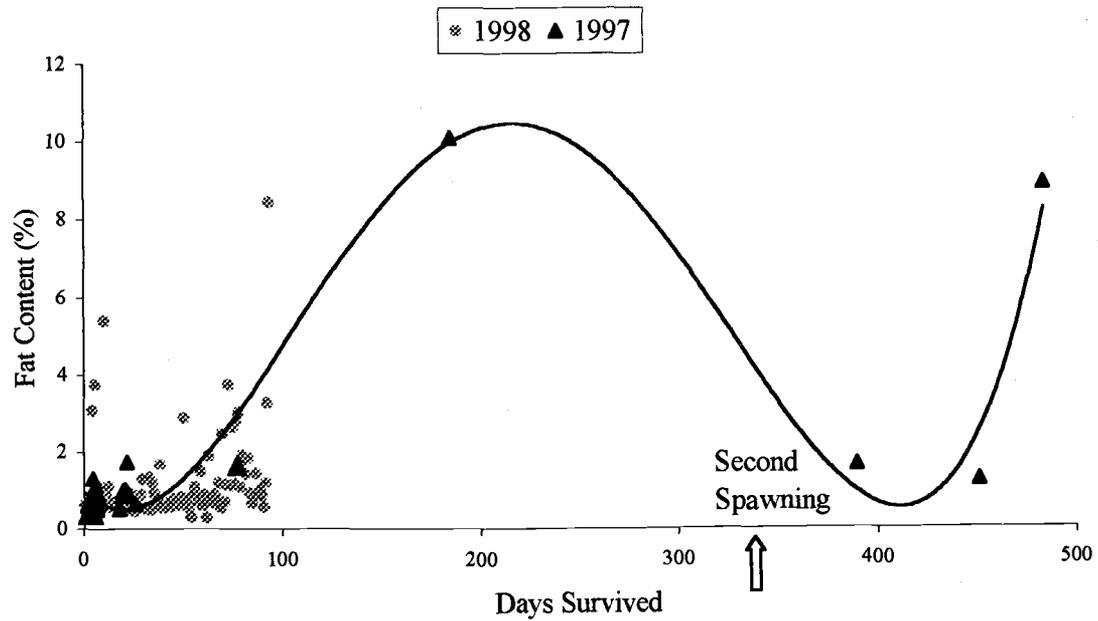


Figure 9. Fat content versus survival time for the 1997 and 1998 cohorts ( $n = 38$  and  $94$ , respectively). The line suggests an annual cycle for the 1997 data.

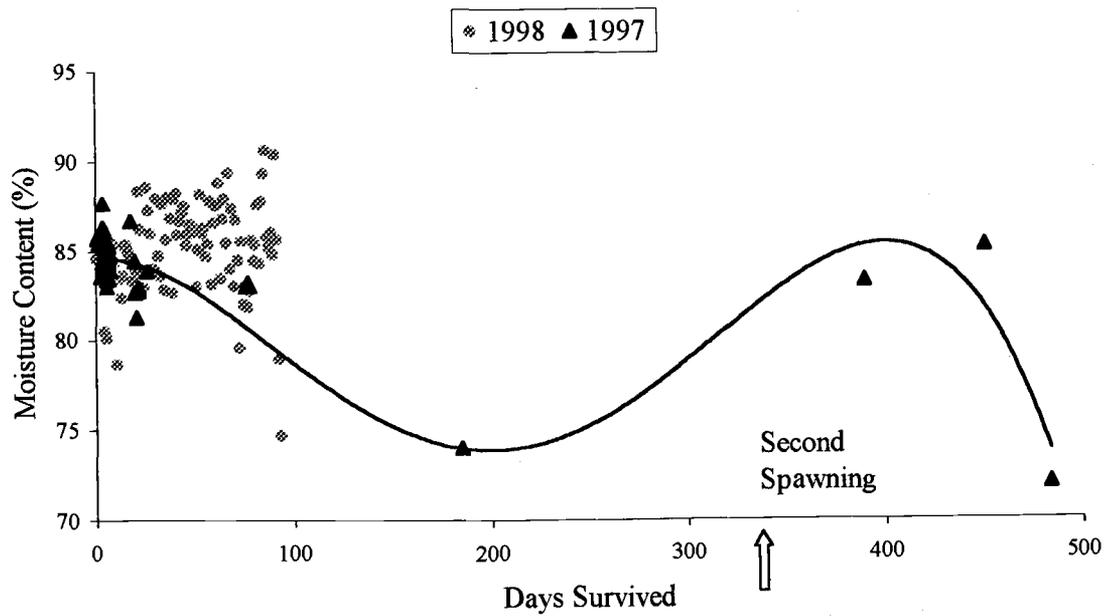


Figure 10. Moisture content versus survival time for the 1997 and 1998 cohorts ( $n = 38$  and  $94$ , respectively). The line suggests an annual cycle for the 1997 data.

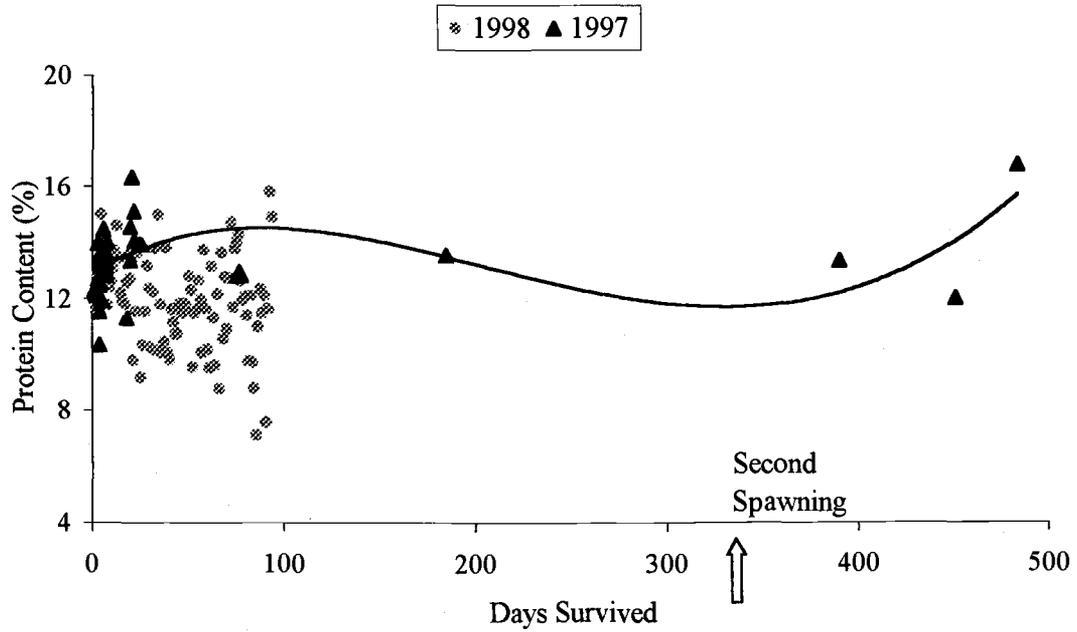


Figure 11. Protein content versus survival time for the 1997 and 1998 cohorts ( $n = 38$  and  $94$ , respectively). The line suggests an annual cycle for the 1997 data.

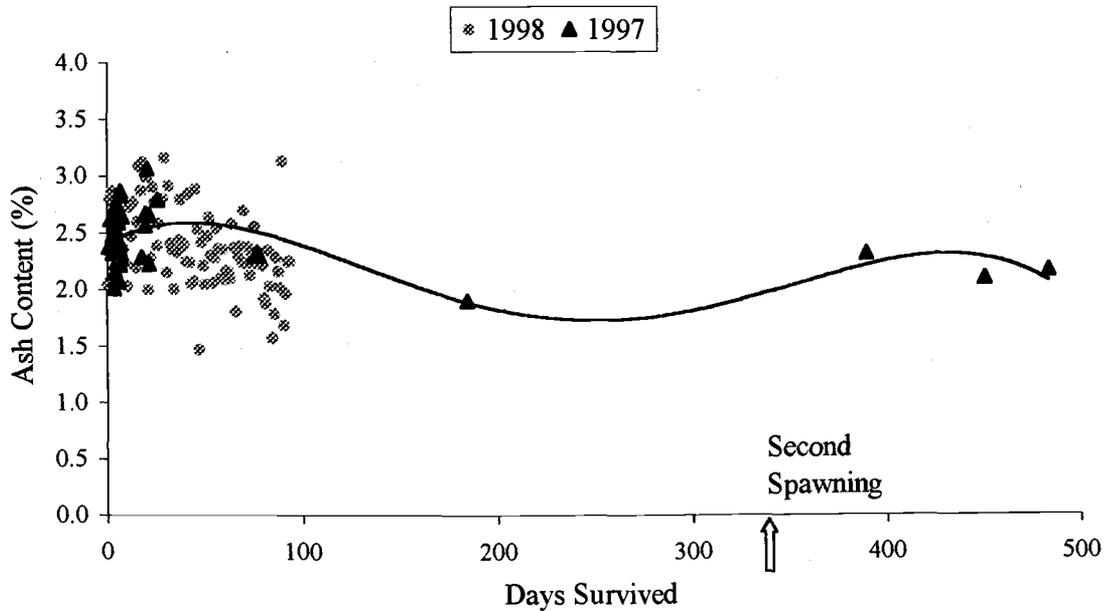


Figure 12. Ash content vs. survival time for 1997 and 1998 cohorts ( $n = 38$  and  $94$ , respectively). The line suggests an annual cycle for the 1997 data.

For the 1997 cohort, percent weight change increased from a negative value to a positive value, i.e., a weight gain, for all fish that lived at least 77 days after spawning (Figure 13). The fish that spawned two years in a row increased body weight by an average of 277% between the first and second spawnings (i.e., in October 1999 and 2000, respectively).

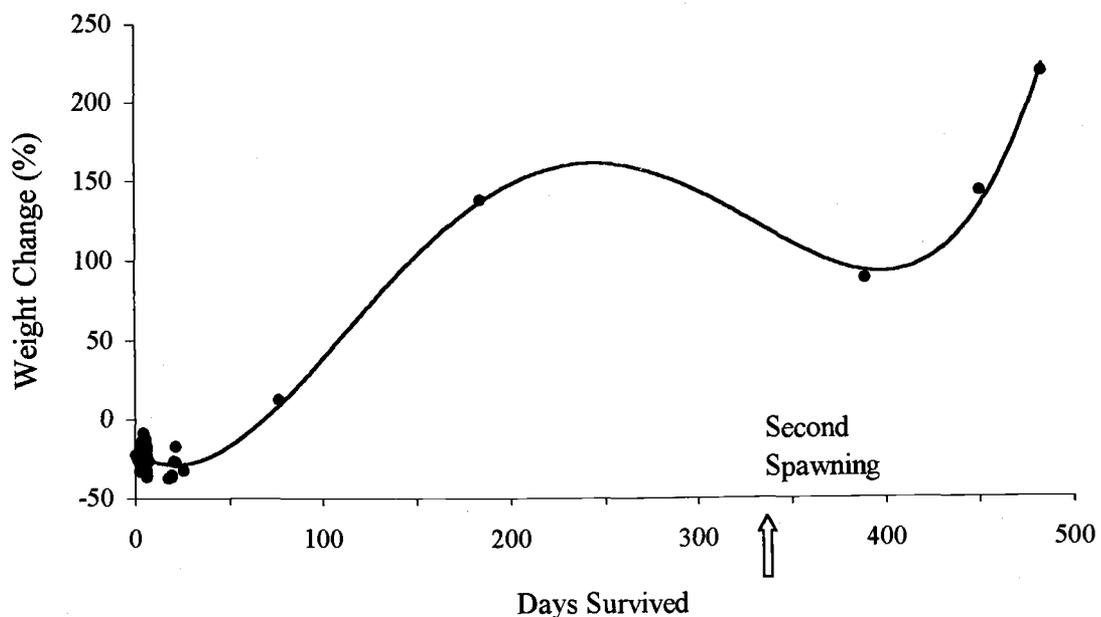


Figure 13. Percent weight change versus survival time for the 1997 Cohort ( $n = 38$ ).  $N = 33$  below 0% weight change, i.e., a weight loss. The line is for illustrative purposes only.

For the 1997 cohort, there was a significant relationship between percent weight change and viscerosomatic index (multivariate regression,  $p = 0.001$ ). In addition, there was a significant relationship between weight change and force-feeding treatment compared to the control (multivariate regression,  $p = 0.006$ ). Ash content was significantly affected by the sham treatment compared to the control (multivariate regression,  $p = 0.049$ ).

For the 1998 cohort, there was a significant relationship between the response measurements, i.e., viscerosomatic index and proximate composition and survival time (multivariate regression, p-values in Table 5). There was one influential case with a Cook's Distance of 7.5. The case was one of two fish that lived to the end of the 120-day experiment and was the only fish in the 1998 cohort that could be considered healthy based on fat content, e.g., 8.4%. Weight data was not analyzed for the 1998 cohort due to ascites from BKD.

Table 5. Summary of p-values for the relationship between the response measurements and survival time with the influential case included and excluded (n = 93) for the 1998 cohort.

Response	Viscerosomatic index	Fat content	Moisture content	Protein content	Ash content
Influential case included	<0.001	0.019	0.324	0.051	<0.001
Influential case excluded	0.001	0.131	0.041	0.016	<0.001

## FERTILIZATION TRIALS

There was no significant difference in the production of eyed-embryos between the reconditioned males (1997 cohort) and 4-year old, anadromous males (ANOVA,  $p = 0.152$ ), after accounting for female affects (Figure 14). The mean production of eyed-embryos from the reconditioned males was 96.9% (95% CI from 95.7% to 98.2%) and the mean production of eyed-embryos from the 4-year old, anadromous males was 95.7% (95% CI from 94.7% to 96.8%) (Table 6).

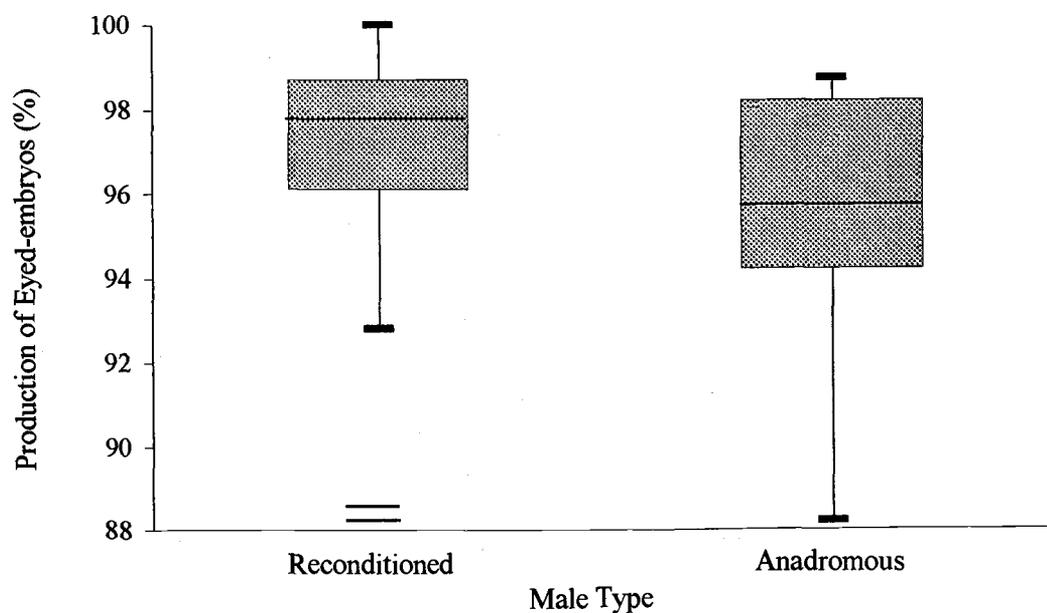


Figure 14. Mean production of eyed-embryos between reconditioned male spring chinook salmon (1997 Cohort) and 4-year-old, anadromous, male spring chinook salmon by male type ( $n = 27$  isolets per type). The shaded box represents the middle 50% of the data. The vertical lines represent the upper and lower 25% of the data. The line within the box represents the median. The separated lines (“-”) are outliers.

Table 6. Mean production of eyed-embryos for individual and male type for reconditioned spring chinook salmon males and 4-year old, anadromous, spring chinook salmon males.

Male type and number	Mean production of eyed-embryos (%)	95% CI (from - to)
Reconditioned No. 1	96.2	93.5 - 99.0
Reconditioned No. 2	97.1	95.5 - 98.7
Reconditioned No. 3	97.5	94.7 - 100.0
Reconditioned group value	96.9%	95.7 - 98.2
Anadromous No. 1	97.0	95.5 - 98.6
Anadromous No. 2	95.6	93.8 - 97.4
Anadromous No. 3	94.6	92.1 - 97.1
Anadromous group value	95.7%	94.7 - 96.8

## DISCUSSION

This is the first report of successful reconditioning and spawning in successive years by stream-type, spring chinook salmon. The reconditioned salmon matured a second time, 355 days after their first spawning as mature male parr. The three spring chinook salmon that spawned two years in a row can be considered "rare events of interest."

The results of this experiment were similar to those of McBride et al. (1965), who found that force-feeding was not a prerequisite for resumption of feeding for sockeye salmon (*Oncorhynchus nerka*). Although, McBride et al. (1965) suggest that force-feeding hastened the recovery of the fish, it also was associated with a marked increase in weight. In addition, McBride et al. (1965) stated that upon successful reconditioning, the reversible changes may have been more directly related to starvation than with sexual changes. Therefore, the increase in weight after spawning may be due more to survival time and fish-specific reconditioning rather than as a direct result of the force-feeding treatment.

The fertilization trials indicate that the sperm from reconditioned males is viable. The fertilization rate of 96.9% for the reconditioned stream-type male chinook salmon parr in this experiment are higher than the mean fertilization rate of 93.3% found by Unwin et al. (1999) for ocean-type mature male chinook salmon parr spawned in successive years. However, the 96.9% fertilization rate is similar to typically iteroparous species, such as Atlantic salmon and steelhead trout, *O. mykiss* (Fleming 1998). The fish from the 1997 cohort that spawned two years in a row represent a respawn rate of 7.9%, which may be comparable to the average respawn rate of 10-11% for Atlantic salmon and steelhead trout (Fleming 1998).

Endogenous and exogenous factors act simultaneously to influence body composition (fat, moisture, protein and ash) (Shearer 1994). However, a new factor may be added having both endogenous and exogenous elements, which influences both proximate composition and maturity: reconditioning after spawning (Figure 15).

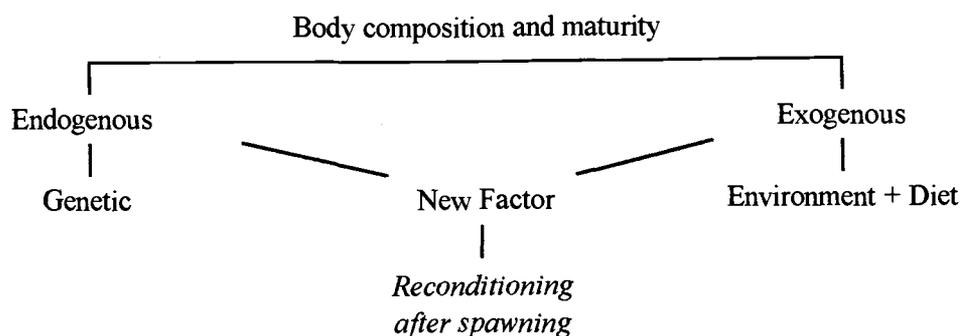


Figure 15. Factors affecting body composition and maturity (adapted from Shearer 1994).

Reconditioning refers to the physiological, morphological and energetic changes that allow mature male parr to spawn two years in a row, although no mechanism has been identified to explain rematuration (Unwin et al. 1999). In this experiment, reconditioning was indicated by a change in proximate composition to a healthier condition after spawning. Fat content, weight gain and survival time indicated that the reconditioning process was observable in the 1997 cohort at 77 days and appeared to be part of an annual cycle. Successful reconditioning was determined by an increase fat content and survival time, followed by maturation and spawning in successive years. This pattern of annual depletion and replenishment has been documented in Atlantic salmon (Rowe et al. 1991).

One question about reconditioning after spawning is whether reconditioned fish can achieve a similar proximate composition to healthy fish. The three fish that were considered to be in the process of reconditioning (two fish from the 1997 cohort, at 185 days after first spawning and 120 days after second spawning, and one fish from the 1998 cohort at 120 days after first spawning) had a proximate composition of 73.6% moisture, 15.1% protein, 9.1% fat and 2.1% ash. The typical proximate composition for salmonids (*Oncorhynchus nerka*, *O. mykiss*, *O. tshawytscha* from 2-300 grams) is approximately 72.0% moisture, 16.0% protein, 9.3% fat and 2.7% ash (Groves 1970; Shearer 1994; Shearer et al. 1997). Therefore, it appears that reconditioning mature male parr can re-establish proximate composition levels similar to healthy, unspawned salmonids.

Fat content was selected as the main indicator of reconditioning. Fat content is an indicator of a salmonids' ability to mature, spawn, recondition and respawn (Crim et al. 1992), due to the inverse relationship between energy use and subsequent survival (Berg et al. 1998), especially for mature parr (Simpson 1992). However, there are some cautions about using fat content as the only indicator of reconditioning. Fat content generally increases with fish size (Shearer 1994) and differs within different tissues upon reconditioning following starvation (Weatherley and Gill 1981).

The energy expended for maturation and reproductive development is derived from body fat (Crim et al. 1992). A minimum level of at least 10% fat content is required in the spring (Rowe et al. 1991) to trigger maturation or rematuration in Atlantic salmon (Rowe and Thorpe 1990). This seasonal pattern of depletion and replenishment of fat occurs in fasted fish, such as the 50-90 days of fasting that occurred in this experiment, thereby delaying the restoration of fat reserves (Rowe et al. 1991). Higher fat reserves may increase post-spawning survival (Rowe and Thorpe 1990).

Based on fat content and survival time after spawning, the three fish that were in the process of reconditioning had fat contents of 10.1%, 8.9% and 8.4%, respectively. Their mean fat content of 9.1% was almost an order of magnitude (9.1 times) greater than the 1.0% mean fat content (95% CI from 0.9% to 1.2%) for the non-reconditioning fish from the 1997 and 1998 cohorts combined ( $n = 129$ ). This means the fish that were in the process of reconditioning had a higher fat content than the fish that were not reconditioning. With a severely reduced or atrophied gut, such as from starvation and spawning, the utilization of available nutrients may not have been possible in non-reconditioning fish.

Jonsson et al. (1997) suggest that the fat content of semelparous salmon is between 1.0-1.5% upon death after spawning and that a fat content of less than 2% may be close to the lower limit for survival of Pacific salmonids. A fat content that is too low (i.e., 1.0–2.0%) at spawning and/or the inability to increase fat after spawning may be the main reason for failure to recondition (Figure 9). Gardiner and Geddes (1980) found that young salmon that did not feed in captivity became moribund at a moisture content of 84.25% (Figure 10).

Results from the 1998 cohort cannot be statistically compared to the results from the 1997 cohort because the experimental designs were different, e.g., there were three treatment groups in three tanks for the 1997 cohort and two treatment groups in one tank for the 1998 cohort. In addition, most of the 1998 cohort had gross (i.e., clinically lethal) levels of bacterial kidney disease, *Renibacterium salmoninarum*.

Bacterial kidney disease created a catastrophic endpoint for the 1998 cohort after spawning. The BKD mortality rate for the non-maturing fish in the 1998 cohort was 2.2% during the same time period (i.e., September 2000 through February 2001). The lower BKD mortality rate for the non-maturing fish indicates that spawning may have initiated the gross,

post-spawning levels of BKD. Protein content may also have been reduced by BKD due to changes in amino acids utilized by *R. salmoninarum* (C. Banner, ODFW, pers. comm.).

Even though most of the fish from the 1998 cohort died of BKD, the proximate analysis results can be considered valid. Pirhonen et al. (2000) found an increased food intake in fish infected with BKD under conditions of previous starvation, such as in this experiment. Furthermore, fish in this experiment were observed eating two different types of food (e.g., beef liver and pellets) up to the day before they expired. In addition, fish had food in their digestive system upon autopsy.

Fish that originated from the Grande Ronde River appeared to survive longer than fish from the Lostine River or Catherine Creek. Although the reason for their apparent robustness is unknown, there could be several possible explanations. Since the spring chinook salmon population in the Grande Ronde River is at the greatest risk of extinction, the survival of post-spawned mature male parr may be a survival mechanism (B. Knox, ODFW, pers. comm.), which provides some physiological advantage compared to the other stocks. If this is true, then it may be possible that other salmonid populations in the Pacific Northwest may respond in the same way. Another possibility is that the reproductive strategy of male spring chinook salmon in the Grande Ronde River may be originating a partial shift (Jonsson and Jonsson 1993) from semelparity to iteroparity via precocious maturation, especially if the requirements for early maturation in fresh water are met (Thorpe 1987). This could be an advantage by increasing the effective population size and conserving genetic variability (Martinez et al. 2000), especially for small stocks (Saunders and Schom 1985).

Unwin et al. (1999) state that repeat spawning in chinook salmon appears to occur only after initial maturation as parr in fresh water, suggesting a link between anadromy and semelparity. The natural occurrence and fate of wild mature male spring chinook salmon parr

in the Pacific Northwest is unknown: whether they die, residualize or smolt and migrate to the sea after spawning. However, it is possible that altered environmental conditions, hatchery practices and downriver mortality (hydroelectric dams and fishing pressure) may be selecting for an increasing number of mature male parr in stream-type salmonid populations.

The Oregon Department of Fish and Wildlife has stated that we are in an emergency situation where dramatic and unprecedented efforts will be needed to prevent the extinction of Grande Ronde River Spring Chinook Salmon (ODFW 1995). ODFW (1995) also stated that the results of research in captive broodstock programs will have broad applicability to other anadromous salmonid recovery efforts and rely on new knowledge to make decisions and adapt program approaches. While mature male parr have been used as captive broodstock since at least 1943 (Thorpe and Morgan 1980), reconditioning and spawning of male spring chinook salmon in successive years could be used to expand the gene pool for breeding in any given year and aid in the recovery of the Grande Ronde River Basin and other endangered *Oncorhynchus* populations.

## FUTURE RESEARCH

Since there are few published studies designed to identify the variables that increase the potential for repeat sexual maturation of spring chinook salmon or the factors affecting post-spawning survival of mature male parr, future research should consider the following recommendations to improve the outcome of reconditioning programs for chinook salmon. First, fish should not be taken off food prior to spawning. Observations of hand-spawned mature male parr indicated that they fed immediately after spawning as soon as food was available (unpublished data). The practice of taking mature fish off feed in captive broodstock programs is not necessary (or desirable) for mature male parr, which may feed up to, during (Armstrong et al. 2001) and after spawning.

Second, there appears to be no advantage to force-feeding. The added stress may not be worth any questionable improvement in reconditioning. Third, the choice of feed may determine whether or not individual fish resume feeding. Soft foods of small size are probably more palatable and beneficial to fish after spawning. In addition, the use of beef liver stimulates an aggressive feeding response in previously spawned fish.

Fourth, every effort should be made to minimize the threat of bacterial kidney disease, which may be the single greatest threat to hatchery and captive broodstock programs in the Pacific Northwest. The use of vaccines and aggressive culling should provide additional protection against BKD. Fifth, the choice of fish stock may be the most important factor in a successful reconditioning program. If there are stock differences, the resources of any program should be spent on the stock with the greatest probability of success.

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