

GRANDE RONDE BASIN SPRING CHINOOK SALMON CAPTIVE BROODSTOCK PROGRAM

1995 - 2002 Project Status Report

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EXECUTIVE SUMMARY

The Grande Ronde Basin once supported large runs of chinook salmon *Oncorhynchus tshawytscha* and estimated peak escapements in excess of 10,000 occurred as recently as the late 1950's (U.S. Army Corps of Engineers 1975). Natural escapement declines in the Grande Ronde Basin have been severe and parallel those of other Snake River populations. These declines have primarily been attributed to increased mortality associated with downstream and upstream migration past eight dams and reservoirs in the Snake and Columbia rivers.

Catherine Creek, Grande Ronde River and Lostine River were historically three of the most productive populations in the Grande Ronde Basin with redd numbers as high as 505 in Catherine Creek (1971), 304 in the Grande Ronde River (1968) and 261 in the Lostine River (1956). However, productivity of these populations has been poor for most recent brood years, with redd numbers dropping to 30 and 31 redds combined in these streams in 1994 and 1995, respectively. The Minam and Wenaha rivers are tributaries of the Grande Ronde River located primarily in wilderness areas and redd counts there also decreased dramatically beginning in the early 1970's to 25-40% of the number seen in the 1960's. No hatchery fish have been released into either of these streams so these populations will be used as controls for evaluating our supplementation efforts in Catherine Creek, upper Grande Ronde River and Lostine River.

The Grande Ronde Basin Captive Broodstock Program was initiated because these chinook salmon populations had reached critical levels where dramatic and unprecedented efforts were needed to prevent extinction and preserve future options for use of endemic fish for artificial propagation programs for recovery and mitigation. In 1995, the Oregon Department of Fish and Wildlife (ODFW), U.S. Fish and Wildlife Service and Nez Perce Tribe (NPT) began development of captive broodstocks from local natural populations in Catherine Creek, Grande Ronde River and Lostine River for genetic conservation and natural production enhancement. The Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and National Marine Fisheries Service (NMFS) became cooperators in this program at a later date. A number of factors led to the development and implementation of this program, including: increased emphasis on natural production and recovery of endemic populations; consultations and requirements resulting from listing of Grande Ronde chinook salmon populations under the Endangered Species Act; our lack of success in using non-local hatchery stocks for supplementing Grande Ronde chinook populations; and preferred strategies for use of artificial propagation identified in the NMFS draft recovery plan. In addition, under the *U.S. v. Oregon* Grande Ronde chinook salmon dispute resolution in 1996, development of local broodstocks was also recommended by an Independent Scientific Review Panel as a prudent management alternative. This program was designed to quickly increase numbers of returning adults, while maintaining the genetic integrity of each endemic population and is providing substantial new knowledge for the use of artificial propagation to enhance natural production. Only recently have captive breeding programs been attempted with Pacific salmon. The captive broodstock program management goals are to prevent extinction of these stocks, maintain the genetic diversity and identity of the stocks in the program and nearby streams, ensure population persistence and provide methodologies to be used in other captive broodstock programs. In addition to the captive broodstock program, there is a conventional broodstock program which is part of the Lower Snake River Compensation Plan (LSRCP) and collects returning adult salmon for

spawning at Lookingglass Fish Hatchery.

The program has a threshold population goal of 150 spawning adults returning to each of Catherine Creek, upper Grande Ronde River and Lostine River annually. We consider spawning escapements below this threshold to pose a high and unacceptable risk to the persistence of these populations. We developed the captive and conventional broodstock programs in an attempt to alleviate this risk.

The captive broodstock program began in 1995, with collection of the 1994 cohort. The conventional program began in 1997 in Lostine River (no further collections were made until 2000 due to low returns) and in 2001 in Catherine Creek and Grande Ronde River. The captive broodstock program proved to be successful in producing F_1 smolts more quickly than anticipated and comprised all of the hatchery-reared chinook salmon released in the Grande Ronde Basin in 2000 and 2001.

Management strategies for integration of captive broodstock and conventional supplementation, as well as genetic risk containment, were originally developed in 1998. These strategies were modified in late 2002, when the co-managers negotiated management strategies for each stream which were implemented beginning in 2003. The three different strategies adopted represent a continuum from aggressive hatchery intervention in the upper Grande Ronde River to more conservative intervention in Catherine Creek. The strategies define smolt production levels by origin, proportion of natural and hatchery returns that can be retained for broodstock, proportion of broodstock permitted to be natural origin fish and the proportion of adults released to spawn naturally that can be of hatchery origin. We will also maintain the Minam and Wenaha rivers as wild salmon streams (i.e., no hatchery supplementation) and will continue to monitor for straying of hatchery fish into these systems.

Prior to beginning parr collections, we developed a production model based on estimates of survival at each stage of the chinook salmon life cycle and production estimates from the scientific literature and Oregon Department of Fish and Wildlife data. We used this model to establish survival and performance benchmarks and determine the number of parr needed to be collected to reach our goals. Captive broodstock progeny are released as smolts at a similar size to natural fish (approximately 20 g and 125 mm FL). Smolts are acclimated for 20-30 days prior to release, at acclimation sites supplied with river water and located within the area where the majority of spawning occurs to encourage imprinting to this area, promote homing and increase survival.

There are three components to the captive broodstock program: Monitoring and Evaluation, Fish Health and Fish Culture, each with its own set of objectives but sharing the common objective of developing innovative methodologies for rearing, spawning and disease prevention/treatment. All aspects of the program are monitored and evaluated for their performance relative to the benchmarks for success that were developed at the inception of the program. Progeny of the captive broodstock program become part of the LSRCP production for each of the program streams. The LSRCP has its own objectives to attain its goal of releasing 900,000 smolts into the Grande Ronde Basin each year with a survival rate of 0.65%, resulting in 5,820 adults returning to the basin annually. Lastly, there are M&E objectives for the captive broodstock F_1 and F_2 generations that will be assessed and accomplished under the LSRCP.

We attempt to collect 500 spring chinook salmon parr from each of the program streams in August/September. The fish are transported to Lookingglass Fish Hatchery (1994-2001

cohorts) or Wallowa Fish Hatchery (2002 and future cohorts) where they are reared under one of two pre-smolt growth regimes: “accelerated” or “natural” growth. At smoltification (early May) the fish are transported to either Bonneville Fish Hatchery or Manchester Marine Laboratory for post-smolt rearing in either freshwater or saltwater, respectively. This creates up to four combinations of pre-smolt and post-smolt treatments: Freshwater Accelerated, Freshwater Natural, Saltwater Accelerated and Saltwater Natural. Upon maturation, saltwater fish are transported to Bonneville Fish Hatchery, where spawning is conducted within populations and treatments and all fish are spawned within a matrix in which collected eggs and sperm are divided to allow cross fertilization by up to four different mates. All mortalities (including spawned fish) are inspected by fish health specialists to determine cause of death or disease state. There are several infectious diseases that could occur in these chinook salmon with bacterial kidney disease (BKD) and external fungus being the most problematic. These diseases are treated prophylactically and when outbreaks occur, with erythromycin or azithromycin (experimental) for BKD and formalin or hydrogen peroxide for fungus.

There are numerous uncertainties associated with captive broodstock programs and this program remains experimental. As such, it has an extensive monitoring and evaluation component, which is used to determine the effectiveness of experimental approaches and standard practices. We assess the program at key periods in the production cycle and have divided the cycle into four periods: Captive Juvenile Period, Captive Adult Period, F_1 Generation Period and F_2 Generation Period. Each period is further subdivided into discrete phases. Data collected are critical for evaluating treatment and overall program performance. We measure an array of variables in each period of the cycle which allow us to: compare our experimental treatments, develop relationships between treatments and performance, monitor the basic progress in fish culture, detect areas of concern that may need our immediate attention and judge the adequacy of the benchmarks we have used to design the overall captive broodstock program. Offspring produced by the captive broodstock program are integrated into the Lower Snake River Compensation Plan hatchery supplementation program. After release as smolts, the F_1 generation is allowed to complete its life cycle in the wild and produce the F_2 generation by spawning with naturally-spawned fish. An increase in wild spawning fish (due to the F_2 generation) will be the final measure of success for this program.

We have collected eight cohorts (1994-2001) of spring chinook salmon juveniles from Catherine Creek and Lostine River in 1995- 2002 and six cohorts from the upper Grande Ronde River. Each year, we collected 500 (or nearly) fish from Catherine Creek and the Lostine River. Only 110 fish were collected from the Grande Ronde River in 1995 (1994 cohort) and no fish were collected from the 1995 and 1999 Grande Ronde River cohorts.

Due to various problems at Lookingglass Fish Hatchery (chiller and water supply), we were unable to achieve acceptable pre-smolt treatments to allow us to achieve different growth rates between treatments for the 1995-1998 cohorts. We were able to achieve treatment groups for the 1999 and 2000 cohorts. Pre-smolt growth for the accelerated growth treatment group is still slower than expected - the expected growth rate may be unattainable. Post-smolt growth has been slower than anticipated. At spawning, captive broodstock females have been approximately 70% as large as fish reared in nature and males are only half as large (partly due to a large percentage of males maturing at ages 2 and 3). Freshwater Accelerated and Freshwater Natural females were larger than Saltwater Natural females. Mean length of mature males did not vary

among treatment groups. We appear to be seeing fish reaching a critical length of 500-550 mm at maturity, as there is little difference in mean length of females or males maturing at ages 4, 5 and 6.

We assumed 50% survival from parr to spawn and we exceeded this goal for each of the first four cohorts. Bacterial kidney disease was the largest source of pre-spawn mortality, causing at least 30-52% of the pre-spawn mortalities. Mean parr-to-smolt survival (97%) was higher than the expected rate of 95%. The expected smolt-to-adult survival rate is 55% and the mean (62.6%) did not differ from the expected rate. Mean smolt-to-adult survival for the Freshwater Natural group (70%) was higher than the expected rate, while survival rates for the Freshwater Accelerated (61%) and Saltwater Natural (57%) did not differ from expected.

Males matured at a younger age and females matured at a slightly older age than anticipated. We predicted a mean age of maturation of 4.1 years with approximately 6% of the females maturing at age 3, 78% at age 4 and 16% at age 5. There were fewer mature age 3 females and more age 6 females than expected. Females from the Freshwater Accelerated group matured at a younger mean age (4.1 years) than those of the Saltwater Natural (4.2 years) which was younger than the Freshwater Natural treatment group (4.3 years). For males, we expected a mean age of maturation of 3.8 years, with 2% maturing at age 2, 35% at age 3, 48% at age 4 and 15% at age 5. Males matured at a substantially younger age than expected and there were fewer mature age 5 males than expected. Males from the Freshwater Accelerated and Freshwater Natural groups matured at a younger mean age (3.0 years) than those of the Saltwater Natural treatment group (3.2 years). Captive broodstock salmon spawned an average of four weeks later than wild salmon.

We expected fecundities to be approximately 1,200, 3,000 and 4,000 eggs for females at ages 3, 4 and 5, respectively, approximating that of wild fish. Mean fecundities for ages 3, 4, 5 and 6 females were 1421, 1865, 1769 and 1369 eggs / female. Mature females of ages 3 and 6 are rare in this program. Mean fecundity was higher in the Freshwater Natural and Freshwater Accelerated groups than the Saltwater Natural treatment group. Fecundity was positively related to length, weight and K - females of ages 4, 5 and 6 were larger than age 3 females. Captive broodstock females also had fewer eggs / kg body weight than conventional broodstock females.

We assumed 75% egg fertility (percent of total eggs reaching the eyed stage) and mean fertility rate (78.4%) did not vary from expected. Mean female fertility was higher in the Freshwater Accelerated group than in either the Freshwater Natural or Saltwater Natural groups. Mean male fertility was higher in the Freshwater Accelerated group than either the Freshwater Natural or Saltwater Natural groups. Use of fresh semen resulted in a mean fertilization rate of 79.4%, while using cryopreserved semen resulted in only 34.0% fertilization. Mean eyed egg-to-smolt survival has been 75% for the 1998, 1999 and 2000 cohorts and stocks, lower than our expected value of 80%. To date, only two years of captive broodstock F_1 's have returned as adults. We have exceeded 0.1% smolt-to-adult survival (expected) for the 1998 cohort (0.2% - 0.79%), even without the age 5 returns.

Protocols for the program have evolved as we have modified our fish culture, fish health and monitoring practices to reduce stress and improve survival. We have reduced the number of times that we handle the fish and have improved our ability to prevent and treat BKD. In addition, we expect that the use of ultrasound or near infrared spectroscopy to examine fish for maturation will allow us to conduct a single maturity sort and determine sex of each maturing

fish early in the year (March/April), instead of conducting two or three maturity sorts and later sorting to determine sex of mature fish.

The problems with chillers and water supply at Lookingglass Fish Hatchery appear to be solved and the 1999 and 2000 cohorts have shown growth rates resulting in mean size at smoltification of natural growth fish being similar to that of wild smolts in this system and the accelerated growth fish are significantly larger. Moving pre-smolt rearing to Wallowa Fish Hatchery should permanently resolve this problem, as the natural growth fish will be reared in spring water with natural seasonal temperature fluctuations. We have also modified the experimental design (adding a Saltwater Accelerated treatment) so that the fish are now reared at equal densities at both BOH and MML. We have used erythromycin, injected and oral, as a prophylaxis and to treat outbreaks of BKD. Post-smolt growth has been substantially lower than expected. With increased experience and improved ability to identify maturing fish and determine their sex (using ultrasound or near infrared spectroscopy) we have improved adult survival. We can now develop spawning matrices earlier, which improves the efficiency and success of spawning and maintains the highest amount of genetic diversity possible within our program. The captive broodstock database (a large number of spreadsheets and databases in various formats) is nearly finished being compiled and organized into a relational database which can be accessed by all who need it for data summarization, statistical analyses and reporting.

We still have some things to learn. Bacterial kidney disease is the largest source of mortality in captive broodstock fish and we often spawn females that are highly infected with *R. salmoninarum* (based on ELISA). Subsequently, we have culled eggs, sometimes at a rate exceeding 25%, in an effort to decrease the prevalence of BKD in the F_1 generation. However, the risks of vertical and horizontal transmission are poorly understood in hatcheries and are even less understood in the wild. Culling entire lots of eggs removes the genetic contribution of that female and reduces or eliminates the genetic contribution of the males which fertilized her eggs. This is counter to the genetic conservation goal of this project. Therefore, we need to further examine the risk of raising progeny from high BKD females.

The co-managers (ODFW, NPT and CTUIR) of the chinook salmon populations in this program have developed a Grande Ronde Hatchery Management Plan which will be implemented in 2003 and provides for the disposition of captive broodstock fish when production exceeds management needs. In 2002, we released 2001 cohort parr into outlet streams: Lostine River fish into Bear Creek, Grande Ronde River fish into Sheep Creek and Catherine Creek fish into Lookingglass Creek. Another option is to release excess mature adults into their natal stream to spawn naturally, preferably with wild fish. However, captive broodstock fish have been spawning approximately three weeks later than wild fish so we need to determine the reasons for this delayed spawning and correct it before any adults can be released. If we are able to consistently produce more smolts than targeted for this program, a reduction in the number of parr collected will be warranted.

INTRODUCTION

Historic and Present Population Status

The Grande Ronde Basin once supported large runs of chinook salmon *Oncorhynchus tshawytscha* and estimated peak escapements in excess of 10,000 occurred as recently as the late 1950's (U.S. Army Corps of Engineers 1975). Natural escapement declines in the Grande Ronde Basin have been severe and parallel those of other Snake River populations. Reduced productivity has primarily been attributed to increased mortality associated with downstream and upstream migration past eight dams and reservoirs in the Snake and Columbia rivers. Reduced spawner numbers, combined with human manipulation of previously important spawning and rearing habitat in the Grande Ronde Basin, have resulted in decreased spawning distribution and population fragmentation of chinook salmon in the Grande Ronde Basin (Figure 1; Table 1).

Escapement of spring/summer chinook salmon in the Snake River basin included 1,799 adults in 1995, less than half of the previous record low of 3,913 adults in 1994. Catherine Creek, Grande Ronde River and Lostine River were historically three of the most productive populations in the Grande Ronde Basin (Carmichael and Boyce 1986). However, productivity of these populations has been poor for recent brood years. Escapement (based on total redd counts) in Catherine Creek and Grande Ronde and Lostine rivers dropped to alarmingly low levels in 1994 and 1995. A total of 11, 3 and 16 redds were observed in 1994 in Catherine Creek, upper Grande Ronde River and Lostine River, respectively, and 14, 6 and 11 redds were observed in those same streams in 1995. In contrast, the maximum number of redds observed in the past was 505 in Catherine Creek (1971), 304 in the Grande Ronde River (1968) and 261 in 1956 in the Lostine River (Tranquilli et al 2003). Redd counts for index count areas (a standardized portion of the total stream) have also decreased dramatically for most Grande Ronde Basin streams from 1964 - 2002, dropping to as low as 37 redds in the 119.5 km in the index survey areas in 1995 from as high as 1,205 redds in the same area in 1969 (Table 1). All streams reached low points (0-6 redds in the index areas) in the 1990's, except those in which no redds were found for several years and surveys were discontinued, such as Spring, Sheep and Indian creeks which had a total of 109 redds in 1969.

The Minam and Wenaha rivers are tributaries of the Grande Ronde River located primarily in wilderness areas. Chinook salmon numbers in these two streams (based on redd counts) also decreased dramatically beginning in the early 1970's (Table 1). Since then there have been a few years of increasing numbers of redds but counts have generally been 25-40% of the number seen in the 1960's. No hatchery fish have been released into either of these streams and we monitor them during spawning ground surveys for the presence of hatchery strays. These populations will be used as a type of control for evaluating our supplementation efforts in Catherine Creek, upper Grande Ronde River and Lostine River. In this way, we can attempt to filter out the effects of downstream variables, over which we have no control, when we interpret the results of the captive broodstock program as the F₁ and F₂ generations spawn and complete their life cycles in the wild.

The Grande Ronde Basin Captive Broodstock Program was initiated because these chinook salmon populations had reached critical levels where dramatic and unprecedented efforts were needed to prevent extinction and preserve any future options for use of endemic fish for

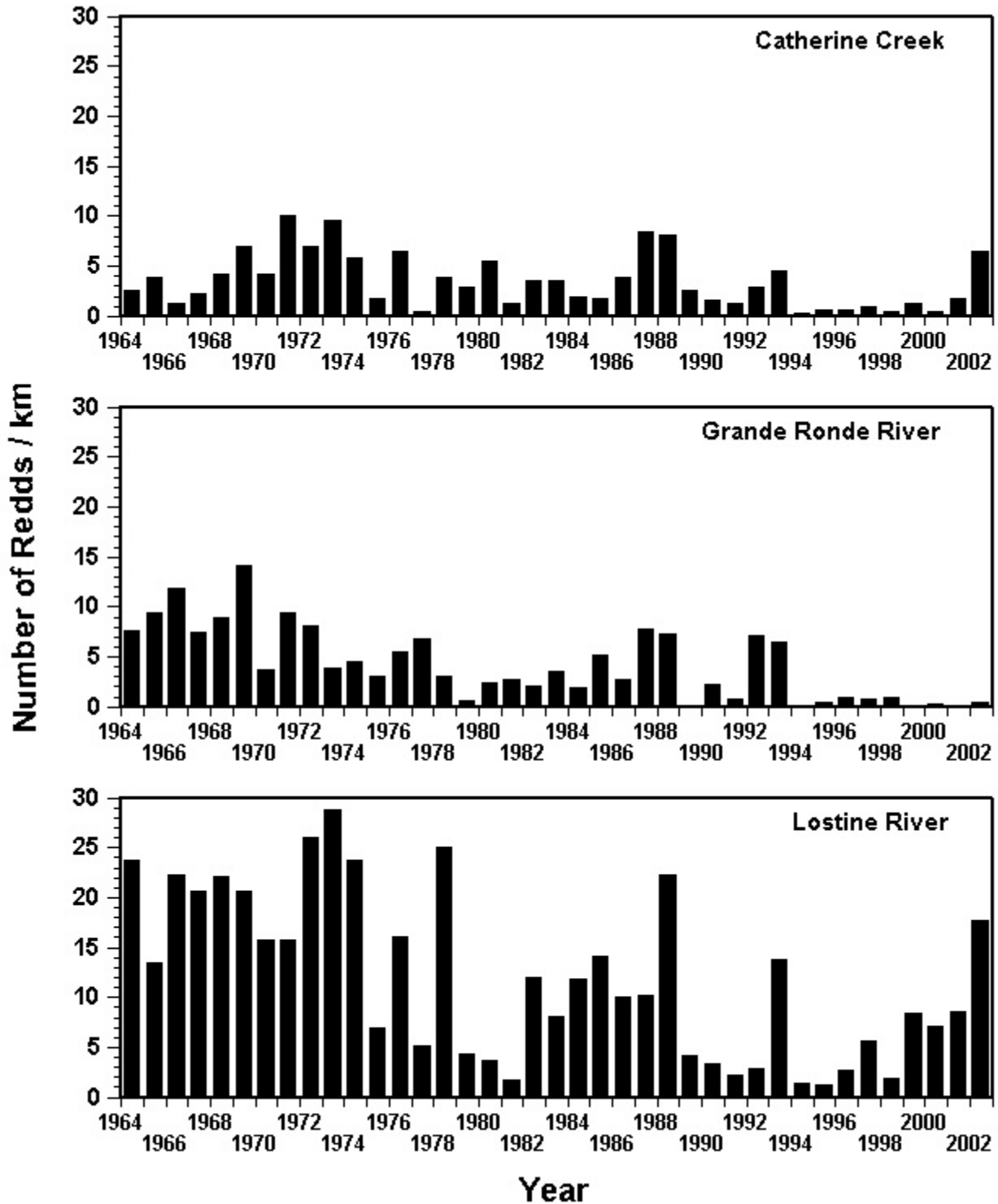


Figure 2 Number of spring chinook salmon redds / km within index areas on Catherine Creek (12.1 km), Grande Ronde River (13.7 km) and Lostine River (4.8 km surveyed), 1964-2002. Note: in Grande Ronde River, 22.5 km were surveyed in 1964, 12.1 km in 1966, 29 km in 1967, 33.8 km in 1968 and 16.1 km in 1971; 1989 survey conducted after flash flood; 1990 supplemental survey used as index count since original index count was conducted early.

Table 1. Annual spring chinook salmon redd counts within index areas on some Grande Ronde River Basin streams, 1964-2002.

Stream	River km surveyed	Year									
		1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Bear Creek	10.5	24	15	12	11	40	23	25	30	55	16
Hurricane Creek	5.6	28	17	1	3	20	9	17	23	18	10
Wallowa River	7.2	35	32	14	15	11	17	14	12	5	11
Spring Creek	1.6	20	6	6	4	1	1	0	0	4	2
S. F. Wenaha River	9.7	165	79	278	185	128	254	279	164	71	205
Lostine River	4.8	114	65	107	99	106	99	76	76	125	138
Little Lookingglass Cr.	6.4	--	--	--	--	--	--	--	--	--	--
Lookingglass Creek	10	141	101	210	92	92	165	188	149	63	101
Catherine Creek	12.1	41 ^a	47	15	27	51	85	51	121	85	116
N. F. Catherine Cr.	4.8	--	--	--	31	15	43	19	28	38	73
S. F. Catherine Cr.	2.4	--	--	--	17	7	19	3	86	21	33
Grande Ronde River	13.7	172 ^b	128	143 ^b	216 ^b	304 ^b	194	51	129	110	52
Sheep Creek	10.1	--	4	--	24 ^c	13 ^c	106	74	58	69	21
Minam River	13.4	151	126	121	50	107	181	175	109	138	118
Little Minam River	2.4	25	27	25	7	10	7	8	11	19	9
Indian Creek	4.8	--	--	--	--	10	2	10	0	19	7

Stream	River km surveyed	Year									
		1974	1975	1976	1977	1978	1979	1980	1981	1982	1983
Bear Creek	10.5	21	33	17	12	25	4	8	4	12	6
Hurricane Creek	5.6	11	2	0	0	11	0	0	1	9	7
Wallowa River	7.2	7	1	15	2	18	0	1	0	1	5
Spring Creek	1.6	0	0	--	--	--	--	--	--	--	--
S. F. Wenaha River	9.7	49	30	20	60	77	5	24	20	27	23
Lostine River	4.8	114	33	77	25	120	21	18	8	58	39
Little Lookingglass Cr.	6.4	--	--	--	--	--	--	--	--	--	--
Lookingglass Creek	10	27	28	40	32	25	13	29	7	26	7
Catherine Creek	12.1	70	21	78	6	47	36	66	16	42	43
N. F. Catherine Cr.	4.8	17	9	13	4	7	0	3	3	14	11
S. F. Catherine Cr.	2.4	19	12	21	--	26	5	0	3	7	4
Grande Ronde River	13.7	61	42	75	92	42	7	32	38	29	49
Sheep Creek	10.1	19	22	18	--	--	0	8	8	18	5
Minam River	13.4	51 ^d	50 ^d	52	14	137	9	10	14	22	21
Little Minam River	2.4	22	13	--	--	--	--	--	--	--	--
Indian Creek	4.8	1	0	0	--	11	--	--	--	--	--

^a 15.9 km surveyed in Catherine Creek in 1964.

^b 22.5 km surveyed in the Grande Ronde River in 1964, 12.1 km in 1966, 29 km in 1967 and 33.8 km in 1968.

Table 1 continued.

Stream	River km surveyed	Year									
		1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Bear Creek	10.5	11	6	10	10	5	2	1	2	0	9
Hurricane Creek	5.6	0	20	5	17	9	2	0	4	1	19
Wallowa River	7.2	12	3	7	15	7	0	0	1	0	1
Spring Creek	1.6	--	--	--	--	--	--	--	--	--	--
S. F. Wenaha River	9.7	12	36	68	62	98	9	31	28	58	46
Lostine River	4.8	57	68	48	49	107	20	16	11	14	66
Little Lookingglass Cr.	6.4	--	--	--	--	--	--	--	--	--	31
Lookingglass Creek	10	--	12	5	18	53	18	19	7	21	89
Catherine Creek	12.1	23	22	47	103	99	31	19	15	36	54
N. F. Catherine Cr.	4.8	1	3	8	14	38	6	6	3	5	7
S. F. Catherine Cr.	2.4	4	7	21	35	39	1	7	1	0	2
Grande Ronde River	13.7	26	70	37	106	99	0 ^e	31 ^f	10	97	88
Sheep Creek	10.1	18	30	6 ^c	7	0	0	0	0	5	0
Minam River	13.4	23	116	63	90	87	33	53	37	16	63
Little Minam River	2.4	--	--	--	--	--	--	--	--	3	6
Indian Creek	4.8	--	--	--	--	--	--	--	--	0	2

Stream	River km surveyed	Year									
		1994	1995	1996	1997	1998	1999	2000	2001	2002	
Bear Creek	10.5	0	0	0	0	1	0	0	1	9	
Hurricane Creek	5.6	0	0	3	9	0	6	12	7	8	
Wallowa River	7.2	0	0	0	2	0	0	0	0	1	
Spring Creek	1.6	--	--	--	--	--	--	--	--	--	
S. F. Wenaha River	9.7	12	3	28	26	24	5	55	88	65	
Lostine River	4.8	7	6	13	27	9	40	34	41	85	
Little Lookingglass Cr.	6.4	11	1	0	0	0	0	0	0	0	
Lookingglass Creek	10	14	2	23	21	1	0	0	0	0	
Catherine Creek	12.1	4	7	8	12	5	16	5	22	79	
N. F. Catherine Cr.	4.8	0	0	1	7	4	1	2	10	6	
S. F. Catherine Cr.	2.4	0	0	0	2	0	0	0	1	1	
Grande Ronde River	13.7	1	5	13	10	12	0	4	2	6	
Sheep Creek	10.1	0	0	--	--	--	--	--	--	--	
Minam River	13.4	5	10	57	36	31	26	63	102	131	
Little Minam River	2.4	0	3	5	5	4	5	0	8	12	
Indian Creek	4.8	0	--	--	--	--	--	--	--	--	

^c 16.1 km surveyed in 1967, 7.2 km in 1968 and 14.5 km in 1986.

^d 16.1 km surveyed in the Minam River in 1974 and 1975.

^e 1989 Grande Ronde survey conducted after flash flooding on 8 August 1989.

^f Supplemental survey data used in 1990 because original index count was conducted too early.

artificial propagation programs for recovery and mitigation. This program was designed to quickly increase numbers of returning adults, while maintaining the genetic integrity of each endemic population.

Background

Grande Ronde Basin chinook salmon populations dropped to alarmingly low levels in 1994 and 1995 (Figure 1; Table 1). Although present escapement levels and recent trends indicate that Grande Ronde Basin spring chinook salmon populations show some improvement, these populations (particularly the upper Grande Ronde River) remain in imminent danger of extinction. The initial management plan under the Lower Snake River Compensation Plan (LSRCP) program called for releases of hatchery-reared fish into four chinook salmon populations in the basin: Catherine Creek and Wallowa, Lostine and upper Grande Ronde rivers. In 1995, the Oregon Department of Fish and Wildlife (ODFW), U.S. Fish and Wildlife Service and Nez Perce Tribe (NPT) began development of captive broodstocks from local natural populations in Catherine Creek, Grande Ronde River and Lostine River for genetic conservation and natural production enhancement. The Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and National Marine Fisheries Service (NMFS) became cooperators in this program at a later date. Development of local broodstocks was also recommended by an Independent Scientific Review Panel under the U.S. v. Oregon Grande Ronde chinook salmon dispute resolution in 1996 (Currens et al. 1996). The decision to begin a captive broodstock program was a result of a number of factors including: increased emphasis on natural production and recovery of endemic populations; consultations and requirements resulting from listing of Grande Ronde chinook salmon populations under the Endangered Species Act; our lack of success in using non-local hatchery stocks for supplementing Grande Ronde chinook populations; and preferred strategies for use of artificial propagation identified in the NMFS draft recovery plan. This program is providing substantial new knowledge for the use of artificial propagation to enhance natural production.

Captive breeding programs have been used extensively in recovery efforts for some fishes and other vertebrates. Only recently has this type of propagation approach been attempted with Pacific salmon. Similar broodstock programs are underway for a number of other listed salmonids including: Sacramento River winter chinook salmon, Salmon River and Tucannon River spring chinook salmon and Redfish Lake sockeye salmon *Oncorhynchus nerka*. We have used the knowledge and experience gained in these other programs, as well as the results of the captive broodstock comprehensive review conducted by Flagg and Mahnken (1995), to develop fish culture, research, monitoring and evaluation protocols for this program.

Recovery of these populations is dependent upon improved juvenile and adult survival through reservoirs and dams on the Snake and Columbia rivers. Project success is dependent upon achieving adequate survival, growth, maturation and gamete viability and reproductive success objectives of captive broodstock fish and performance of F₁ and F₂ generations. In order to promote recovery, we must first insure survival of these stocks into the future. Our initial efforts using captive broodstock were focused on ensuring short term persistence of the populations. Recently, the emphasis for the upper Grande Ronde and Lostine programs has shifted to supplementation and meeting smolt production goals (CTUIR et al 2002).

The Biological Requirements Work Group (1994) developed “threshold” escapement levels for use in their analyses, based on considerations of demographic and genetic risk. These

threshold levels represent escapement levels at which qualitative changes in processes are likely to occur and below which uncertainties about processes or population enumerations are likely to become significant. For spring chinook salmon populations, they decided on a level of 150 naturally spawning adults annually for small populations. Lostine River, Catherine Creek and upper Grande Ronde River are all classified as small populations. Our take of 500 parr each year from each stream is based on producing 150,000 F_1 generation smolts to attain an annual threshold population goal of 150 spawning adults returning to each stream. Escapement for Lostine River, Catherine Creek and upper Grande Ronde River chinook salmon exceeded 150 adults (threshold) nearly 100% of the time during 1964-1974. However, from 1975-1994 escapement in each of these streams exceeded the threshold only 54-61% of the time and escapement has exceeded 150 fish 0-56% of the years since 1994. Reduction of spawning escapements below this threshold indicates a high and unacceptable risk to the persistence of these populations, thus, we developed the captive and conventional broodstock programs to attempt to alleviate this risk.

The conventional broodstock program, part of LSRCF, collects returning adult salmon for spawning at Lookingglass Fish Hatchery and subsequent rearing of offspring to the smolt stage, at which time they are returned to the river from which their parents were collected. Captive broodstock progeny are included as part of the overall production for the LSRCF program.

The captive broodstock program began in 1995, with collection of the 1994 cohort. The conventional program began in 1997 in Lostine River (no further collections were made until 2000 due to low returns) and in 2001 in Catherine Creek and Grande Ronde River. The captive broodstock program proved to be successful in producing F_1 smolts more quickly than anticipated and comprised all of the hatchery-reared chinook salmon released in the Grande Ronde Basin in 2000 and 2001.

Management strategies for integration of captive broodstock and conventional supplementation, as well as genetic risk containment, were originally developed in 1998. However, co-manager agreement was not reached until these strategies were modified in late 2002, when management strategies for each stream were finalized (CTUIR et al 2002). These strategies will be implemented beginning in 2003. The three different strategies adopted represent a continuum from aggressive hatchery intervention in the upper Grande Ronde River to more conservative intervention in Catherine Creek with the Lostine River program falling between the two. The management strategies define smolt production levels by source (captive and conventional), proportion of natural and hatchery returns that can be retained for broodstock, proportion of broodstock that should be natural origin fish, and the proportion of adults released to spawn naturally that can be of hatchery origin. The upper Grande Ronde River has the lowest number of returning adult chinook salmon and will be managed to achieve an annual smolt production goal and maximize the rate of increasing numbers of natural spawning fish, regardless of origin. Up to 250,000 smolts from both captive and conventional broodstock programs will be released annually. Adult collections for the conventional program in the upper Grande Ronde River will be more aggressive, with the purpose of collecting enough adults (within our Endangered Species Act permit) to maximize smolt production at the permitted level of 250,000 (Table 2). Also, this stream will be managed to increase the number of adults spawning in the wild by not limiting the percentage of hatchery fish released above the weir. Adult collections for both the Catherine Creek and Lostine River conventional broodstock programs will be more conservative and follow the original adult sliding scale developed in 1998 in which no more than

Table 2. Sliding scale for collection of adults for conventional broodstock spawning in Catherine Creek, Grande Ronde River and Lostine River. Note: Hatchery collections may not include any captive broodstock progeny.

Estimated adult escapement	Catherine Creek			Grande Ronde River			Lostine River		
	Percent retained for broodstock		Percent hatchery above weir	Percent retained for broodstock		Percent hatchery above weir	Percent retained for broodstock		Percent hatchery above weir
	Wild	Hatchery		Wild	Hatchery		Wild	Hatchery	
≤ 250	≤40%	≤40%	–	≤50%	Number needed	No restriction	≤40%	≤40%	–
251-500	≤20%	≤20%	≤70%	≤50%	for collection		≤20%	≤20%	≤70%
>500	≤20%	0	≤50%	≤50%	goal)		≤20%	0	50%

40% of the estimated hatchery and wild escapement may be collected for broodstock and no more than 70% of the fish released above the weirs may be of hatchery origin (NPT 1998; ODFW 1998). Captive broodstock offspring will be used to supplement conventional broodstock production. Production of captive broodstock progeny in excess of those needed for each program stream will be released into Grande Ronde Basin tributaries previously identified and agreed upon by the co-managers. We will also maintain the Minam and Wenaha rivers as wild salmon streams (i.e., no hatchery supplementation) and will continue to monitor for straying of hatchery fish into these systems.

Collection of juveniles (parr), and their subsequent rearing, for the captive broodstock program will not be immediately affected by this management agreement but release of their progeny will be more conservatively managed in some streams. The captive broodstock program was originally focused on an assurance of survival of the populations, using the threshold annual return goal of 150 adults as an indicator. At initiation of the program, the proposed release of F_1 generation smolts in all three populations was based on a sliding scale and was more aggressive at lower levels of escapements and reduced as natural spawning escapements approached or exceeded 150 adults (Table 3). The proposed releases of captive broodstock offspring would continue at reduced levels for escapements up to 300 adults to provide a safety net for the population. Since the model to estimate eventual adult returns from parr collected for the captive broodstock program is based on a series of assumptions (survival, fecundity, etc.), continuing low level releases of captive broodstock offspring offers some hedge in case our experience results in less fish than we assumed would return. The juvenile sliding scale was eliminated from the management strategies for the upper Grande Ronde and Lostine rivers, where no juvenile sliding scale will be used and a maximum of 250,000 smolts will be released each year. In Lostine River, a maximum of 150,000 smolts can come from the Captive broodstock program while in Grande Ronde River there is a preference for conventional fish but no limit on source of the hatchery fish. The long term combined captive/conventional production goal for Catherine Creek is 150,000 smolts.

Table 3. Proposed sliding scale for release of captive broodstock F₁ generation smolts. This scale assumes 4000 eggs/female, 3.1 adults/redd and 11.9% egg-to-smolt survival for wild fish. This framework is only used for the long term management strategy in Catherine Creek.

Wild chinook salmon			Captive broodstock chinook salmon		
Number of Redds	Number of Smolts	Percent of Total Smolts	Number of Smolts	Percent of Total Smolts	Total Smolts
10	4760	3	150000	97	154760
16	7600	4	150000	96	157616
32	15200	11	120000	89	135232
48 ^a	22850	19	100000	81	122848
65	30950	34	60000	66	90940
80	38080	43	50000	57	88080
100 ^b	47,600	66	25000	34	72600

^a represents 150 adults

^b represents 310 adults

Prior to beginning parr collections for the captive broodstock program, we developed a production model based on estimates of survival at each stage of the chinook salmon life cycle, fecundity and return rates from the scientific literature and Oregon Department of Fish and Wildlife data. We used this model to establish survival and performance benchmarks and determine the number of parr that would be needed to be collected annually to reach our goals. Detailed assumptions used to develop the production program and which serve as benchmarks of success are as follows:

- 1) We anticipated a 1:1 sex ratio at collection for each population.
- 2) We have assumed 50% survival from parr to spawn, based on Smith and Wampler (1995). It is unlikely we will need to collect more than 500 juveniles, based on the 90% parr-to-smolt survival at Lookingglass Fish Hatchery (LFH) during the first year of a program, 80% survival from smolt to three year adult for spring chinook in Washington's Dungeness program (Witczak 1995) and 90% first year survival observed for juvenile Redfish Lake sockeye held at MML (C. Mahnken, NMFS, Seattle, personal communication). Thus, the model is based on a collection of 500 juveniles each year.
- 3) We predicted that approximately 6% of the females will mature at age 3, 78% at age 4 and 16% at age 5 (adapted from Nielson and Geen 1986; Hankin et al. 1993; Burck 1994; Appleby and Keown 1995). We expected that 2% of the males will mature at age 2, 35% at age 3, 48% at age 4 and 15% at age 5.
- 4) Based on Flagg and Mahnken (1995), we expected fecundities to be approximately 1,200, 3,000 and 4,000 eggs for females ages 3, 4 and 5, respectively.
- 5) We assumed 75% embryo viability (see Smith and Wampler 1995) and 80% viable embryo to smolt survival (ODFW, Lookingglass Fish Hatchery, unpublished data). In an earlier model,

we used an estimate of 45% embryo viability but Flagg and Mahnken (1995) suggest that 75% embryo viability is reasonable.

- 6) We assumed a 80% viable embryo to smolt survival (ODFW, Lookingglass Fish Hatchery, unpublished data).
- 7) Typically, chinook salmon reared at and released as smolts from LFH return at a 0.1% smolt-to-adult survival rate.
- 8) We assumed that 10%, 60% and 30% of the smolts released into the wild will return as adults at ages 3, 4 and 5, respectively, based on production data from LFH and additional studies cited above.

Release of captive broodstock progeny will be made as smolts at a similar size to natural fish (approximately 20 g and 125 mm FL). Because of the critical need to promote the survival of these populations into the future, smolts were chosen as the preferred life stage for release, since they have proven to provide a substantial egg-to-adult survival advantage over pre-smolt releases in the Grande Ronde system and is the most effective short term strategy (Messmer et al 1992; 1993). Smolt releases also result in reduced interactions between hatchery and naturally-produced fish, as they will spend little time in freshwater. Smolts are acclimated for 10-30 days prior to release, at acclimation sites supplied with river water and located within the area where the majority of spawning occurs to encourage imprinting to this area, promote homing and increase survival. Acclimation sites are located on Lostine River at river kilometer (RK) 19.3, upper Grande Ronde River at RK 319.5 and Catherine Creek at RK 48.4.

Excess captive broodstock program adults and eggs, fry, parr or smolts (i.e., those above that required to reach smolt goals) may be released into suitable, underseeded habitat. Previously identified sites include: Wallowa River (9 RK 64-76), Hurricane Creek (RK 0-8), Bear Creek (RK 0-14) and Prairie Creek (RK 0-8) in the Wallowa River drainage; Sheep Creek (RK 0-8) in the upper Grande Ronde River drainage; and Little Catherine Creek (RK 0-3) in the Catherine Creek drainage. Additionally, Catherine Creek fish will be used in an effort to reestablish natural spawning of chinook salmon in Lookingglass Creek.

We will reassess our model assumptions for the captive broodstock program to determine whether the number of juveniles collected from the wild each year should be modified, while considering the gene conservation and genetic diversity objectives of the program. The use of fish not needed in the primary program will provide a reasonable outlet that in the short term recognizes the limits of hatchery space, uses the fish where there is some chance of success and puts fish in habitat that is spatially separated from the majority of spawning and rearing areas. The use of outplanted adults would allow the maximum amount of natural selection in the habitat as well as provide nutrients to the stream from decomposing carcasses.

Goals and Objectives

This program was initiated as a conservation measure in response to severely declining runs of chinook salmon in the Grande Ronde Basin. Our management goals are four-fold:

- 1) Prevent extinction of the Catherine Creek, Lostine River and upper Grande Ronde River chinook salmon populations.
- 2) Maintain the genetic diversity and identity of the Catherine Creek, upper Grande Ronde River and Lostine River populations, as well as that of unsupplemented wild Minam and

Wenaha rivers populations.

- 3) Ensure a high probability of population persistence well into the future once the causes of basin wide population declines have been addressed.
- 4) Provide a future basis to reverse the decline in abundance of endemic Grande Ronde Basin chinook salmon populations.

The specific objectives for attaining these goals vary with each component of the captive broodstock program (i.e., Monitoring and Evaluation, Fish Health and Fish Culture) and the associated LSRCP program. An objective for each component of the program is to develop innovative methodologies for rearing, spawning and disease prevention/treatment. All aspects of the program are monitored and evaluated for their performance relative to the benchmarks for success that were developed at the inception of the program (Oregon Department of Fish and Wildlife 1996).

Fish Culture is the base of the captive broodstock program - the fish culturists care for the fish and monitor them by daily observation. Some Fish Culture objectives vary among hatcheries because of differences in their roles in the program. Hatchery objectives include:

- 1) Maintain a healthy environment to promote good health and high survival (all hatcheries).
- 2) Rear fish in a manner to achieve the desired growth rates (LFH/WFH).
- 3) Rear fish to maturation and successfully segregate maturing and immature fish at the proper time (BOH and MML).
- 4) Use proper spawning protocols to achieve genetic diversity objectives and high gamete survival (BOH).

Fish Health monitoring is an essential component for maintaining health of the captive broodstock and resulting offspring. They administer prophylactic treatments and treat specific diseases observed by culturists. Objectives of fish health monitoring include:

- 1) Determine etiology of captive broodstock morbidity and mortality.
- 2) Implement prophylactic and therapeutic treatments, as needed.
- 3) Monitor fish culture practices and fish handling for situations that may contribute to impaired fish health or exacerbate disease.
- 4) Implement prophylactic erythromycin treatments for bacterial kidney disease under INAD protocols.

There are also monitoring and evaluation components to this program that are designed to determine the best methodologies to be used in captive broodstock programs. The primary objective of the Monitoring and Evaluation (M&E) component is to assess specific objectives of the Grande Ronde Basin Captive Broodstock Program. In addition, we have research objectives to determine the effect of two pre-smolt and two post-smolt rearing environments on performance of captive broodstock fish and to monitor and evaluate the performance of captive broodstock offspring in the hatchery (F_1 only) and in nature (F_1 and F_2). Additional evaluations of innovative methodologies have been added, such as a study that is currently underway to assess the effectiveness of azithromycin and erythromycin to prevent vertical transmission of BKD. Lastly, through our results and in conjunction with other captive broodstock programs, we

will assess the role of using captive broodstocks in recovery efforts of salmonids listed under the Endangered Species Act (ESA):

- 1) Assess and compare the effects of the natural and accelerated pre-smolt rearing strategies on growth, survival, maturation and reproductive success.
- 2) Assess and compare the effects of the freshwater and saltwater post-smolt rearing strategies on growth, survival, maturation and reproductive success.
- 3) Determine the success of our smolt transfer protocol.
- 4) Monitor and assess growth, development, life stage specific survival and maturation for all populations and treatments.
- 5) Assess the success in achieving genetic conservation goals.
- 6) Assess the effectiveness of matrix spawning protocols and success at meeting performance benchmarks for production of captive broodstock offspring.
- 7) Identify and evaluate the effectiveness of innovative methodologies for rearing, spawning and disease prevention/treatment.
- 8) Monitor and compare aspects of life history and production performance between captive and conventional broodstock programs.
- 8) Develop and maintain a comprehensive database for the program.

The captive broodstock program was originally implemented strictly as a population conservation effort. As it has begun to produce offspring, the captive broodstock program has been integrated with the Grande Ronde Basin Spring Chinook Salmon Lower Snake River Compensation Plan mitigation efforts. Progeny of the captive broodstock program become part of the LSRCP production for each of the program streams. The LSRCP has a goal of releasing 900,000 smolts into the Grande Ronde Basin each year with a survival rate of 0.65%, to produce 5,820 adults returning to the LSRCP area annually. The objectives of the LSRCP for spring chinook salmon in the Grande Ronde Basin are:

- 1) Establish an annual return of 5,820 fish.
- 2) Establish adequate broodstock to meet annual production needs.
- 3) Restore and maintain natural spawning populations of spring chinook salmon in the Grande Ronde Basin.
- 4) Reestablish historic tribal and recreational fisheries.
- 5) Maintain endemic wild populations of spring chinook salmon in the Minam and Wenaha rivers.
- 6) Minimize the impact of hatchery programs on resident stocks of game fish.

Lastly, there are M&E objectives of the captive broodstock program that will be assessed and accomplished under the LSRCP monitoring program. These objectives involve performance, in the hatchery and/or in nature, of the F_1 and F_2 generations of the captive broodstock fish. Standard hatchery evaluation variables and protocols are used to assess performance of the pre-smolt (in hatchery) and smolt-to-adult (in nature) stages of the F_1 generation. For monitoring F_1 generation adult performance and the entire F_2 generation, we rely on standard evaluation variables and protocols used for monitoring wild salmon populations, as well as genetic analyses to determine the parental heritage of wild-spawned offspring. These evaluations will be

conducted at key periods in the production cycle (e.g., incubation, juvenile rearing, adult return, spawning - see Methods; Monitoring and Evaluation):

- 1) Determine and compare the effects of parental post-smolt rearing strategies (saltwater vs. freshwater) on survival, hatching time, fry survival, growth rates, condition, size distribution, fry-smolt survival, smolt outmigration performance.
- 2) Determine and compare smolt-to-adult survival, catch distribution, run timing, age structure at return, size-at-age, sex ratio, pre-spawn survival in nature, spawn timing, spawning distribution in nature and spawning success with naturally-spawned chinook salmon in the same streams. Straying of captive broodstock progeny will also be assessed.
- 3) Assess egg-to-smolt survival, juvenile tributary migration patterns, parr and smolt production, smolt migration patterns, smolt-to-adult survival, adult run timing, adult age structure at return, size and age at maturation, sex ratios, pre-spawn survival in nature, spawning distribution in nature and productivity (progeny to parent ratios) for supplemented populations. Compare these factors between supplemented and prior unsupplemented years, using unsupplemented streams (Minam and Wenaha rivers) as controls.

Significance to Regional Programs

Captive broodstock projects for Snake River spring/summer chinook salmon are supported by recommendations in the Snake River Salmon Recovery Team (SRSRT 1994), NMFS draft recovery plan (NMFS 1995) and the Northwest Power Planning Council's (NPPC) Fish and Wildlife Program (NPPC 1994). This project addresses numerous objectives identified in the 1994 Fish and Wildlife Program including: 7.1B which addresses conservation of genetic diversity; 7.2 which identifies the need for improvement of existing hatchery production; 7.3B which directs implementation of high priority supplementation projects; 7.4A which specifies the need to evaluate and implement new production initiatives; and 7.4D which directs implementation of captive broodstock programs. The NMFS draft recovery plan states "captive broodstock and supplementation programs should be initiated and/or continued for populations identified as being at imminent risk of extinction, facing severe inbreeding depression or facing demographic risks". The recovery plan also states "considering the critical low abundance of the Grande Ronde spring/summer chinook salmon, impacts to listed fish should be avoided and LFH should be operated to prevent extinction of local populations. Consequently, indigenous broodstock should be immediately transferred to LFH (natural fish collected in 1995) and production should be maximized to supplement natural populations." The goal of the Grande Ronde Basin Chinook Salmon Captive Broodstock Program is to prevent extinction of the three program populations, provide a future basis to reverse the decline in population abundance and ensure a high probability of population persistence. Use of non-local broodstock is inconsistent with sound conservation principles and development of local broodstocks was recommended by an Independent Scientific Review Panel under the U.S. v. Oregon Grande Ronde chinook salmon dispute resolution in 1996 (Currrens et al. 1996). This captive broodstock program is directed by the conceptual premise that identifies maintenance of genetic variation, within and between populations, and of life history characteristics essential for long term fitness and persistence. This premise is an integral part of the LSRCP in-kind and in-place mitigation program.

Relationships to Other Programs

This captive broodstock project is one of the first such production projects in the Columbia Basin and is completely integrated with the LSRCP. Embryos produced from spawned captive brood become a source for smolt production under the LSRCP for the Grande Ronde Basin Chinook Salmon. Additionally, this captive broodstock project was designed as a large scale adaptive management program examining three production strategies: a) accelerated pre-smolt rearing with post-smolt freshwater rearing, b) natural pre-smolt rearing with post-smolt freshwater rearing and c) natural pre-smolt rearing with post-smolt saltwater rearing. A fourth strategy (accelerated pre-smolt rearing with post-smolt saltwater rearing) was added with collection of the 2000 cohort. The project is also closely integrated with other hatchery, habitat and research projects in the Grande Ronde Basin such as:

- 1) Bonneville Fish Hatchery (BOH) Operations; Bonneville Power Administration funding: The captive broodstock production facility was completed at BOH in May 1998. Fish culturists at BOH oversee the freshwater post-smolt production program and we share equipment and personnel with BOH.
- 2) LSRCP Hatchery Operations and Evaluations: This captive broodstock project is completely integrated with the LSRCP Program. LSRCP facilities and personnel are implementing the production, evaluations and fish health monitoring for the captive brood program and extensive sharing is occurring between the programs. In addition, ongoing research under LSRCP will provide information to assess the success of the captive broodstock project in the F_1 and F_2 generations.
- 3) The Northwest Power Planning Council's Fish and Wildlife Program: The Council's 1994 report calls for initiation of captive broodstocks and associated research. Measure 7.4.D.2 requests that Bonneville Power Administration "fund captive broodstock demonstration projects identified under the coordinated habitat and production process."
- 4) Captive broodstock and supplementation projects for Snake River spring/summer chinook salmon: These projects are supported by Snake River Salmon Recovery Team recommendations (SRSRT 1994) and NMFS (1995) draft recovery plan. NMFS draft recovery plan states, "captive broodstock and supplementation programs should be initiated and/or continued for populations identified as being at imminent risk of extinction, facing severe inbreeding depression, or facing demographic risks," and further states, "considering the critical low abundance of Grande Ronde spring/summer chinook salmon, impacts to listed fish should be avoided and LFH should be operated to prevent extinction of local populations. Consequently, indigenous broodstock should be immediately transferred to LFH (natural fish collected in 1995) and production should be maximized to supplement natural populations."
- 5) Early life history of spring chinook salmon in the Grande Ronde Basin: This project provides data on migration and survival of hatchery and naturally produced fish that is essential for evaluating the success of the captive broodstock project.
- 6) Manchester Marine Laboratory (NMFS): This facility rears fish through the captive adult period, in seawater, for all three populations.
- 7) Chinook Salmon Captive Propagation Technical Oversight Committee (TOC: Bonneville Power Administration): The committee, comprised of biologists from Oregon, Idaho and Washington, U.S. Fish and Wildlife Service, national Marine Fisheries Service, Shoshone-

Bannock Tribe and University of Idaho, meets approximately six times per year. The TOC allows these biologists to coordinate with other researchers (as required under our ESA permit) and discuss the current status of captive broodstock programs for chinook salmon in Idaho, Oregon and Washington. Programs in Idaho and Washington have been designed differently than that in Oregon and it is helpful to discuss problems and successes in this forum to improve our respective programs.

- 8) Protect and Enhance Anadromous Fish Habitat in Grande Ronde Basin Streams (ODFW): This program develops and implements projects to protect, enhance and restore riparian and in stream habitat for anadromous fishes. Streams enhanced by this project may be colonized or stocked with chinook salmon produced by the captive broodstock program.
- 9) Grande Ronde Supplementation: Lostine River O&M and M&E (NPT): Operate adult and juvenile acclimation facilities and conduct monitoring and evaluation in the Lostine River to implement the Lostine component of the Grande Ronde Basin Endemic Spring Chinook Supplementation Program. This is a collaborative effort with the ODFW Captive broodstock program. The juvenile acclimation facility is used to release captive broodstock progeny and the adult trap captures returning progeny. Captive broodstock progeny have returned for two years.
- 10) Facility O&M and Program M&E for Grande Ronde Anadromous Salmonids (CTUIR): The purpose of this project is to develop, implement and evaluate integrated conventional and captive broodstock hatchery programs to prevent extinction and stabilize and restore anadromous threatened salmonid populations in-place and in-kind in the upper Grande Ronde River and Catherine Creek. This is a collaborative effort with the ODFW Captive broodstock program. Acclimation sites and adult weirs are used for release and capture of Captive broodstock progeny.
- 11) Artificial production (ODFW, NPT and CTUIR): The Captive broodstock program is designed to work with conventional hatchery programs in these streams. Maximum numbers of hatchery fish (combined captive and conventional) have been established for these streams. Each program is also designed to minimize the likelihood of domestication by allowing all progeny of captive broodstock fish to spawn in the wild.
- 12) Grande Ronde Model Watershed: This program brings relevant interest groups together to address watershed restoration and declining ESA listed anadromous fish populations in the Grande Ronde Basin. Watershed enhanced by this project may be colonized or stocked by chinook salmon produced by the captive broodstock program.
- 13) Northeast Oregon Hatcheries Planning and Implementation (ODFW): The captive broodstock project is an integral element of this program which incorporates both conventional and captive broodstock strategies..
- 14) ODFW/CTUIR and USFS Habitat Restoration and Enhancement Projects: Habitat restoration is critical to increasing the numbers of chinook salmon in these streams. We expect that chinook salmon produced by the captive broodstock program will utilize the restored habitat and increase the rate at which the populations grow.
- 15) NPT Captive Broodstock Artificial Propagation: This is a collaborative effort between NPT and ODFW to implement the captive broodstock program on the Lostine River.
- 16) Genetic Evaluation and Monitoring of Snake River Salmon and Steelhead: The genetics M&E project provides genetics analyses and interpretation for the captive broodstock program.



Figure 3 Grande Ronde Basin, Oregon and Washington, with major tributaries, hatcheries, weirs and acclimation sites used in the captive broodstock program.

DESCRIPTION OF STUDY AREA AND FACILITIES

Grande Ronde Basin

The Grande Ronde River drainage basin covers approximately 10,700 km² of northeast Oregon (Figure 2). The Grande Ronde Basin headwaters are above 2,100 m in the Blue and Wallowa mountains, with some peaks in the Wallowa Mountains reaching 3,000 m, and joins the Snake River at RK 272, upstream from Asotin, Washington. The Grande Ronde River is fed largely by snow melt and peak runoff occurs from April through June. Catherine Creek and Lostine River are two of its larger tributaries. Catherine Creek enters the Grande Ronde River at RK 224, near the town of Cove, Oregon. Lostine River joins Wallowa River at RK 26, near the town of Wallowa, and Wallowa River flows into Grande Ronde River at RK 132. Salmon from the Grande Ronde Basin must migrate up to 1,120 km between the spawning/rearing grounds and the Columbia River estuary. This migration takes them through eight dams and their associated

reservoirs on the lower Snake and Columbia rivers.

Lookingglass Fish Hatchery

Lookingglass Fish Hatchery is located 4 km upstream from the mouth of Lookingglass Creek, a tributary of Grande Ronde River (RK 136). The Captive broodstock program uses 12 Canadian troughs for juvenile rearing and chillers (323 L/min total capacity) for temperature control. Water temperature is monitored automatically in all tanks with an integrated System Control and Data Acquisition system. It has a pathogen-free water supply using well water and unfiltered (not pathogen-free) stream water can be used in case of emergency to maintain fish. It also has a diesel powered emergency electrical backup system. Lookingglass Fish Hatchery reared the 1994-2001 cohorts of captive broodstock fish to smolt and will continue to rear the captive broodstock F₁ generation from fry to smolt.

Wallowa Fish Hatchery

The captive broodstock program will use Wallowa Fish Hatchery (WFH) for captive broodstock pre-smolt rearing instead of LFH beginning with collection of the 2002 cohort in August 2003. Wallowa Hatchery is located one mile west of Enterprise, Oregon, on Spring Creek (RK 1), a tributary to the Wallowa River (RK 66.8) which is a tributary of Grande Ronde River (RK 132). The captive broodstock program will use 12 semi-square tanks to rear the fish. Water sources include gravity flow spring water with a capacity of 296 Lpm, and two wells, each with a capacity of pumping 296 Lpm. Well temperature is 13° C and water temperature in the spring fluctuates seasonally between 5-11° C. Water sources can be blended to provide temperature control. The wells are equipped with alarms and a back-up generator. The well is pathogen-free and the artesian spring has been filled with rock to prevent fish colonization. We reared sentinel chinook salmon in the spring water for a full year without discovering any reportable pathogens in the fish.

Bonneville Fish Hatchery

Bonneville Fish Hatchery is located below Bonneville Dam on Columbia River (RK 234). The Captive Broodstock Facility is located at the hatchery and includes a 972 m² building with rearing and spawning facilities, office and storage. There are fifteen 6.1 m diameter rearing tanks and four 3.05 m diameter rearing tanks. Water comes from either a well or Tanner Creek and temperature ranges from 8.9-11.1° C. Dissolved oxygen is maintained between 7-10.7 ppm and there is an alarm system for drops in dissolved oxygen and low or high water levels. Effluent is filtered, to meet standards for effluent, first by a rotary filter that collects all particles >21 µm and then by an ultraviolet water purification system.

We also house equipment and storage facilities (liquid nitrogen) for cryopreserved semen at the Bonneville Captive Broodstock Facility. The 1.5 m stainless steel container holds 860 L of liquid nitrogen and 102,300 0.5 mL straws. We currently have over 500 semen samples cryopreserved.

Manchester Marine Laboratory

Manchester Marine Laboratory is a National Marine Fisheries Service lab located on Puget Sound near Port Orchard, Washington. In addition to other facilities, this lab accommodates the Oregon and Idaho Snake River chinook and sockeye salmon captive

broodstock program's saltwater rearing. A 400 m² building houses six 4.1 m circular tanks and a 1,280 m² building houses twenty 6.1 m diameter rearing tanks. Portable tanks (0.8-2.3 m²) are also available for use. Salmon here are raised in 7-13° C saltwater that is filtered through sand and ultraviolet filters. Dissolved oxygen is maintained between 9-10 ppm and oxygen is continuously bubbled into the tanks to insure adequate dissolved oxygen levels in case of water system failure.

METHODS

The following are general methods and schedules that were followed for collecting, rearing and spawning of each cohort. Variations may have occurred during a specific year and some methodologies have been modified as the program evolved. See each year's Annual Operating Plans for details of annual rearing strategies.

Fish Collection

We attempt to collect 500 spring chinook salmon parr from each of Catherine Creek, Grande Ronde River and Lostine River in August and September. Under ESA Permits 1011 and 1149, we are permitted to collect 500 or 25% of the available parr, whichever is less, from each of Catherine Creek, upper Grande Ronde River and Lostine River. The number to be collected is determined based on an estimate of parr abundance which is based on the number of redds counted during spawning ground surveys in the stream the previous spawning season. To estimate the number of available parr, we assume 4,000 eggs / redd (Oregon Fish Commission 1964; Galbraeth and Ridenour 1964) and 11.9% survival from egg to smolt (Carmichael and Boyce 1986). Information from the ODFW Early Life History crew and reconnaissance surveys are also used to estimate fish abundance in each section of the stream and fish distribution for each year.

Collections are made from throughout the drainage to incorporate as much genetic variability as possible into the captive broodstock. Fish are collected using a method in which snorkelers herd fish into a seine. Fish are then transferred into 19 L carboys until they are transferred into 151 L coolers containing well water. To reduce stress, fish collections are made only when stream water temperature is $<15^{\circ}\text{C}$.

Captive Juvenile Phase

At the end of each day's collection efforts, the fish are transported to LFH (to WFH with the 2002 and future cohorts) where they are measured for length and weight, checked for external parasites and randomly assigned to one of two pre-smolt treatment groups: accelerated or natural growth. Beginning with the 2000 cohort, one half of the fish were reared under each pre-smolt growth regime. Previous cohorts were divided into thirds, with one-third of the fish being reared under the accelerated growth regime (destined to be Freshwater Accelerated group) and two-thirds were reared under the natural growth regime (Freshwater Natural and Saltwater Natural groups).

Experimental treatment of natural and accelerated groups differs based on water temperature and amount of food fed (which is also based on water temperature and the ability of fish to metabolize food at a given temperature; Table 4). The "natural" growth treatment group is raised under a simulated natural growth regime that is designed to produce smolts that are of a size similar to that seen in wild salmon from the Grande Ronde Basin (approximately 120 mm TL and 20 g). Temperature for natural growth groups decreases to 5°C (the lowest that we are able to chill water), simulating a natural decrease in winter water temperature. The accelerated growth treatment maintains the fish at approximately 14°C throughout the winter and the fish are fed to satiation to encourage maximum growth. A naturally increasing photoperiod is important

Table 4. Photoperiod and temperature regimes for captive broodstock parr reared at Lookingglass Fish Hatchery. Note: beginning date of treatment is for the 1999 cohort and varies slightly each year.

Beginning date of treatment	Photoperiod			Temperature (°C)	
	Time on	Time off	Total hours of light	Natural treatment	Accelerated treatment
17 Aug	423	1928	15.1	12.2	12.2
31 Aug	442	1903	14.3	12.2	12.2
14 Sep	500	1835	13.6	12.1	12.2
28 Sep	517	1808	12.8	11.6	12.2
12 Oct	535	1742	12.1	10.0	12.2
26 Oct	553	1720	11.4	6.0	12.2
09 Nov	611	1701	10.8	6.0	12.2
23 Nov	629	1649	10.3	6.0	12.2
07 Dec	644	1643	10.0	6.0	12.2
21 Dec	654	1646	9.9	6.0	12.2
04 Jan	658	1657	10.0	6.0	12.2
18 Jan	654	1712	10.3	6.0	12.2
01 Feb	642	1731	10.8	6.0	12.2
15 Feb	624	1750	11.4	6.0	12.2
01 Mar	601	1809	12.1	6.0	12.2
15 Mar	536	1827	12.8	6.0	12.2
29 Mar	509	1846	13.6	6.8	12.2
12 Apr	443	1905	14.4	7.6	12.2
26 Apr	417	1924	15.1	9.4	12.2
10 May	355	1943	15.8	11.0	12.2

for a complete and coordinated smoltification in chinook salmon (Hoffnagle and Fivizzani 1998), so all treatments are reared under a simulated natural photoperiod that is adjusted every two weeks.

The captive broodstock fish are fed Moore-Clarke Nutra Plus food of an appropriate size for the size of the fish - sizes 1-3 crumbles and 1.5 mm pellets. Daily ration is 2.6% of body weight at 12° C and decreasing to 1.1% of body weight every second day at 6° C. In November, three months after capture, the parr are implanted with a Passive Integrated Transponder (PIT) tag to individually identify them.

We have employed a set of protocols to prevent diseases that are known threats to the program. First, a subsample of the incoming parr are visually checked for the presence of parasitic copepods. Second, the parr receive a prophylactic treatment for BKD by either an intraperitoneal injection of erythromycin on the day of capture, an intraperitoneal injection of azithromycin on the day of capture or a 10 day azithromycin medicated feeding as soon as possible after collection and adjustment to feeding. Additional prophylactic treatments (the number has varied) with erythromycin are given to the fish through the year. The 1998, 1999 and

2000 cohorts were given an injection of a BKD vaccine (Renogen). However, this inoculation was discontinued with the 2001 cohort because it has not appeared to be sufficiently effective in our program to warrant the additional handling and stress. Also, to prevent vibriosis, a vibrio inoculation is given to all fish at least two weeks prior to transfer to saltwater. Although vibriosis is a disease of saltwater-reared fish only, the inoculation is given to all fish to maintain consistency of treatment between experimental groups.

Captive Adult Phase

Rearing from smolt to adult is accomplished in either freshwater (BOH) or saltwater (MML). Beginning with the 2000 cohort, one half of the fish (50% of the natural growth group and 50% of the accelerated growth group) were/will be transferred to each of BOH and MML. In previous years, one third (one half of the natural growth group) of the fish were reared in saltwater and two thirds (one half of the natural growth group and all of the accelerated growth group) were reared in freshwater.

At smoltification (early May) the fish are transported to either BOH or MML. For the first three years of this program, we conducted salinity tolerance tests to determine the best time to transfer the fish to saltwater. Beginning in late April, six fish from each stock were placed in a tank containing 35 ppt artificial saltwater. If all six fish of a stock, survived for one week, then the entire population was transferred. If there was mortality, then another six fish were tested the following week. This method did not work well and no groups ever had 100% survival. One group was transferred as late as mid-June and suffered high mortality at MML, probably because they had smolted and were reverting back to freshwater physiology when they were finally transferred to saltwater. Currently, transfer of the majority of the saltwater fish is preceded by the transfer of ten sentinel fish to ensure that they have smolted and will thrive in saltwater. Sentinels are transferred to MML in early May and placed in 278 L tanks filled with freshwater. After they have been placed in the tank, saltwater is added to the tank at a rate of 7.6 L / min, replacing the freshwater. The fish are fed after two days and are observed closely for feeding behavior and signs of acclimation. If the sentinel fish survive and are actively feeding within seven days, then the remainder of the saltwater fish are transferred. If not, an additional ten sentinels are transferred and the process is repeated until the sentinels adapt well to saltwater. This method has worked very well to insure successful transfer to saltwater and we have seen little mortality due to incorrect timing of transport.

Fish at both BOH and MML are reared in separate tanks for each population and cohort, except for remaining five- and six-year old fish which are combined within each population. All fish are reared on a simulated natural photoperiod. At age 2, a Visual Implant (VI) tag is inserted in each fish for use as a secondary tag in case of loss of the PIT tag. The fish are fed according to their size and observed for general health.

The fish are also administered erythromycin as a prophylactic treatment for BKD at least twice each year (approximately December and June). The dose of erythromycin is 100 mg / kg fish weight / day with fish pills comprising about 30% of the feed for 28 days with a seven day withdrawal period before further handling or other stress. Although vibriosis is only a saltwater disease, in order to maintain comparable treatment of all groups, all fish are inoculated against *Vibrio* sp. Other diseases are treated, as needed.

The fish are sampled for growth (length and weight) and general condition during quarterly sampling in which 25 fish or 25% (whichever is greater) of each population, cohort and

treatment are examined. Once each year, all fish are examined, weighed and measured. For handling, all fish are anesthetized using MS-222 and are sometimes treated with hydrogen peroxide (1:3500 for one hour) after handling if fungal infection is a concern.

Bonneville Fish Hatchery

At BOH, captive broodstock fish are reared in pathogen-free, well water that ranges in temperature from 8.9-11.1° C. Water flows into the tanks at a rate of 270-795 L/min, depending on the density of fish in the tanks, which has ranged from 0.28-9 kg / m³. The highest densities occur when a cohort reaches four years of age and has not suffered much mortality. The fish are fed Moore Clarke 2-8.5 mm pellets at rates ranging from 2% of body weight for small fish to 0.37% for the largest fish.

Manchester Marine Laboratory

At MML, the fish are reared in filtered seawater from Puget Sound. Temperature ranges from 7-13° C (chillers maintain temperature at or below 13° C). Flow into the tanks ranges from 95-284 L/min, depending on the number and size of fish in the tank. Rearing density is kept below 8 kg / m³. The fish are fed Moore-Clarke 2.5-8.5 mm pellets and at a rate of 0.5-2% of body weight / day. Automatic feeders feed the fish approximately eight times each day.

Maturation and Spawning

The goal of the spawning protocol employed in this captive broodstock program is to maximize genetic diversity in the F₁ generation while minimizing the effects of gametes with low viability and the risk of losing gametes to donor mortality. Our approach considers the total spawning population, multiple age classes and use of cryopreserved semen, as well as balancing the logistic limitations associated with spawning. All fish are spawned within a matrix and spawning is conducted within populations and treatments. Furthermore, we are concerned about potential sibling crosses and inbreeding. Based on five years of captive brood spawning in this program, we may now assume that most females will mature at ages 4 and 5 and that most males will mature at ages 2, 3 and 4 - this dramatically reduces the likelihood of sibling crosses. We use the following decision-making process to spawn the captive broodstock. These protocols have been and will continue to be modified as we learn more about the process, but will follow similar principles. The general spawning protocol is as follows, developed during five years of spawning captive broodstock fish at BOH.

Sorting of maturing fish

Initially, we relied solely on visual determination of maturation in the fish. We examined the fish for changes in coloration, body shape and secondary sexual characteristics. This required handling the fish several times over the summer - as often as monthly from May through August. This method worked but the added stress on the fish (both maturing and immature) likely increased pre-spawning mortality.

We are now developing a method for sorting maturing from immature fish in the spring so that salmon reared in saltwater can be transferred to freshwater at the approximate time that wild fish from these streams are entering freshwater (March/April) and all maturing fish can be taken off feed, as they would in the wild. This should reduce stress on the fish (by reducing handling) and provide a more natural maturation cycle, particularly for fish reared in saltwater.

Rearing in saltwater beyond April/May is likely to stress kidney osmotic function and may affect egg quality. We are using ultrasound and testing near infrared spectroscopy to allow us to determine maturation status and sex of each fish. We are striving to be able to conduct a single, accurate and complete maturity sort in March/April for each year. During the first maturity sort at BOH in May 2001, ultrasound was used to determine maturity status and sex of each fish and discovered many maturing fish that would have been classified as immature by visual examination at that time. Use of ultrasound in March/April and May 2002 was successful but some fish were incorrectly classified. We began to evaluate the use of near infrared spectroscopy for early determination of maturation and sex in 2002. The need for additional maturity sortings in June or July is determined by each facility's fish culturist. A final maturity sorting is conducted in mid-August, regardless of the outcome of previous maturity sortings. Fish characterized as maturing during maturity sorts are transferred to tanks designated for holding maturing fish at BOH. These tanks are supplied with unfiltered water from Tanner Creek to give the fish a more natural temperature regime for maturation.

Immediately following the August maturity sorting, an inventory and erythromycin injection of all maturing fish is conducted by BOH personnel. The first ripeness sort is conducted during mid- to late August and we attempt to estimate the sex ratio (female:male) for each population and treatment at this time (we anticipate that ultrasound or NIR will provide conclusive sexing information when the fish are determined to be maturing). These estimated sex ratios are used to determine the type and number of spawning matrices to be used for each population/treatment during spawning. Additional ripeness sorts continue on a weekly basis throughout the spawning season. These sorts provide information on the number of fish available for spawning each week for each cohort, population, treatment and sex.

Spawning matrix development

Our objective is an equitable contribution to the next generation by all mature fish, within disease and survival constraints but without resorting to culling healthy eggs to equalize family contributions. We have focused on equalizing each parent's contribution to the next generation by maximizing the number of family groups (individual male x female combinations used in spawning) in each matrix, ensuring female fertilization by more than one male, preferring that males fertilize eggs from more than one female, and maximizing family group numbers in each matrix for a given number of spawners (i.e., a 2 x 2 matrix is preferred over a 1 x 3 matrix). The spawning matrix ratio and age distribution of the spawners is used to assign fish of a specific age, sex and treatment to each matrix. Our goal is to emphasize crosses between different age classes to reduce sibling crossing. We begin by assigning females, then males to matrices. When we have to use more than one fish from a given age class, we initially target mates from a different age class and then target mates from the age class with the greatest number of fish. For example, if we were using a matrix that called for 3 males, our preference would be 1 male from each age class. Our second choice in this example would be to have 2 males from the age class with the greatest number of fish and 1 male from a second age class. Our last choice would be to have 3 males from one age class, especially the same age class as the female.

Based on genetic and logistic considerations, we prefer equal numbers of males and females in each matrix, e.g., 4 x 4, 3 x 3 or 2 x 2 matrices (in that order). One-by-one (1 x 1) matrices and any matrix with only one male are not used. The female:male ratio (X) will fall into one of 11 categories and each category is associated with a particular spawning matrix (Table 5).

If accurate estimates of sex ratios for a population (i.e., the stock and treatment within which spawning will be conducted, e.g., Catherine Creek freshwater) are available, the ‘preferred matrix development protocol’ is employed throughout the spawning season. Population sex ratios should be made prior to the first spawn (we expect to improve this by using ultrasound or NIR). Ripeness sorts are conducted on a weekly basis throughout the spawning season which provide information on fish available for spawning each week by cohort, population, treatment and sex: these are not population sex ratios and are not used to determine sex ratios for matrix development purposes. If accurate sex ratio estimates are not available a back-up protocol is used.

The sex ratios for each population and treatment, as determined prior to the first spawn, are the target sex ratios used to develop spawning matrices throughout the spawning season. During each week of spawning these sex ratios are used for developing successive matrices until there are too few fish of either sex available to meet the target sex ratio for the respective population/ treatment combination. At this time, the criteria for the ‘Back-up matrix development protocol’ is employed. For example, if the target sex ratio is 3:2 (female:male) and there are 19 fish to spawn (11 females and 8 males), then the first three matrices would fall into category ‘D’ (3 x 2 matrices) which would leave 2 females and 2 males which would fall into category ‘F’ and would be spawned in a 2 x 2 matrix.

A back-up protocol is employed when too few fish of either sex are available to meet the target sex ratio under the ‘preferred matrix development protocol’ or the population sex ratio is unknown. Ripeness sorting is conducted throughout the spawning season to provide information on cohort, population, treatment and sex of fish available for spawning. Each week, when this process is completed, we determine the female:male ratio by population and treatment of fish that are ready to spawn. For each population and treatment, we assign a spawning category (A-K; Table 5) and develop the first matrix based on the spawning matrix ratio associated with that spawning category (generally we expect to be in categories E, F or G). After the first matrix is assigned, we recalculate the female:male ratio of the remaining spawners for that population and treatment and use the appropriate matrix to spawn. This is an iterative process that occurs after each successive matrix assignment.

The preferred ratio is one that falls in Category F (e.g., 1:1 sex ratio). Under Category F we will spawn fish in either a 4 x 4, 3 x 3 or 2 x 2 (female x male) matrix. Since 1 x 1 matrices will not be used, we may have to use one of the two smaller matrix configurations to avoid the possibility of 1 x 1 matrices. For example, when the sex ratio calls for use of Category F and ten fish are available (e.g., 5 females and 5 males) we will use one 3 x 3 matrix and one 2 x 2 matrix rather than one 4 x 4 and one 1 x 1 matrix.

Use of cryopreserved semen

Cryopreserved semen is used whenever there are fewer than two fresh males available for a spawning matrix (e.g., Categories A-C) - except in rare circumstances, at least one fresh male is used with every female. Whenever cryopreserved semen is used, each female is spawned with as many males as possible - up to four males (e.g., one fresh male and three cryopreserved semen samples). For example, if there is only one female and one fresh male from a given population and treatment for a given matrix, then three cryopreserved semen samples of the same population and treatment are used to make a 1 x 4 matrix. If there is more than one female, but only one fresh male for a matrix, then the fresh male is used with each female and cryopreserved semen

Table 5. Spawning categories with associated sex ratios (X) for development of spawning matrices.

Spawn category	Spawning population sex ratios (female/male)	Spawning matrix ratio	Spawning criteria and comments
A	$X > 77.5/22.5$	4 : 1	4 x 4; 1 fresh and 12 cryo (1 fresh with 3 cryo males/female); 50% eggs with fresh
B	$77.5/22.5 > X > 69.5/30.5$	3 : 1	3 x 4; 1 fresh and 9 cryo (1 fresh with 3 cryo males/female); 50% eggs with fresh
C	$69.5/30.5 > X > 63.0/37.0$	2 : 1	Matrix matches spawning matrix ratio; if cryo is used, 2 x 4; 1 fresh and 6 cryo (1 fresh with 3 cryo males/female); 50% eggs with fresh
D	$63.0/37.0 > X > 58.5/41.5$	3 : 2	Matrix matches spawning matrix ratio
E	$58.5/41.5 > X > 55.0/45.0$	4 : 3	Matrix matches spawning matrix ratio
F	$55.0/45.0 > x > 45.0/55.0$	1 : 1	Matrix matches spawning matrix ratio
G	$45.0/55.0 > X > 41.5/58.5$	3 : 4	Matrix matches spawning matrix ratio
H	$41.5/58.5 > X > 37.0/63.0$	2 : 3	Matrix matches spawning matrix ratio
I	$37.0/63.0 > X > 27.0/73.0$	1 : 2	Matrix matches spawning matrix ratio
J	$27.0/73.0 > X > 22.5/77.5$	1 : 3	Matrix matches spawning matrix ratio
K	$22.5/77.5 > X$	1 : 4	Matrix matches spawning matrix ratio

from three separate males is used for each female in the matrix to make a series of 1 x 4 matrices. This results in the use of one fresh male and as many as 12 cryopreserved semen samples to fertilize the eggs from a maximum of four females. If sufficient cryopreserved semen is not available to develop a four-male matrix, then cryopreserved semen from a single male may be used to fertilize the eggs from more than one female. When one fresh male is used in a matrix with cryopreserved semen, the eggs from each female in the matrix are divided as follows: 50% of the eggs are fertilized by the fresh male and 50% are fertilized by cryopreserved semen - i.e., 16.7% of the eggs will be fertilized by each of the three cryopreserved semen samples (Table 5).

Due to large differences in the fertilization rates between fresh and cryopreserved semen (see Analyses, below), we are examining this allocation of eggs between fresh and cryopreserved males. The present allocation results in a potential decrease in genetic diversity of the F_1 generation because cryopreserved males actually fertilize only approximately 34% of the eggs while fresh males fertilize approximately 80%. The present allocation system gives priority to female contribution to the F_1 generation and, hence, to smolt production, at the expense of cryopreserved male contribution. However, increasing the contribution of cryopreserved males will decrease the female contribution (and production). We are hoping to develop a strategy that will be more equitable to cryopreserved males while not seriously reducing female contribution.

Selection of cryopreserved semen to be used for spawning is done as follows. First, determine the population, treatment and cohort needed for the matrix. Second, randomly select a cryopreserved semen sample from all available samples for the appropriate population, treatment

and cohort. Lastly, activate part of the semen sample and check it for motility (present or absent). If motility is present, this sample will be used in the matrix. If motility is absent this sample will not be used in the matrix and another sample will be randomly selected.

If too few fresh males or cryopreserved semen samples are available to accomplish a two-male matrix, we attempt to use recycled males to make up the difference. Recycled males are live-spawned males that are held alive to make up for an expected insufficient cryopreserved semen supply. If too few cryopreserved semen samples and recycled males are available to achieve a two male matrix and if conventional broodstock (adults collected from nature to be spawned in the hatchery) males are available from appropriate populations, we would consider using them to achieve the two-male matrix. If all of these options combined do not allow us to achieve a two-male matrix we will modify the spawning matrix to ensure that all eggs are fertilized using whatever matrices are necessary. These scenarios have not yet occurred in this program and all efforts will be made to avoid them.

Spawning procedures

Mature fish are given an injection of erythromycin and placed in separate tanks designated for maturing fish at BOH based on population and age. Maturing fish from MML are transferred to BOH (transported in 3:1 freshwater:saltwater) to finish maturing in freshwater, as wild fish would do. At BOH, the fish are held in unfiltered Tanner Creek water (4.4-11.1° C) to expose them to chemical cues for maturation and a more natural seasonal and diel temperature regime. Mature fish are treated with formalin or hydrogen peroxide to combat fungal infection three times each week from the first mature sort through the first spawn. In the past, formalin was used at a concentration of 1:6,000 for one hour. In 2001 and 2002, hydrogen peroxide (1:3,500 for one hour) was, and will likely continue to be, used instead of formalin.

Spawning occurs from early September through mid-October (as fish mature). Ripeness sorts are conducted each Monday, beginning on the last week of August to separate ripe from green fish - based on the ability to expel milt from males and softness of the abdomen of females. Fish are identified using PIT and VI tags and an additional tag (jaw tag) is applied for quick and accurate visual identification during spawning and to insure identification in case both the PIT and VI tags are lost. Ripe males are placed in labeled nets and ripe females are placed in fish tubes (PVC, approximately 15 cm diameter and 1 m long) to prevent them from spawning in the tanks and allow easy retrieval of specific females for spawning. Fish are spawned only within populations and treatments and not usually within cohorts, to prevent sibling crosses (see matrix development sections above).

Males are spawned first and each is anesthetized and confirmed as being ripe before being taken from the tank. They are scanned for its PIT tag, VI tag or jaw tag (in that order) to obtain corresponding information from the database and fork length and weight are measured. The male is then placed on the spawning rack, wiped with an iodophor solution (200 ppm), dried and semen collected in a paper cup. Once semen is collected, the male is killed with a sharp blow to the head, unless it is to be recycled. All semen samples are tested for motility prior to combining semen with eggs - motility is evaluated as present or absent based on microscopic observation of sperm cells. The semen is divided into two, three or four labeled cups, depending on the number of females in the matrix. The cups are then placed in order in the refrigerator. This process continues for each male until semen has been collected from all males for a specific matrix.

Following semen collection, females are removed from the spawning tubes, anesthetized,

given a final check for ripeness and, if ripe, are killed by a blow to the head. They are scanned for their PIT tag, VI tag or jaw tag, weighed, length measured and placed on the spawning rack according to the mating order of the matrix. Their tails are cut to bleed the fish (to prevent blood from being mixed with the eggs and interfering with fertilization) and the fish are wiped down with an iodophor solution and then dried. The female's abdomen is cut open and the eggs fall into a colander and the eggs are divided into pre-weighed, labeled buckets. Buckets containing eggs are weighed and a sample of 20 eggs is collected from each female to calculate mean egg weight for fecundity estimation. If, after the eggs have been collected, the female displays gross signs of BKD (kidney is swollen and pus-filled), then the eggs from that female are culled (after being weighed) as a disease control measure. Cups of sperm are removed from the refrigerator and placed next to the appropriate bucket of eggs along with a cup of fresh well water. Sperm is poured on the eggs and the well water added to activate the sperm. After 30 seconds, excess sperm and water are decanted from the eggs. A 75 ppm iodophor solution is used to rinse the eggs and the buckets are filled with the solution, lids placed on the bucket and put aside to water harden for 40 minutes, undisturbed. The rest of the females on the spawning rack are spawned in the same manner to complete the matrix. After 40 minutes of water hardening, the eggs are transported to Oxbow Fish Hatchery (OFH), where they are placed in incubators.

Egg incubation and F₁ rearing

Incubation of eggs to the eyed stage is conducted at OFH. The eggs are incubated separately by family group to the eyed stage, at which time dead eggs are removed and live eggs are combined into maternal groups and shipped to Irrigon Fish Hatchery (IFH) for hatching and initial rearing. Also at eye-up, some eggs are culled to reduce the risk of horizontal and subsequent vertical transmission of BKD. Generally, eggs from females with ELISA values ≥ 0.8 are culled, although this level varies with egg production, space available at the hatcheries and management concerns.

At IFH, eggs are incubated at a range of temperatures designed to affect development so that all eggs will hatch at approximately the same time and all fish will be of a similar size. Eggs are incubated in stacks with eggs from females with lower ELISA values in the top rows and those from higher ELISA values in lower rows, to further reduce BKD transmission. Initial rearing is conducted in circular tanks at IFH until space becomes available at LFH after the previous cohort is released. Again, fish are segregated by BKD ELISA classifications, whenever possible. Fry are then transported to LFH and placed directly into raceways, in which they are reared. They are released at smoltification, after being acclimated, into their parents' natal stream.

Health Monitoring and Disease Treatments

All mortalities (including spawned fish) are inspected by fish health specialists to determine cause of death or disease state. There are several infectious diseases that could occur in the spring/summer chinook salmon maintained in the Oregon captive broodstock project. Among the infectious diseases that could or have occurred are: bacterial kidney disease, erythrocytic inclusion body syndrome (EIBS), bacterial gill disease (BGD), systemic gram negative infections [bacterial cold water disease (CWD), columnaris, enteric redmouth disease (ERM), aeromonad-pseudomonad septicemia (APS) and furunculosis] and infectious hematopoietic necrosis (IHN). External fungus on the body or gills is always a threat and

infestations by ectoparasites are also possible. By far, the most survival-threatening infection has proven to be BKD.

Because there are no reliable non-lethal or non-invasive techniques for sampling any of the agents causing the infections or infestations listed above, monitoring of morbidity and mortality is critical. This monitoring provides the primary basis for the need for antibiotic or chemical treatment of diseases. Daily observations of the fish by hatchery personnel and periodic inspections by fish pathology personnel help to identify conditions requiring treatment before clinical disease occurs. While there are capabilities for invasive sampling for some disease agents, these pose a greater risk and stress than can be justified for routine monitoring purposes.

Bacterial kidney disease

Kidneys of mortalities and moribund fish are assayed by ELISA and/or DFAT. Erythromycin treatments, via medicated feed, pills or injection, are initiated if a weekly mortality rate of $\geq 1.2\%$ (3/250) attributable to BKD occurs in any rearing unit. This does not apply if the fish have received a treatment within the prior 30 days.

Fish are treated for BKD by antibiotics and may be treated through feeding or injection. Dietary treatments (i.e., medicated feed and pills as aquamycin or erythromycin) are given at a dosage of 100 mg/kg body weight/day for 21 consecutive days. Fish are monitored closely for any signs of toxicity and are not handled for 7-14 days following the treatment. Erythromycin treatment of BKD by injection is at a dose of 20-30 mg/kg body weight. In fish with gross indications of BKD up to 40 mg/kg is used (maximum documented safe dose). Injections are given in the dorsal sinus or intraperitoneally. Unless an emergency treatment is required, all injections are given along with scheduled handling events. The order of preference for method of erythromycin treatment of BKD is:

- 1) Moore-Clarke Aquamycin medicated feed
- 2) Fish pills
- 3) Injections

The decision on which form of treatment to be used was based on the disease state, life stage of the fish in question, efficacy, availability and logistics. A new drug, azithromycin, may also be used to treat BKD in the future.

The 1998-2000 cohorts were vaccinated against BKD using the vaccine (Renogen, Aqua Health, Ltd.). The following factors were taken into consideration:

- 1) The manufacturer recommends that fish weigh at least 10 g.
- 2) Fish should not be treated with antibiotics within 30 days of vaccination, since the vaccine employs a live bacteria (closely related to *R. salmoninarum*, the causative agent of BKD) that will be killed by antibiotics and result in no immune response against *R. salmoninarum*.
- 3) Insure that physiological or environmental factors, such as temperature, do not interfere with the successful development of BKD resistance by the fish.
- 4) Vaccination should occur during a regularly scheduled handling event, to avoid additional stress to the fish.

In maturing fish, injectable erythromycin is given, via dorsal sinus or intraperitoneal injection at a dosage of 20-30 mg/kg body weight, during the maturity sort in July and repeated during the August sort. All mortalities of maturing fish are evaluated for BKD and erythromycin toxicity. If toxicity is prevalent or if other Gram-negative infections are indicated, injectable oxytetracycline is given.

Hatchery and pathology personnel monitor fish for external lesions at all opportunities and fish sorted as mature are monitored daily. Any rearing unit in which a fungus-infected fish was observed is immediately treated with three consecutive days of formalin flushes for one hour. Persistent fungus problems, such as in maturing adults, may require a weekly regimen of treatments on Monday, Wednesday and Friday, with minimal feedings on non-treatment days if the fish are non-maturing.

Other diseases

Monitoring for cold water disease, columnaris, enteric redmouth disease, aeromonad-pseudomonad septicemia and furunculosis is done by streaking smears from kidneys of morbidities and mortalities on TYE or TYES and TSA agar plates incubated at 18°C. Dietary or parenteral oxytetracycline treatment would be initiated if a weekly mortality rate of $\geq 1.0\%$ due to any single agent, occurs in a tank or raceway. The same treatment would be initiated if external lesions typical of CWD were observed. Romet would be used for furunculosis if oxytetracycline resistance were indicated. Oxytetracycline for CWD and ERM must be administered under a prescription. Parenteral oxytetracycline should be given by intraperitoneal injection at 10-20 mg/kg body weight.

Monitoring for bacterial gill disease is conducted by culturing smears from gills of all morbidities and mortalities and by daily observations by hatchery personnel for signs typical of BGD. Anytime BGD is suspected, wet mounts of gill tissue from moribund or fresh-dead fish will immediately be made and smears from gills collected on sterile cotton swabs will be made on TYE agar plates incubated at 18°C. If gill disease bacteria are observed microscopically or if gill disease bacteria are isolated, chloramine-T treatments according to Investigational New Animal Drug (INAD) protocols, will begin immediately in the rearing unit involved. The treatment regimen will depend on the degree of BGD determined.

If mortality reaches $\geq 1.2\%$ per week, without identification of etiological agents or causes, or if signs consistent with IHN virus are observed, assays for IHN and other viruses from morbidity and mortality will be conducted according to methods in the Fish Health Section Bluebook (Thoesen 1994). There are no treatments for IHN. Management of the disease could be attempted through density reduction if possible. Otherwise, the fish may be euthanized.

Hatchery personnel monitor the fish daily for signs of ectoparasitic infestation, such as flashing. If signs of ectoparasites are observed, fish pathology personnel will collect gill clip and skin scrape samples from freshly dead or moribund fish. Also, a subsample of incoming parr is visually inspected for the presence of *Salmincola* sp., a parasitic copepod. If copepods are present, a subsample is visually inspected at each handling event. If numbers of *Salmincola* sp. exceed five / side or if the majority of fish examined approach this level, treatments will be initiated: Ivermectin, physical removal or other technique. Treatments for most other ectoparasites will be in the form of formalin flushes as described for fungal treatments.

Most treatments listed above are standard and quite specific in some cases. It is often

necessary, however, to make adjustments from standard protocols to accomplish recovery of fish from infections. This would also be expected for captive broodstock fish. Indeed, other captive broodstock programs have encountered unexpected and even previously unknown diseases and fish health problems. Such situations may call for the use of innovative therapies.

By rearing the Captive broodstock fish in pathogen-free water at BOH and MML, the risk of introducing infectious agents back into the Grande Ronde River basin is very low. Their progeny undergo similar evaluations before they are released and are monitored for health and disease under currently established monthly and preliberation protocols.

There are two areas of concern relative to transfers to and from MML, where fish are raised in filtered Puget Sound sea water. First, marine infections and diseases might reduce survival. Second, acquisition of infections or diseases might preclude their transfer back to BOH. If pathogen-free water is 100% maintained, this should not pose a risk. Sand filtration and ultra-violet light treatment of influent salt water at MML, should prevent this. However, all fish are vaccinated against vibriosis at least 10 days prior to transfer of fish to MML. Vaccination is by intraperitoneal injection of a commercially available vibriosis bacterin, following the manufacturer's recommendations. If viral hemorrhagic septicemia virus (VHSV) or the Rosette agent is detected in the Oregon captive brood fish at MML, a decision of what to do with the fish will be made at that time.

All spawned fish (male and female) are sampled individually for the presence of *Renibacterium salmoninarum*. A sample of ovarian fluid is collected from each female spawned and semen is collected from a subsample of males (some males do not produce enough milt for a sample) to detect the presence of culturable viruses. Subsamples of tissues from fish from each population and cohort are examined for culturable viruses, EIBS and *Myxobolus cerebralis*. Levels of sampling will be determined by the pathology staff based on an assessment of infection risk and new sampling will be implemented, as necessary. Spawned fish will be visually examined externally and internally and lesions, fungus or other anomalies noted.

Monitoring and Evaluation

The Captive broodstock program is experimental. As such, it has an extensive monitoring and evaluation component, which is used to determine the effectiveness of experimental approaches and standard practices. As with all parts of this program, it is an ongoing process but it also will include the final tasks to be accomplished - assessing the effectiveness of using captive broodstock techniques in recovery of ESA listed salmonids. Specific annual objectives include:

- 1) Collect 500 parr for captive broodstock annually from Catherine Creek, Lostine River and upper Grande Ronde River populations.
- 2) Collect tissue samples from all collected fish for genetic analyses.
- 3) Monitor growth, development and survival to smoltification for each stock and treatment group.
- 4) Mark all individuals in each cohort and population with primary (PIT tag) and secondary (VI tag) marks.
- 5) Determine when fish are ready to be transferred to seawater.
- 6) Rear all fish to maturation.
- 7) Implement prophylactic treatments for bacterial kidney disease under INAD protocols.

- 8) Assess growth, survival and age at maturation for all stocks and treatments.
- 9) Determine etiology of captive broodstock morbidity and mortality.
- 10) Monitor fish culture practices and fish handling for situations that may contribute to impaired fish health or exacerbate disease.
- 11) Develop and implement complex matrix spawning protocols and oversee and facilitate the spawning of all ripe fish.
- 12) Coordinate Endangered Species Act permit activities and participate in captive broodstock planning and oversight.
- 13) Develop and maintain a comprehensive database for the program.
- 14) Analyze and summarize data and prepare reports and presentations to disseminate our findings.

Each captive broodstock cycle begins when 500 natural parr are collected from each stream in August/September, approximately 12 months after fertilization. Pre-smolt rearing for the 1994-2001 cohorts was conducted at Lookingglass Fish Hatchery and will be conducted at Wallowa Fish Hatchery beginning with the 2002 cohort. Post-smolt rearing is at either Bonneville Fish Hatchery or Manchester Marine Laboratory.

The first experimental treatments are conducted during pre-smolt rearing. We rear the fish under one of four (originally three) treatments: two pre-smolt and two post-smolt treatments. One pre-smolt treatment is a simulated natural growth regime, in which we grow the fish at a rate as close to that resembling natural as possible. This is accomplished by lowering the water temperature, from 12° C to 6° C, to simulate the winter temperature decline that occurs in natural streams during the winter. We also reduce feeding rates to coincide with this reduction in temperature. The other pre-smolt treatment is an accelerated growth regime, in which we maintain the water temperature at a steady 12° C in order to grow the fish as large as possible before smoltification. The second treatment begins at smoltification, when a portion of the smolts are transferred to Manchester Marine Laboratory for post-smolt rearing in saltwater and the other portion are transferred to Bonneville Fish Hatchery for post-smolt rearing in freshwater.

This creates four evaluation groups: Freshwater Natural (FN), Freshwater Accelerated (FA), Saltwater Natural (SN) and Saltwater Accelerated (SA). The Freshwater Accelerated (FA) group is reared entirely in freshwater and received the accelerated pre-smolt growth treatment. The Freshwater Natural (FN) and Saltwater Natural (SN) groups received the simulated natural pre-smolt growth treatment and were reared in freshwater or saltwater, respectively, following smoltification. The Freshwater Accelerated (FA) and Saltwater Accelerated Natural (SA) groups received the accelerated pre-smolt growth treatment and were reared in freshwater or saltwater, respectively, following smoltification. Originally, there were three experimental groups and one-third of the fish (SN) were reared at MML, while two-thirds (FA and FN) were reared at BOH. Beginning with the 2000 cohort (captured in 2001), one-half of the fish have been allocated into each pre-smolt growth group and one half of each of those are transferred to each of BOH and MML, creating the fourth evaluation group (SA).

The two primary treatment evaluations are a comparison of fish reared, as juveniles, at either an accelerated rate or a natural rate and a comparison of fish reared exclusively in freshwater to those reared in freshwater as juveniles and in saltwater as adults. Variables other than environmental salinity and juvenile growth rate remain as similar between treatments as possible. For example, at all times, all fish will be reared under a simulated natural photoperiod.

populations and treatment groups will always be kept separate and cohorts will be kept separate until spawning. After spawning, F_1 generation fish resulting from parents of a certain treatment group will be kept separate from those produced from parents of a different treatment group, at least until time of tagging. We will compare various parameters among populations, cohorts, age classes and treatments, as appropriate, using statistical tests at $\alpha=0.05$.

Captive broodstock fish are reared from collection as parr through maturation, spawned and their progeny are reared to the smolt stage and released into the stream from which their parents were collected. These progeny return and spawn naturally and their offspring complete a natural cycle. This cycle typically requires 1-5 years of captive rearing to reach maturity and spawn, 1.5 years of F_1 juvenile rearing to smolt and release, 1-3 years for F_1 adult returns and 1.5 years for natural F_2 smolt production and 1-3 years for F_2 adult returns. Logical evaluation points are during and at the end of these periods of the cycle. Hence, to completely evaluate a cycle requires up to 14 years (Figure 3; Table 6). The experimental design requires a minimum of five cycles, thus requiring 19 years to completion.

There are numerous uncertainties associated with captive broodstock programs. Therefore, we assess the program at key life history phases in the production cycle. We have divided the cycle into four phases: the Captive Juvenile Phase, the Captive Adult Phase, the F_1 Generation Phase and the F_2 Generation Phase. Each phase is further subdivided into discrete periods. Data collected during each phase and period are critical for evaluating treatment and overall program performance. Critical variables measured during each phase/period are described below.

The *Captive Juvenile Phase* begins at collection and ends once fish have been transferred to BOH or MML. It is composed of two periods: pre-smolt growth and smoltification. The primary measures of performance for this period of the cycle are growth, survival, condition, size distribution, smoltification and disease profile. Sampling occurs throughout the period to gather the necessary data.

The *Captive Adult Phase* begins at transfer to either BOH or MML and ends when the fish die - either before or at spawning and is composed of three shorter periods: post-smolt growth, maturation and spawning. Performance during the post-smolt period is assessed primarily by growth, condition, survival and disease profile. A broad array of variables are measured during the maturation period including: external morphology characteristics, date of mature recognition, degree of ripeness, ultrasound characteristics, age, time of maturation, survival and sex ratios. The key performance measures for the spawning period include: age and size at maturity and spawning, spawn timing, egg size, fecundity, sperm viability, fertility and disease profile.

The *F_1 Generation Phase* begins at fertilization of eggs from captive broodstock fish and ends when the resulting fish die. This phase is composed of the incubation, juvenile rearing, smolt release, post-smolt growth, maturation and spawning periods. Many of the standard hatchery evaluation variables are used to assess performance. Important variables include: egg survival, hatching time, fry survival, growth rates, condition, size distribution, fry-smolt survival, smolt outmigration performance, smolt-to-adult survival, catch distribution, run timing, age structure at return, size-at-age, sex ratios, pre-spawn survival in nature, spawning distribution in

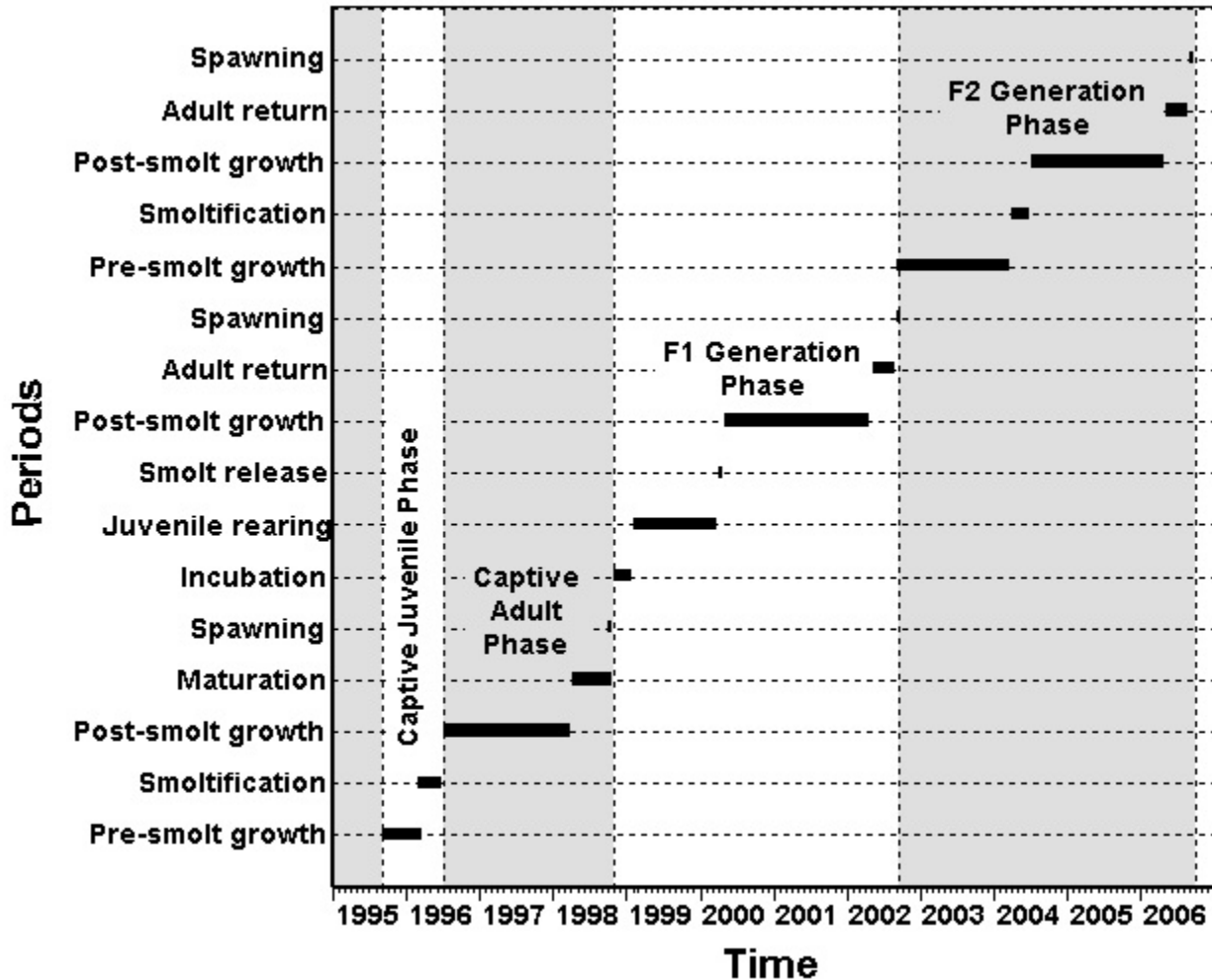


Figure 4 Captive broodstock monitoring and evaluation terminology and example time scale for fish maturing at age four.

nature, spawning success and straying.

The *F₂ Generation Phase* begins once embryos resulting from *F₁ Generation* fish are formed and ends when fish from these embryos die. This phase is composed of the pre-smolt, smolt, post-smolt growth, adult return and spawning periods. During this period we measure variables in the natural environment to assess the natural production performance of captive fish reproducing in nature. Variables include egg-to-fry survival, egg-to-smolt survival, juvenile tributary migration patterns, growth rates, parr and smolt production, smolt migration patterns, smolt-to-adult survival, run timing, age structure at return, size and age at maturation, sex ratios, pre-spawn survival in nature, spawning distribution in nature, spawning success, straying and productivity (progeny to parent ratios).

We measure an array of variables in each period of the cycle which will allow us to compare and evaluate performance of our experimental treatments (FN, FA, SN, SA), monitor basic progress in fish culture, detect areas of concern that may need our immediate attention and

Table 6. Captive broodstock monitoring and evaluation terminology and example time scale for fish maturing at age four.

Phase/period	Example of approximate actual time period for fish maturing at age four
<u>Captive Juvenile Phase: August 1995 - June 1996</u>	
pre-smolt growth period	August 1995 - April 1996
smoltification period	April - June 1996
<u>Captive Adult Phase: June 1996 - September 1998</u>	
post-smolt growth period	June 1996 - April 1998
maturation period	April 1998 - October 1998
spawning period	September - October 1998
<u>F₁-Generation Phase: September 1998 - September 2002</u>	
incubation period	September 1998 - February 1999
juvenile rearing period	February 1999 - March 2000
smolt release period	March - April 2000
post-smolt growth period	April 2000 - April 2002
adult return period	April - August 2002
spawning period	August - September 2002
<u>F₂-Generation Phase: September 2002 - September 2007</u>	
pre-smolt period	September 2002 - March 2004
smolt period	March - June 2004
post-smolt growth period	June 2004 - April 2006
adult return period	April - August 2006
spawning period	August - September 2006

judge the adequacy of benchmarks that we used to design the captive broodstock program. We measure fork length of each fish and weight of a sample of fish at collection and at specific sampling periods to assess the growth profile and condition of captive fish. We implant PIT tags in November (approximately 15 months after fertilization) and VI tags the following summer (approximately 23 months following fertilization) to allow us to track individual fish. As fish mature, we make standardized visual observations of the fish and have begun using ultrasound and testing near infrared spectroscopy to determine maturation and sex at early stages. We record age and size of maturing fish and assess whether information on growth and condition of the fish at earlier life stages can be used to predict age or time of maturity. At spawning, we measure fork length and weight of all spawned fish; determine spermatocrit and sperm viability of all spawned males; determine fecundity and mean egg weight; and calculate the rate of egg

fertilization of each male x female cross. We also calculate overall survival rate during the Captive Juvenile and Captive Adult periods.

The offspring produced by the captive broodstock program are integrated into the LSRCP hatchery supplementation program. For the F₁ generation, we compare hatching time and success and survival rates between green egg, eyed egg, fry and smolt stages. We also measure length and weight through the rearing period for each treatment to assess growth and condition of the fish. Following release of the fish into nature, we will record timing of adult migration and spawning and size and age of return and calculate and compare smolt-to-adult survival rate, stray rate, age-at-return, spawning success, length at age by sex and time-at-return for each treatment group and between hatchery-reared and naturally-produced fish. We will also collect genetic samples to assess spawning success - contribution to the F₂ generation. An increase in the number of wild spawning fish above that which would have been produced without the hatchery program (due to the F₂ generation) will be the final measure of success for this program.

F₁ and F₂ Generations

All spawning is conducted at Bonneville Fish Hatchery - mature captive broodstock fish from MML are transported to BOH where they are held with mature fish from BOH under a simulated natural photoperiod and in Tanner Creek water, so they can experience natural water temperature fluctuations to help synchronize maturation. When fish become ripe, eggs and sperm are collected, mixed to fertilize the eggs and water-hardened in a 75 ppm Argentyne solution. F₁ generation embryos from a given male-female pairing are kept separate from embryos from other pairs at Oxbow Hatchery. Embryos are kept distinguishable by female until the eyed stage. Eyed eggs are incubated and hatched at Irrigon Hatchery and the fry are started on fish feed at swim-up. Fry are transported to LFH for completion of rearing to smoltification.

F₁ generation fry are reared in outdoor raceways supplied with Lookingglass Creek water at LFH. Progeny from each treatment group are fin-marked (adipose-clipped) and coded-wire-tagged during June and July to permit later identification. As many as 50,000 fish are reared in each pond, approximately one half of the density for which the raceways were designed. Fish are reared and sampled according to standard protocols at LFH and targeted for 23 g, or a mean fork length of 125 mm, at their release as yearling smolts. A portion of the fish in each raceway are PIT-tagged to monitor outmigration survival and characteristics.

In March, the fish are transported to acclimation facilities located within the area of their parents' natal stream where natural fish spawn. Fish from each evaluation group within a given population may be mixed together at the time of transportation. Acclimation sites are supplied with ambient stream water and fish at these sites are given supplemental feed. In April, after a 14-30 day period of acclimation, fish are released into the stream.

We anticipate that some of these fish will mature one, two or three summers after they are released. Weirs are installed near the mouth of the Lostine River, on Catherine Creek near the town of Union and on the upper Grande Ronde River upstream of the town of La Grande. All captive broodstock F₁ adults will be allowed to spawn naturally (some may be collected at weirs for use as spawners in unseeded habitat) - no returning adults from the captive broodstock F₁ generation adults will be used for hatchery broodstock.

F₁ generation fish that survive to spawn may reproduce with other natural fish or other F₁ generation fish. The majority of the successful progeny produced from these matings are expected to migrate to the ocean as yearlings and return when they are 3, 4 and 5 years old. We

will monitor the production and life history characteristics of the F₂ generation fish. Standard sampling will be conducted on pre-smolts to determine their relative abundance and to collect morphometric data and tissue for subsequent genetic analysis. Some fish may also be tagged so their migratory behavior can be evaluated. Juvenile migrant traps and weirs are placed in Lostine and upper Grande Ronde rivers and Catherine Creek. The production and timing of fish migrating to and from the ocean will be monitored. Characteristics of each study population will be evaluated prior to, during and (potentially) after the captive broodstock program.

RESULTS

This report summarizes the data collected in this study and provides cursory statistical analyses of these data. Few evaluations of methodologies have been conducted other than those that have been ongoing as part of this project. Comprehensive data analysis for this project will be complicated, since there are many covariates that may affect each variable. Therefore, further analyses will be conducted when the database is ready and sufficient data have been collected to make sound conclusions. Additionally, we are currently reorganizing our database, which will facilitate statistical analyses and reporting of the large amount of data collected by this project.

This captive broodstock program was initiated in the Grande Ronde Basin in 1995. We have collected eight cohorts (1994-2001) of chinook salmon parr from Catherine Creek and Lostine River in 1995- 2002 and six cohorts from the upper Grande Ronde River (in 1995, 1997-1999, 2001 and 2002 - collections were attempted in 1996 and 2000 but were unsuccessful). Each year, we collected 500 (or nearly) fish from Catherine Creek and the Lostine River (Table 7). Only 110 fish were collected from the Grande Ronde River in 1995 (1994 cohort) and no fish were collected from the 1995 and 1999 Grande Ronde River cohorts. Fish were reared at LFH until the yearling smolt stage and then transferred to BOH and MML for the Captive Adult Phase. Fish collected in 2002 (2001 cohort) were transferred to BOH/MML in June 2003.

1994 Cohort

Collections

The 1994 cohort (498 fish) was collected from Catherine Creek from 29 August-1 September 1995 between river kilometers (RK) 29.8-52.0. Grande Ronde River fish (110) were collected from 18-22 September between RK 296-325. Lostine River parr (499) were collected from 14-17 August between RK 1.3-31.0. There were no collection-related mortalities in any of the populations.

Size at Collection

Size at collection of chinook salmon parr varied with population (Figure 4). Grande Ronde River parr were the largest, with a mean fork length of 97.8 mm (range: 80-125) and weight of 11.29 g (5.8-21.8). Lostine River parr were the smallest, with a mean fork length of 80.7 mm (65-118) and weight of 7.26 g (3.3-25.4). Catherine Creek parr had a mean fork length of 93.3 mm (72-110) and weight of 10.34 g (4.9-17.8).

Growth

The Saltwater Natural growth groups were larger until about the age 4 in both the Catherine Creek and Lostine River populations - no Grande Ronde River fish were raised in saltwater (Figure 5). Overall, the populations grew at similar rates.

Catherine Creek

Growth of the Catherine Creek fish was similar between the Freshwater Natural and Saltwater Natural growth regimes (Figure 5). Mean fork length and weight of the Freshwater Natural treatment group was 187.0 mm and 87.68 g in July 1996, 316.2 mm and 471.39 g in July

Table 7. Number of spring chinook salmon parr collected from the 1994-2001 cohorts in Catherine Creek, upper Grande Ronde River and Lostine River annually from 1995-2002, number of mortalities and number surviving to spawn, as of 31 December 2002. Note: 1994 cohort was collected in 1995, etc. "Survived" means that the fish survived to spawn although gametes may not have been collected - e.g., we attempted to spawn some fish too early.

Cohort/variable	Stream		
	Catherine Creek	Grande Ronde River	Lostine River
<u>1994</u>			
Collected	498	110	499
Mortality	138	80	268 ^a
Survived	360	30	231
<u>1995</u>			
Collected	500	0 ^b	481
Mortality	258	0	210
Survived	242	0	271
<u>1996</u>			
Collected	500	500	501
Mortality	153	98	237
Survived	347	402	263
<u>1997</u>			
Collected	500	500	500
Mortality	177	83	158
Survived	323	417	342
<u>1998</u>			
Collected	500	500	498
Mortality	142	267	102
Survived	325	221	368
<u>1999</u>			
Collected	503	0	500
Mortality	138	0	73
Survived	113	0	172
<u>2000</u>			
Collected	503	502	503
Mortality	27	33	71
Survived	17	6	42
<u>2001</u>			
Collected	500	461	500
Mortality	4	5	13
Survived	0	0	0

^a Includes 49 fish that jumped out of the tank soon after collection.

^b One Grande Ronde River chinook salmon parr was collected but returned to the river.

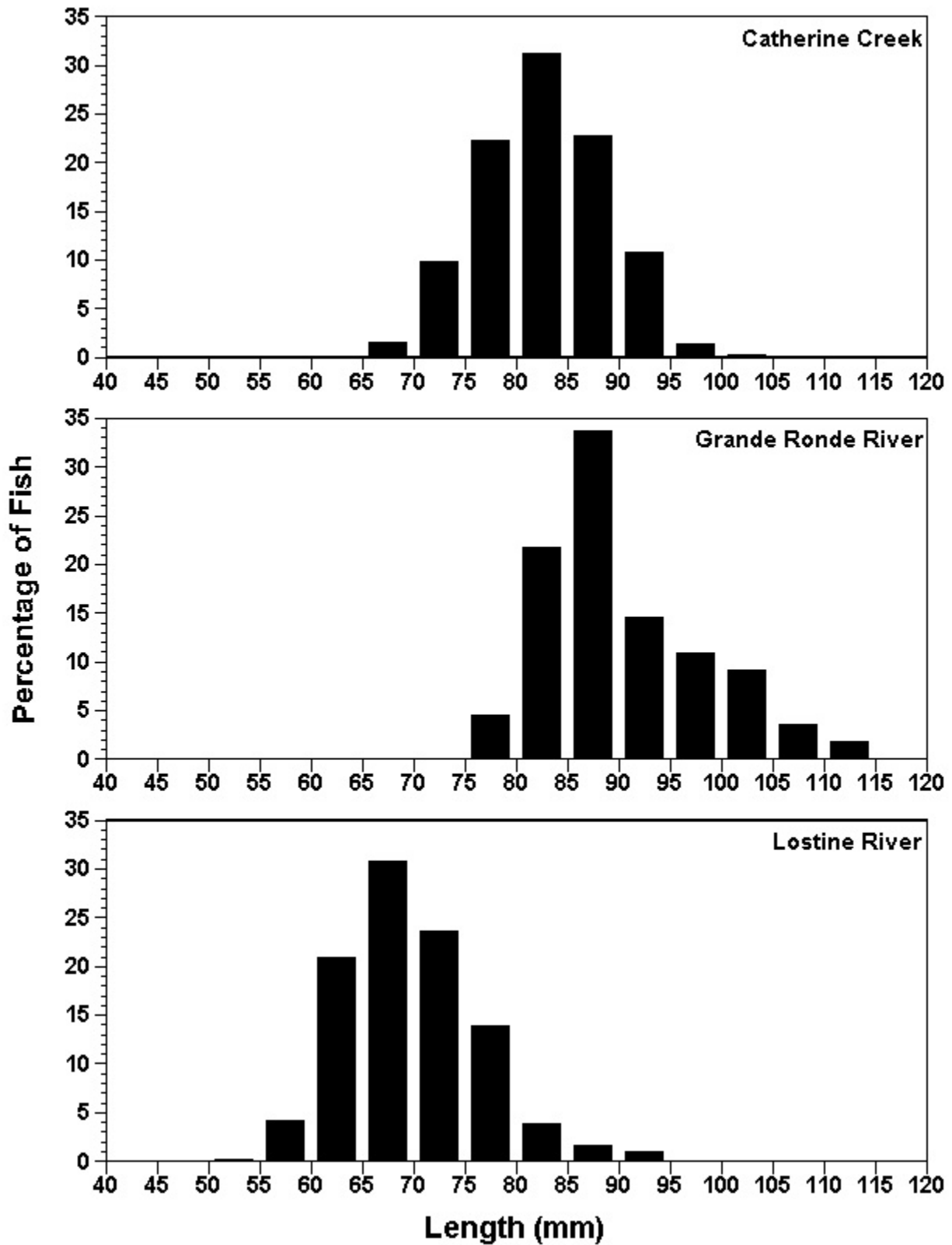


Figure 5 Length frequency distribution for 1994 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1995.

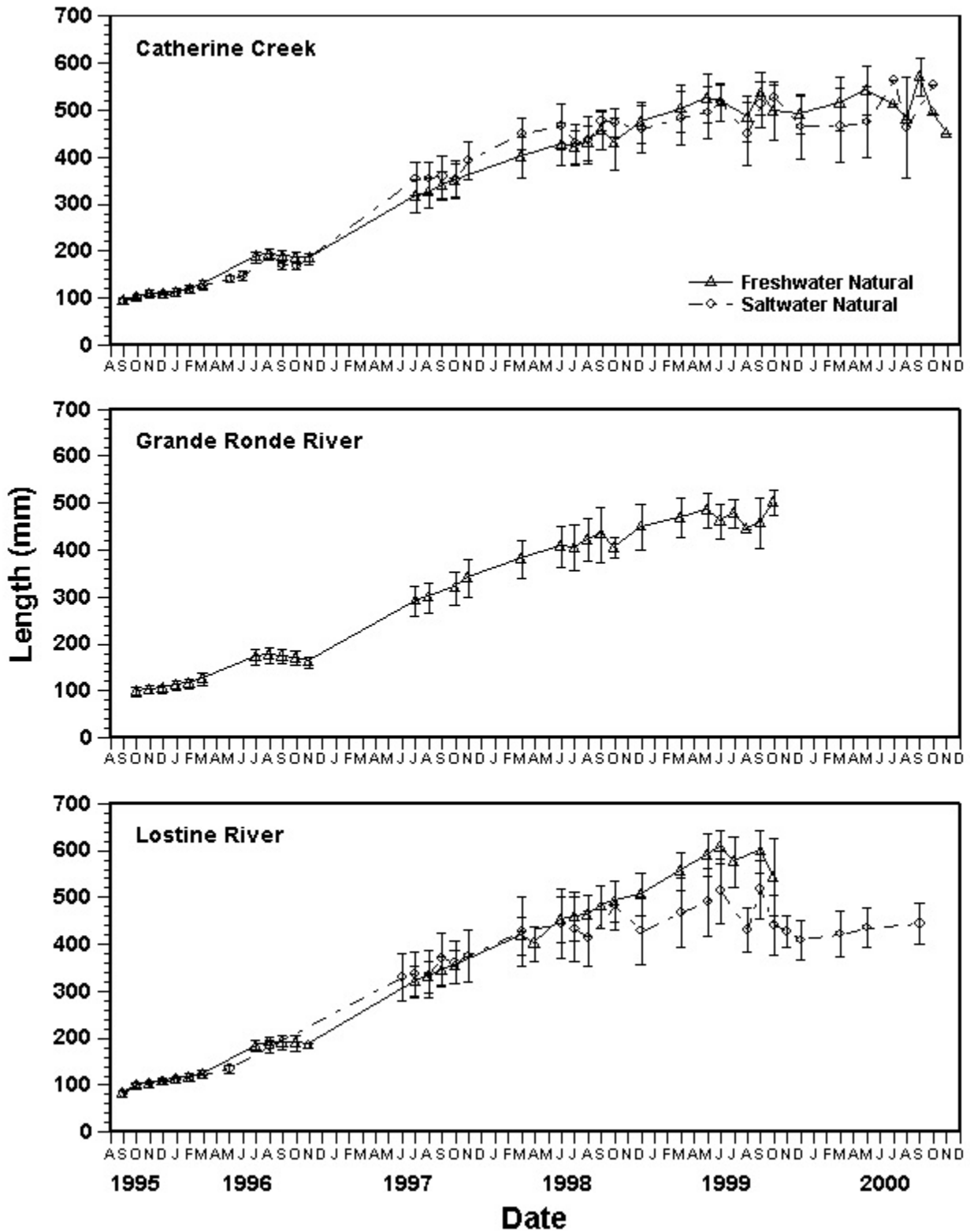


Figure 6 Mean length (± 1 SD) of 1994 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Natural and Saltwater Natural growth regimes, 1995-2000.

1997, 419.2 mm and 1034.12 g in July 1998, 515.8 mm and 2199.85 g in June 1999 and 541.7 mm and 2395.50 g in May 2000.

The Saltwater Natural growth regime fish grew to 148.4 mm and 42.70 g in June 1996, 354.2 mm and 627.20 g in July 1997, 428.3 mm and 1061.70 g in July 1998, 516.0 mm and 2197.67 g in June 1999 and 474.8 mm and 1720.71 g in May 2000.

Grande Ronde River

All Grande Ronde River fish were raised in freshwater. These fish were 170.8 mm and 66.25 g in July 1996, 291.0 mm and 365.49 g in July 1997, 404.6 mm and 1020.73 g in July 1998 and 477.0 mm and 1771 g in July 1999 (Figure 5).

Lostine River

The Freshwater Natural Lostine River fish were larger as they reached maturity than the Saltwater Natural fish (Figure 5). Mean fork lengths and weights of Freshwater Natural fish were 183.9 mm and 85.15 g in July 1996, 320.3 mm and 484.40 g in July 1997, 458.4 mm and 1404.33 g in July 1998 and 574.8 mm and 3214.72 g in July 1999.

The Saltwater Natural fish were 182.5 mm and 81.64 g in August 1996, 336.2 mm and 523.99 g in July 1997, 432.6 mm and 1111.42 g in July 1998, 513.3 mm and 2177.49 g in June 1999 and 435.4 mm and 1213.00 g in May 2000.

Mortality

All of the 1994 cohort fish are now dead: 56.0% were spawned or had semen cryopreserved. Causes of mortality varied among populations, treatments and ages (Table 8). Bacterial kidney disease and unknown causes were the most common sources of non-spawning mortality among all populations, ranging from 5.8-56.0%. The Lostine River stock lost 16.0% of the fish to operational causes.

The Freshwater Natural group had 55.3% and the Saltwater Natural group had 57.9% of the fish survive to contribute gametes (Table 8). Bacterial kidney disease killed at least 22.2% of the Freshwater Natural fish and 5.3% of the Saltwater Natural fish. Unknown causes (some of which were probably BKD) accounted for 7.6% of the Freshwater Natural and 25.1% of the Saltwater Natural mortalities.

Causes of mortality changed with age (Table 8). Gamete collection was the cause of the majority of mortalities of fish at ages 2 (38.9%), 3 (71.4%), 4 (49.6%), 5 (79.3%) and 6 (39.1%). Bacterial kidney disease was a large mortality factor for the ages 4 (33.5%) and 6 (39.1%) fish, particularly those from the Grande Ronde and Lostine rivers. Unknown causes accounted for a large percentage of the mortalities at ages 1-3 (10.6%, 30.9% and 11.8%, respectively). Operational causes were the cause of 84.8% of the age 1 mortalities.

Survival to Spawning and Age of Maturity

All 1994 cohort fish have died and 621 of 1107 parr collected (56.1%) survived to contribute gametes. Within treatments, 55.3% of the Freshwater Natural fish survived to spawn: of those, 40.8% were females, 6.9% were males that were spawned and 52.4% were males that had their semen cryopreserved (Figure 6). In the Saltwater Natural treatment group 57.9% survived to spawn, of which 54.1% were females, 8.7% were males that were spawned and 37.2% were males that had semen cryopreserved.

Table 8. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SN=Saltwater Natural) and age class of 1994 cohort spring chinook salmon. Note: "unknown" mortalities include 1 Catherine Creek mortality which has not yet been examined for cause of death.

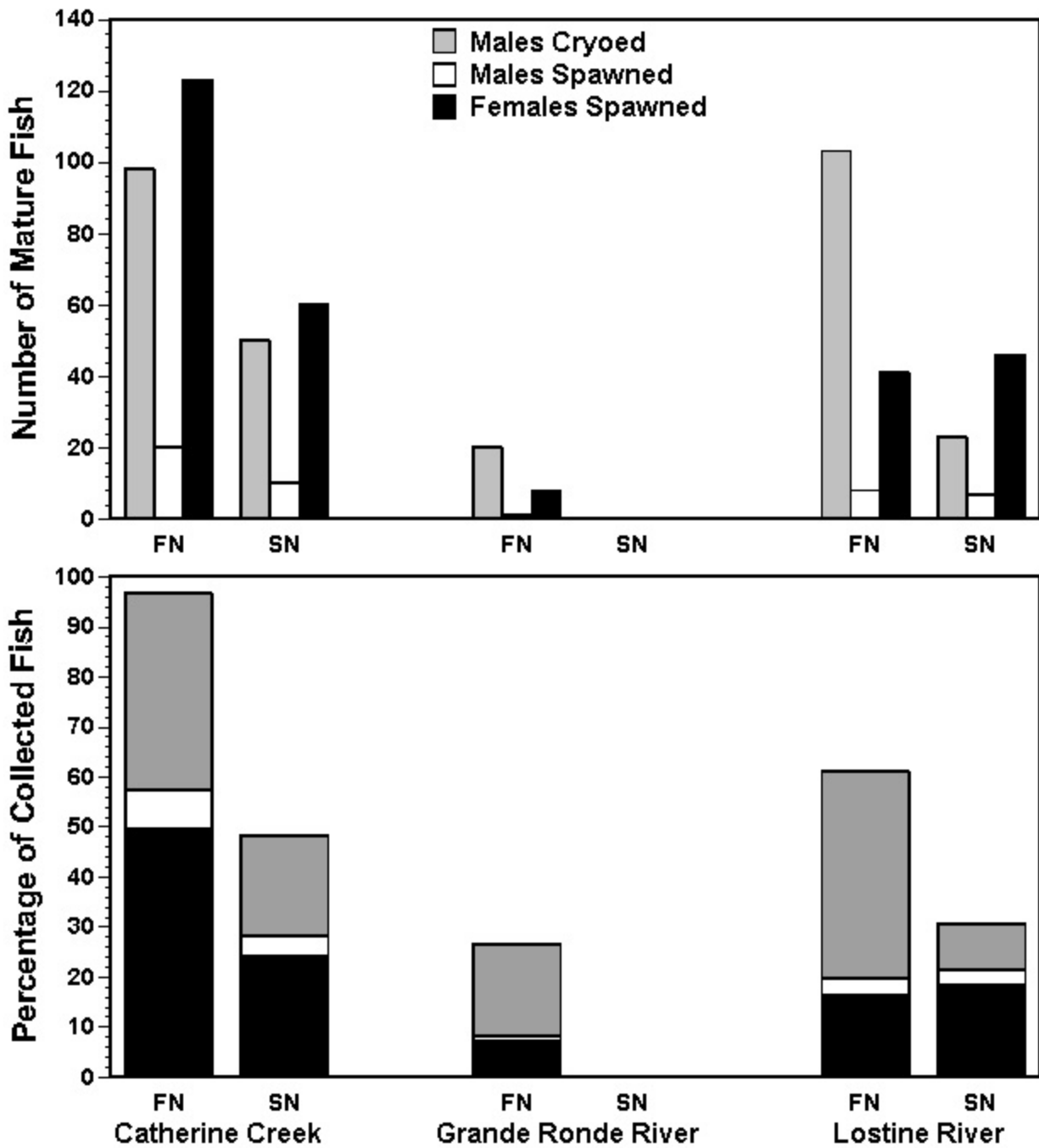
Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	360	72.1	20	4.0	29	5.8	21	4.2	3	0.6	65	13.2
GR	30	27.5	1	0.9	61	56.0	5	4.6	2	1.8	10	9.2
LR	231	46.1	80	16.0	98	19.6	14	2.8	8	1.6	70	14.0
<u>Treatment</u>												
FN	423	55.3	79	10.3	170	22.2	30	3.9	5	0.7	58	7.6
SN	198	57.9	22	6.4	18	5.3	10	2.9	8	2.3	81	25.1
<u>Age</u>												
1	0	0.0	56	84.8	3	4.5	0	0.0	0	0.0	7	10.6
2	68	38.9	19	10.9	23	13.1	9	5.1	2	1.1	53	30.9
3	217	71.4	23	7.6	18	5.9	6	2.0	4	1.3	36	11.8
4	170	49.6	2	0.6	115	33.5	17	5.0	5	1.5	34	9.9
5	159	79.3	0	0.0	20	10.1	6	3.0	2	1.0	13	6.6
6	7	39.1	1	4.3	9	39.1	2	8.7	0	0.0	2	8.7
Total	621	56.0	101	9.1	188	17.0	40	3.6	13	1.2	145	13.1

Sex ratios of spawners shifted with age (Figure 7). Of those fish that matured, 11.04% matured at age 2 and 34.9% at age 3; 100% of these age classes were males from which semen was cryopreserved. At age 4, 27.4% matured (67.0% were females, 24.3% were males that were spawned and 8.7% were males had semen cryopreserved), 25.3% matured at age 5 (95.6% were female and 4.4% were males that were spawned) and 1.1% (all females) matured at age 6.

1995 Cohort

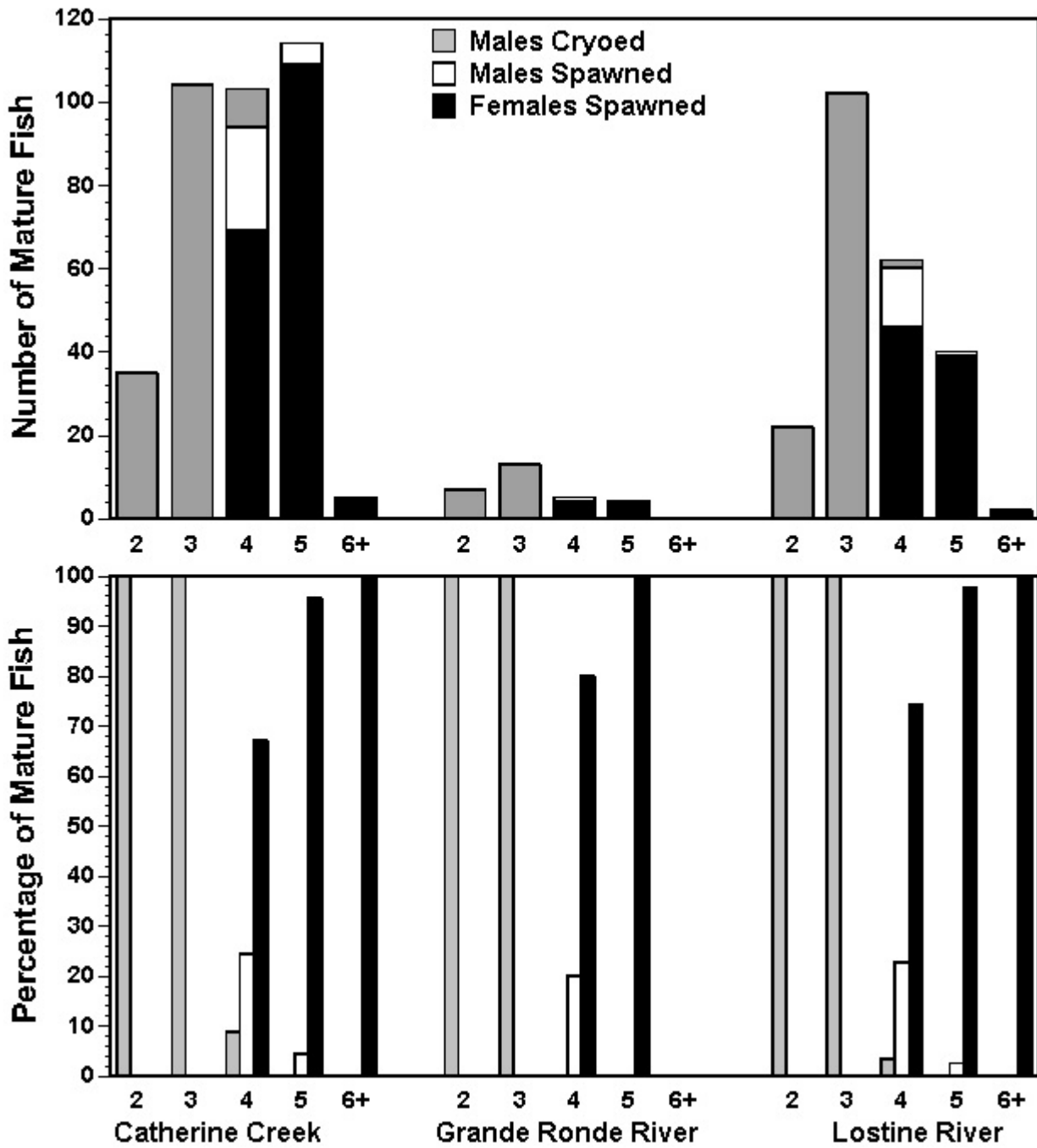
Collections

The 1995 cohort (500 fish) was collected from Catherine Creek from 29 August-1 September 1996 between RK 29.8-52.0. Lostine River parr (481) were collected from 13-16 August between RK 1.3-31.0. We searched for Grande Ronde River fish from 16-18 September between RK 269-325 but only one was found. There were no collection-related mortalities in any of the populations.



Stock and Treatment

Figure 7 Number and percentage of 1994 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.



Stock and Treatment

Figure 8 Number and percentage of 1994 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that matured to contribute gametes (spawn or have semen cryopreserved) at ages 2-6.

Size at Collection

Size at collection of chinook salmon parr varied with population (Figure 8). Lostine River parr were the smallest, with a mean fork length of 79.0 mm (range: 56-103) and weight of 5.98 g (2.0-12.8). Catherine Creek parr had a mean fork length of 85.2 mm (65-108) and weight of 7.97 g (3.2-12.2). Only one 1995 cohort Grande Ronde River parr was captured so it was returned to the river so there was no 1995 Grande Ronde River cohort in the program.

Growth

The Saltwater Natural growth groups generally grew slower, although there was little difference in mean size (Figure 9). Both populations also grew similarly.

Catherine Creek

Growth rate of Catherine Creek fish was similar among the three growth regimes (Figure 9). However, the Freshwater Natural fish were consistently larger and the Saltwater Natural fish were often smaller than the other fish. Mean fork length and weight of the Freshwater Accelerated growth group was 147.9 mm and 40.89 g in July 1997, 270.3 mm and 281.43 g in July 1998, 455.5 mm and 1515.53 g in June 1999 and 495.4 mm and 1924.94 g in May 2000. The Freshwater Natural growth regime fish grew to 142.8 mm and 33.83 g in July 1997, 290.2 mm and 342.70 g in July 1998, 468.0 mm and 1704.16 g in June 1999 and 535.2 mm and 2465.96 g in May 2000. The Saltwater Natural growth regime fish grew to 149.4 mm and 43.12 g in August 1997, 295.9 mm and 370.69 g in July 1998, 419.0 mm and 1175.41 g in May 1999 and 482.2 mm and 1749.65 g in May 2000.

Grande Ronde River

Only one Grande Ronde River BY1995 chinook salmon was captured and it was returned to the river. As a result, there was no 1995 Grande Ronde River cohort in the Captive broodstock program.

Lostine River

Freshwater Natural fish were consistently larger and Saltwater Natural fish consistently smaller than the Freshwater Accelerated group (Figure 9). Mean fork lengths and weights of Freshwater Accelerated fish were 159.9 mm and 51.39 g in July 1997, 327.6 mm and 548.33 g in July 1998 and 498.6 mm, 2044.52 g in July 1999 and 528.7 mm and 2215.33 g in July 2000. The Freshwater Natural fish were 145.4 mm and 35.69 g in July 1997, 335.1 mm and 562.99 g in July 1998, 523.3 mm and 2344.58 g in June 1999 and 598.1 mm and 3292.90 g in July 2000. The Saltwater Natural fish were 158.6 mm and 52.42 g in August 1997, 318.3 mm and 485.14 g in June 1998, 478.2 mm and 1951.12 g in June 1999 and 516.5 mm and 2262.90 g in May 2000.

Mortality

Causes of mortality varied among populations, treatments and ages (Table 9). All of the 1995 cohort fish have died and 52.3% were spawned/cryopreserved: 48.7% of the Catherine Creek and 56.6% of the Lostine River fish. Bacterial kidney disease was the largest cause of non-spawning mortality in the Catherine Creek and Lostine River populations (25.4% and 26.5%, respectively).

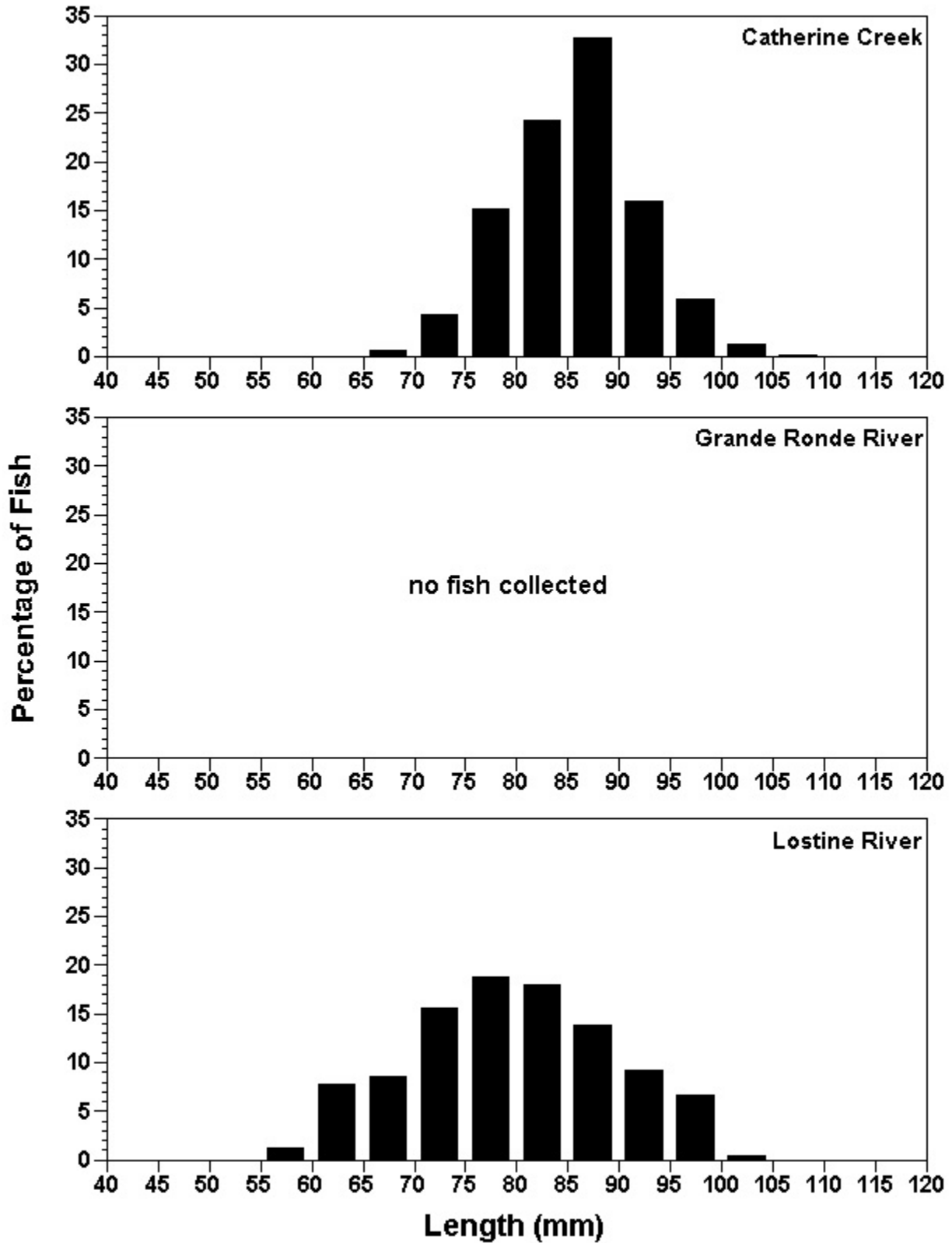


Figure 9 Length frequency distribution for 1995 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1996.

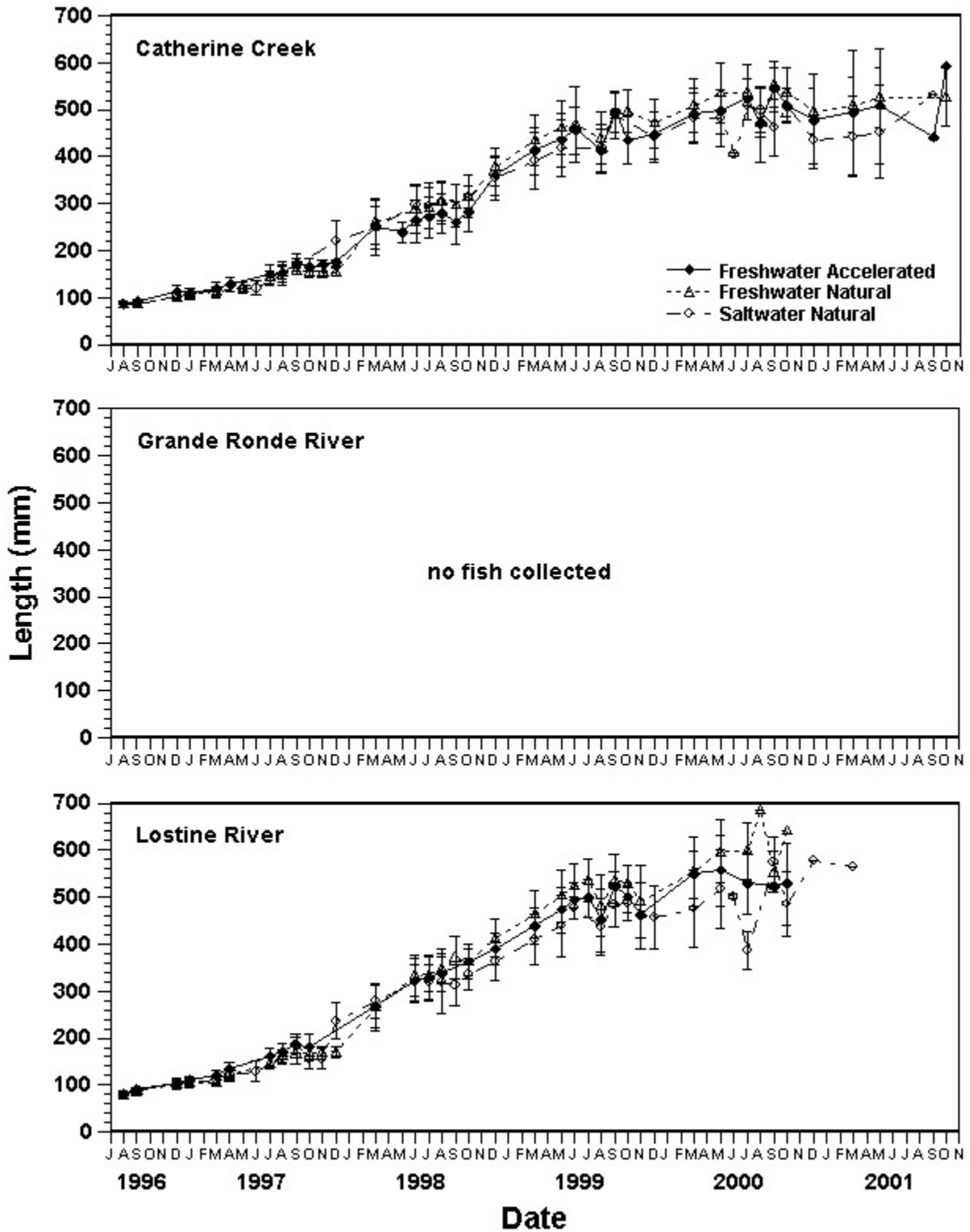


Figure 10 Mean length (± 1 SD) of 1995 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1996-2001.

Table 9. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SN=Saltwater Natural) and age class of 1995 cohort spring chinook salmon. Note: "unknown" mortalities include 3 from Catherine Creek and one from Lostine River which have not yet been examined for cause of death.

Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	242	48.7	23	4.6	126	25.4	12	2.4	9	1.8	88	17.1
GR	none collected											
LR	271	56.6	24	5.0	127	26.5	10	2.1	9	1.9	39	7.9
<u>Treatment</u>												
FA	120	36.8	14	4.3	145	44.5	11	3.4	7	2.1	29	8.9
FN	243	74.5	5	1.5	50	15.3	9	2.8	7	2.1	12	3.7
SN	150	46.2	28	8.6	58	17.8	2	0.6	4	1.2	85	25.5
<u>Age</u>												
1		0.0	4	26.7		0.0		0.0		0.0	11	73.3
2	36	23.4	36	23.4	3	1.9	6	3.9	8	5.2	65	42.2
3	200	50.1	7	1.8	150	37.6	9	2.3	7	1.8	26	6.5
4	211	70.3	0	0.0	63	21.0	6	2.0	3	1.0	17	5.7
5	59	59.0	0	0.0	36	36.0	1	1.0	0	0.0	5	4.0
6	7	77.8	0	0.0	1	11.1	0	0.0	0	0.0	2	11.1
Total	513	52.6	47	4.8	253	25.9	22	2.3	18	1.8	122	12.5

Spawning/cryopreservation accounted for 36.8%, 74.5% and 46.2% of the mortalities in the Freshwater Accelerated, Freshwater Natural and Saltwater Natural treatment groups, respectively (Table 9). Bacterial kidney disease was the major cause of mortality for both the Freshwater Accelerated (44.5%) and Freshwater Natural (15.3%) treatment groups. Unknown causes (25.2%) and BKD (17.8%) were the prominent causes of non-spawning mortality in the Saltwater Natural treatment group.

Causes of mortality changed with age (Table 9). Operational and unknown causes resulted in 26.7% and 73.3% of the age 1 mortalities. Age 2 fish mostly died from unknown (42.2%), gamete collection (jacks; 23.4%) and operational causes (23.4%). Of the age 3 fish, 50.1% died from gamete collection and 37.6% from BKD. Gametes were collected from 70.3%, 59.0% and 77.8% of the age 4, 5 and 6 fish, respectively, while BKD killed 21.0%, 36.0% and 11.1% of the same fish.

Survival to Spawning and Age of Maturity

A total of 513 of 981 parr collected (52.6%) survived to contribute gametes. Within treatments, 36.8% of the Freshwater Accelerated fish spawned, 74.5% of the Freshwater Natural fish and 46.2% of the Saltwater Natural fish (Figure 10). Within the Freshwater Accelerated treatment group, 42.9% were females, 8.4% were males that were spawned and 48.7% were males that had their semen cryopreserved. Of the Freshwater Natural group, there were 43.4% females, 42.6% males that were spawned and 14.0% males that had semen cryopreserved. In the Saltwater Natural treatment group, 43.3% were females, 40.7% were males that were spawned and 16.0% were males that were cryopreserved.

Sex ratios shifted with age (Figure 11). Of the mature fish, 6.9% matured at age 2 and all were males from which their semen was cryopreserved. At age 3, 39.6% of the fish matured: 0.5% were females, 65.0% were spawned males and 34.5% were cryopreserved males. At age 4, 41.0% matured (74.4% females, 19.8% males that were spawned and 5.8% males from which semen was cryopreserved), 11.3% matured at age 5 (98.2% females and 1.8% males that were spawned) and 1.2% survived to spawn at age 6 (66.7% females and 33.3% spawned males).

1996 Cohort

Collections

The 1996 cohort (500 fish) was collected from Catherine Creek from 26-29 August 1997 between RK 29.8-52.0. Water temperature ranged from 9.4-16.0° C. Grande Ronde River fish (500) were collected from 2-4 September between RK 296-325. Water temperature ranged from 9.0-11.5° C. Lostine River parr (501) were collected from 25-27 August between RK 1.3-31.0 and water temperature ranged from 8.0-12.0° C. There were no collection-related mortalities.

Size at Collection

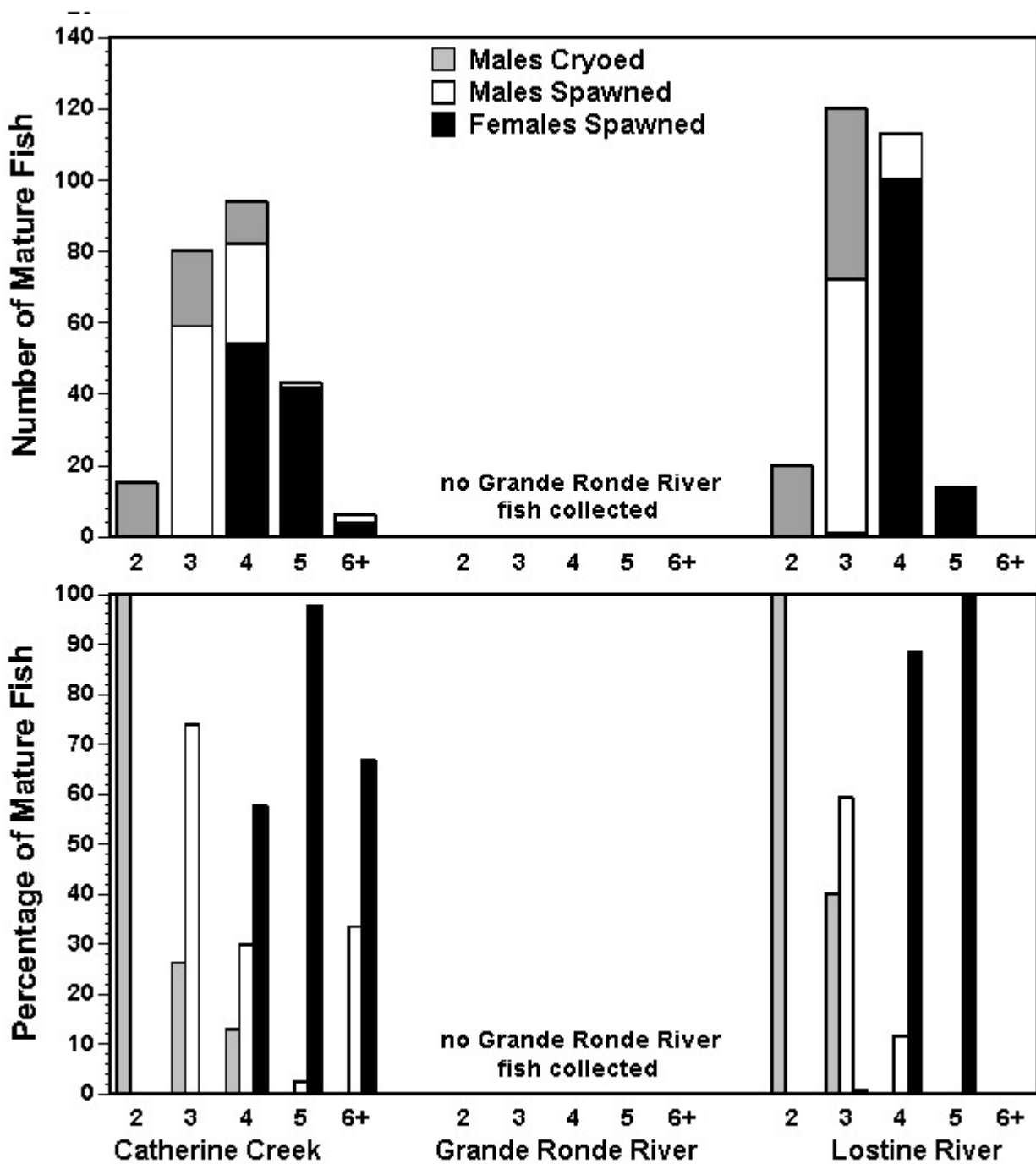
Size at collection of chinook salmon parr varied with population (Figure 12). Lostine River parr were collected in August 1997 and were the largest, with a mean fork length of 81.4 mm and weight of 6.40 g. Grande Ronde River parr were collected in September and were the smallest, with a mean fork length of 65.6 mm and weight of 4.02 g. Catherine Creek parr, collected in August, had a mean fork length of 76.8 mm and weight of 5.42 g.

Growth

The fish in the Saltwater Natural groups were consistently smaller than the other treatment groups in the Catherine Creek and Lostine River populations (Figure 13). Grande Ronde River fish grew similarly in all treatments.

Catherine Creek

Growth of the Catherine Creek fish was similar between the Freshwater Natural and Freshwater Accelerated regimes but growth of the Saltwater Natural group was consistently slower since the fish were transferred to saltwater (Figure 13). Mean fork length and weight of August 1998, 428.5 mm and 1335.43 g in June 1999 and 535.7 mm, 2499.36 g in June 2000 and 610.8 mm and 3261.57 g in May 2001. The Freshwater Natural growth regime fish grew to 205.0 mm and 123.70 g in August 1998, 404.1 mm and 1019.76 g in May 1999 and 548.5 mm, 2607.93 g in June 2000 and 619.7 and 3541.81 g in May 2001. The Saltwater Natural growth regime fish grew to 123.4 mm and 23.31 g in April 1998, 350.6 mm and 667.23 g in May 1999



Stock and Treatment

Figure 11 Number and percentage of 1995 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved at ages 2-5.
 Figure 12 Number and percentage of 1995 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

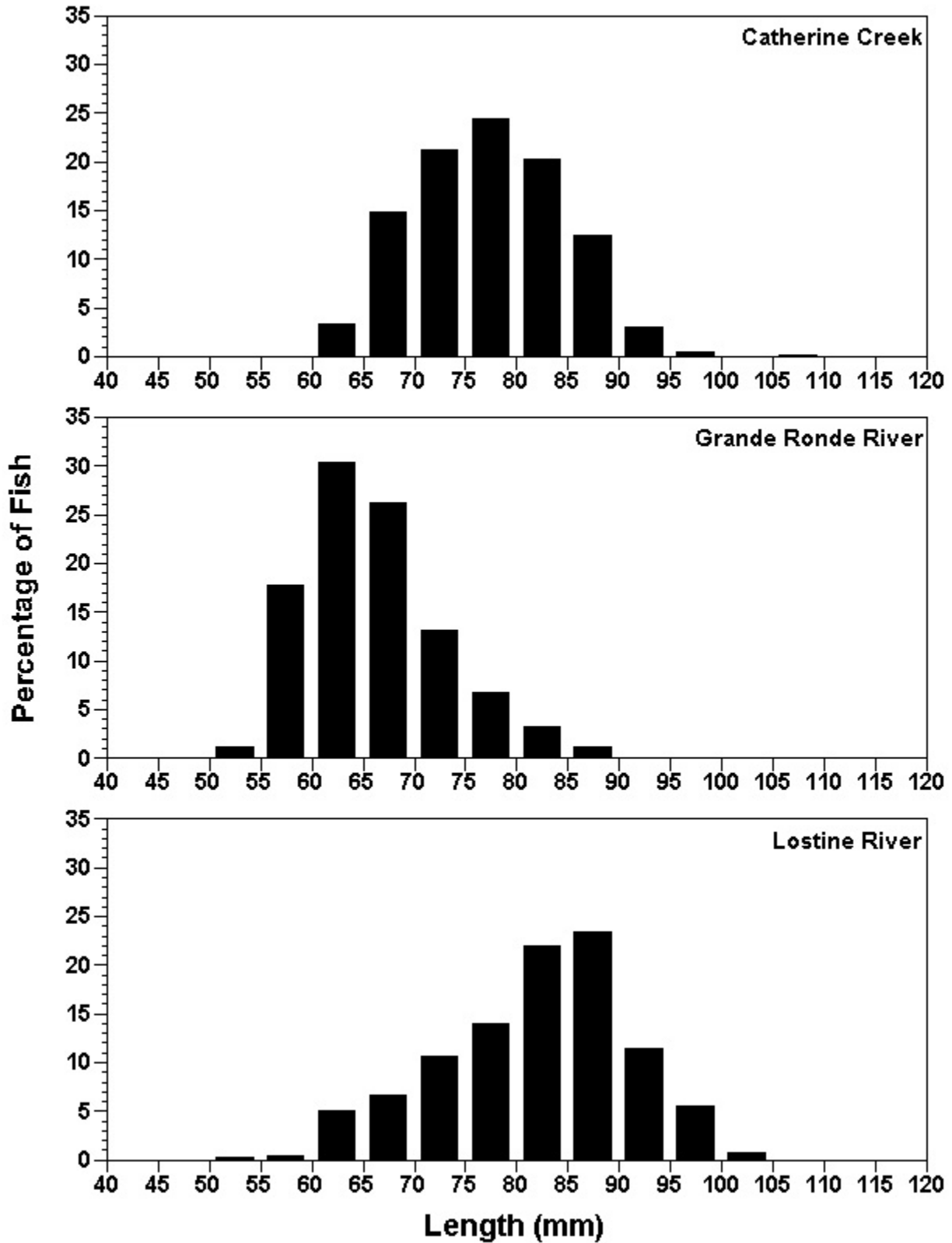


Figure 13 Length frequency distribution for 1996 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1997.

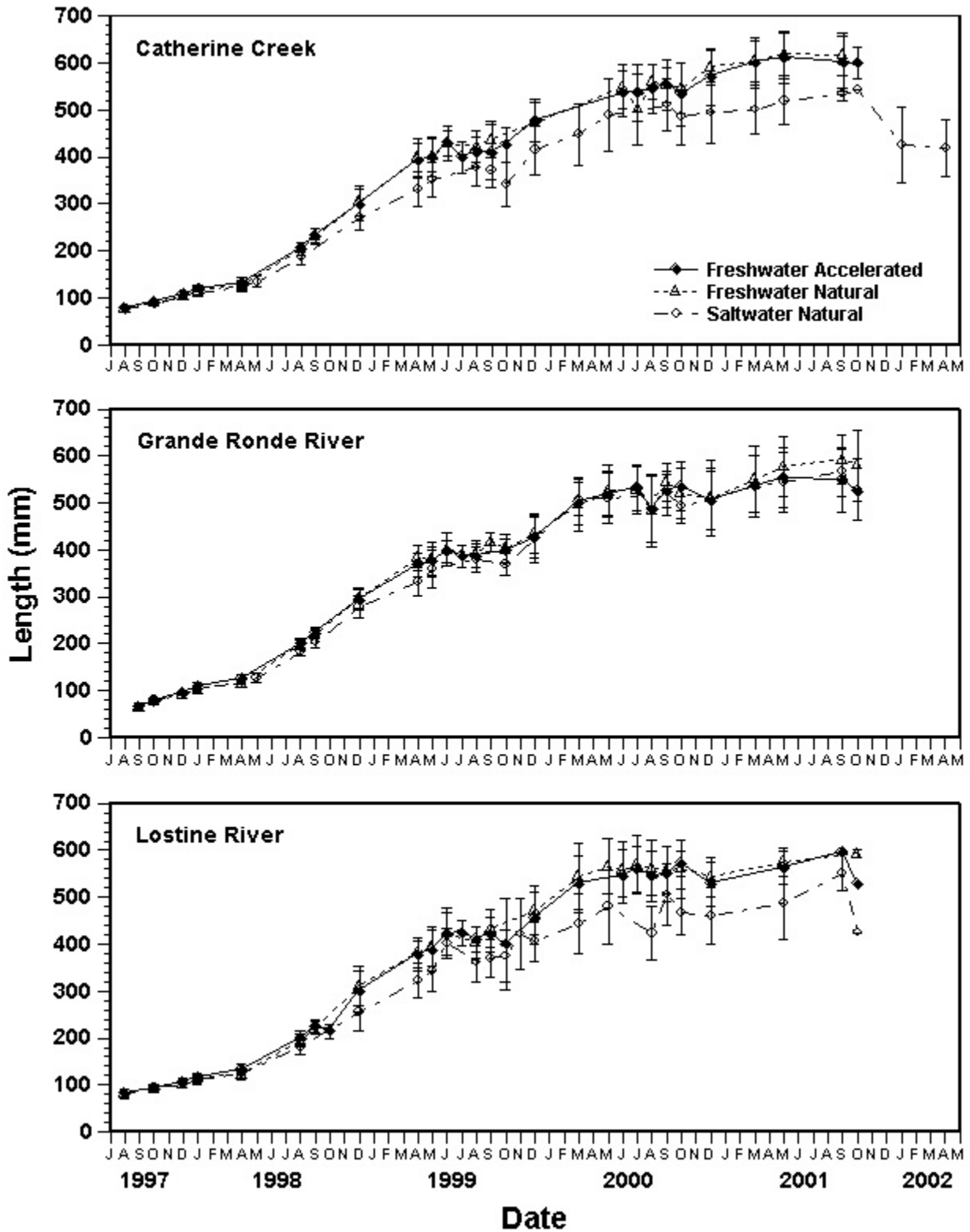


Figure 14 Mean length (± 1 SD) of 1996 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1997-2002.

and 489.4 mm, 1914.51 g in May 2000 and 518.4 mm and 2014.71 g in May 2001.

Grande Ronde River

All treatment groups of the Grande Ronde River grew at similar rates (Figure 13). Mean fork length and weight of the Freshwater Accelerated growth regime was 200.1 mm and 103.02 g in August 1998, 375.1 mm and 831.84 g in May 1999 and 518.3 mm, 2291.28 g in May 2000 and 553.3 mm and 2647.68 g in May 2001. The Freshwater Natural growth regime fish grew to 197.2 mm and 99.00 g in August 1998, 380.9 mm and 897.08 g in May 1999 and 526.6 mm, 2390.38 g in May 2000 and 576.9 mm and 2858.16 g in May 2001. The Saltwater Natural growth regime fish grew to 182.9 mm and 78.14 g in August 1998, 359.3 mm and 714.40 g in May 1999 and 511.2 mm, 2175.99 g in May 2000 and 543.8 and 2544.89 g in May 2001.

Lostine River

The Saltwater Natural Lostine River fish were consistently smaller than either of the freshwater treatment groups since the fish were about three years of age (Figure 13). Mean fork length and weight of the Freshwater Accelerated growth regime was 200.1 mm and 112.58 g in August 1998, 386.9 mm and 837.74 g in May 1999 and 545.2 mm, 2652.43 g in June 2000 and 562.3 mm and 3030.83 g in May 2001. The Freshwater Natural growth regime fish grew to 194.5 mm and 103.48 g in August 1998, 394.8 mm and 903.68 g in May 1999, 558.1 mm and 2798.18 g in June 2000 and 575.3 and 2811.97 g in May 2001. The Saltwater Natural growth regime fish grew to 123.2 mm and 22.29 g in April 1998, 342.7 mm and 627.29 g in May 1999, 479.4 mm and 1730.72 g in May 2000 and 486.2 and 1687.68 g in May 2001.

Mortality

All 1996 cohort fish have died, due to maturation and spawning or some other cause. Causes of mortality varied among populations, treatments and ages (Table 10). Overall, 67.4% of the fish survived to maturity. Of the causes of non-spawning mortality, BKD (15.5%) and unknown causes (9.5%) were the highest.

Of the Catherine Creek fish, 69.4% survived to maturity (Table 10). Bacterial kidney disease (18.2%) and unknown causes (9.0%) were the most prominent causes of mortality. In the Grande Ronde River fish, 80.4% survived to maturation while unknown (8.4%) and other causes (7.2%) were the most common causes of death. Only 1.0% died from BKD. Only 53.0% of Lostine River fish survived to maturation. Bacterial kidney disease caused 27.6% and unknown causes another 11.1% of the mortalities.

Within treatment groups, gametes were collected from 71.1% of the Freshwater Accelerated group, 74.3% of the Freshwater Natural group and 57.7% of the Saltwater Natural group (Table 10). Bacterial kidney disease caused the death of 13.0% of the Freshwater Accelerated group, 17.0% in the Freshwater Natural group and 16.7% of the Saltwater Natural group. Unknown causes accounted for the majority of the remaining mortalities.

Causes of mortality changed with age (Table 10). Unknown (87.5%) and operational (12.5%) were the causes of mortality of age 1 fish. The majority of the mortalities of ages 2-6 were due to gamete collection (52.4%, 71.4%, 69.1%, 60.2% and 33.3%, respectively). Unknown causes resulted in the death of 31.1% of the age 2 fish. Unknown (10.5%) and other (10.9%) were the most common causes of non-spawning mortality in the age 3 fish. Bacterial kidney disease caused 24.0% of the age 4 mortalities, 34.7% of the age 5 mortalities and 33.3%

Table 10. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SN=Saltwater Natural) and age class of 1996 cohort spring chinook salmon. Note: "unknown" mortalities include 1 Catherine Creek mortality which has not yet been examined for cause of death.

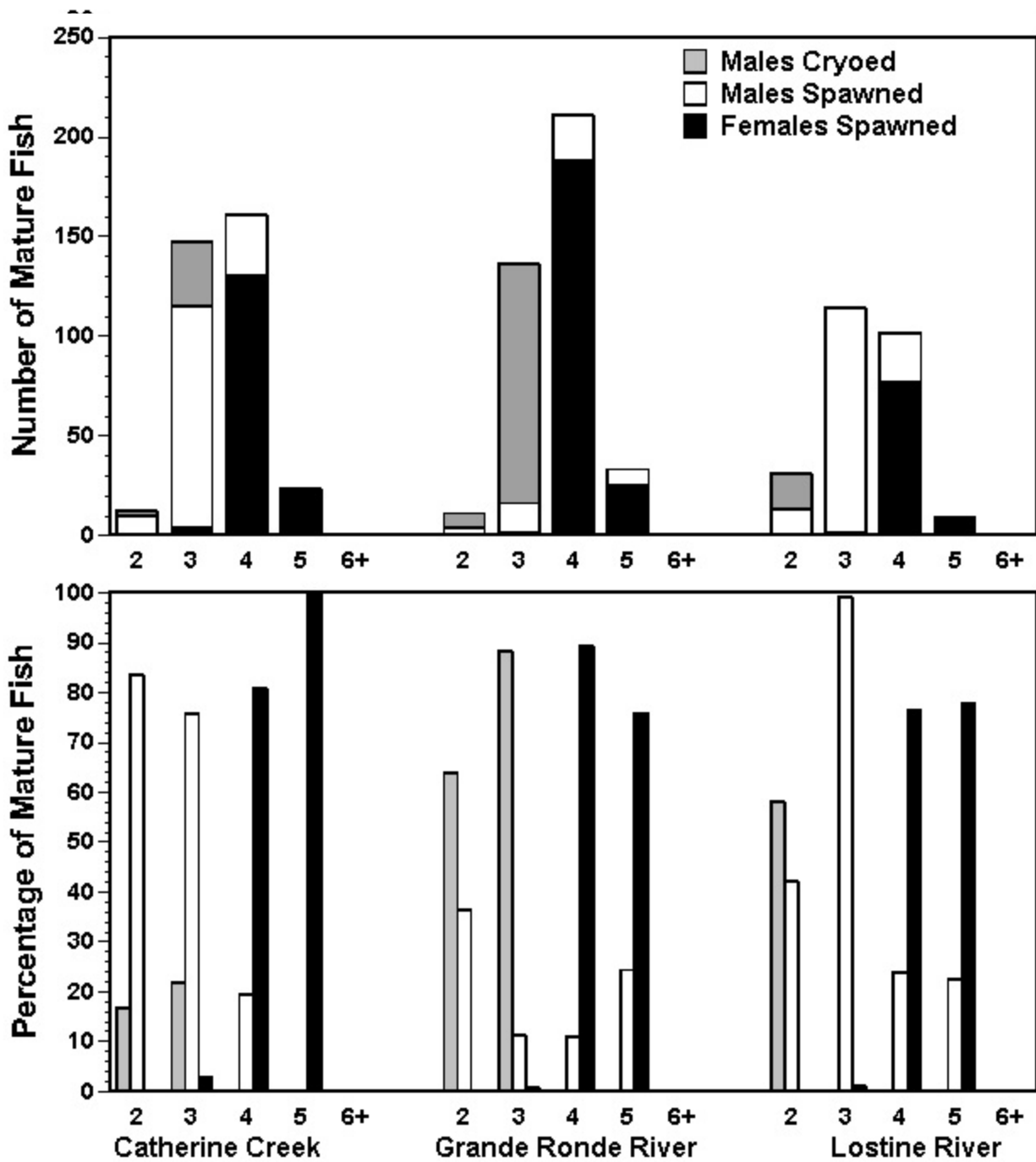
Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	347	69.4	1	0.2	91	18.2	5	1.0	10	2.0	46	9.2
GR	402	80.4	7	1.4	5	1.0	8	1.6	36	7.2	42	8.4
LR	263	53.0	4	0.8	137	27.6	13	2.6	24	4.8	55	11.1
<u>Treatment</u>												
FA	355	71.1	4	0.8	65	13.0	12	2.4	27	5.4	36	7.2
FN	371	74.3	1	0.2	85	17.0	11	2.2	8	1.6	23	4.6
SN	286	57.7	7	1.4	83	16.7	3	0.6	35	7.1	83	16.5
<u>Age</u>												
1	0	0.0	1	12.5	0	0.0	0	0.0	0	0.0	7	87.5
2	54	52.4	9	8.7	3	2.9	3	2.9	2	1.9	32	31.1
3	406	71.4	1	0.2	21	3.7	19	3.3	62	10.9	60	10.5
4	480	69.1	1	0.1	167	24.0	3	0.4	4	0.6	40	5.8
5	71	60.2	0	0.0	41	34.7	1	0.8	2	1.7	3	2.5
6	1	33.3	0	0.0	1	33.3	0	0.0	0	0.0	1	0
Total	1012	67.6	12	0.8	233	15.6	26	1.7	70	4.7	143	9.5

of the age 6 mortalities.

Survival to Spawning and Age of Maturity

A total of 1012 of 1501 parr collected (67.4%) contributed gametes. Within treatments, 69.4% of the Freshwater Accelerated fish, 73.8% of the Freshwater Natural fish and 57.2% of the Saltwater Natural fish spawned (Figure 14). Within the Freshwater Accelerated treatment group, 50.7% were females, 20.7% were males that were spawned and 28.5% were males that had their semen cryopreserved. Of the Freshwater Natural group, there were 45.3% females, 41.7% males that were spawned and 13.0% males that had semen cryopreserved. In the Saltwater Natural treatment group, 43.0% were females, 46.2% were males that were spawned and 10.8% were males that were cryopreserved.

Sex ratios shifted with age (Figure 15). Of the fish that survived to maturity, 5.5% matured at age 2: 50% were spawned and 50% cryopreserved. At age 3, 40.1% of the maturing



Stock and Treatment

Figure 15 Number and percentage of 1996 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved at ages 2-4.

fish matured (1.5% females, 60.2% spawned males and 38.3% cryopreserved males). At age 4, 47.8% matured, of which 83.5% were females, 16.5% were males that were spawned and no males were cryopreserved. At age 5, 6.6% matured: 84.6% were females and 15.4% were spawned males.

1997 Cohort

Collections

The 1997 cohort (500 fish) was collected from Catherine Creek from 17-19 August 1998 between river kilometers (RK) 29.8-52.0 and up to RK 4.8 on the North fork of Catherine Creek and to RK 0.4 on the South Fork. Water temperature ranged from 8.0-12.0° C. Five hundred Grande Ronde River fish were collected from 8-10 September between RK 296-325. Water temperature ranged from 7.0-13.0° C. Five hundred Lostine River parr were collected from 24-26 August between RK 1.3-31.0. There were no collection-related mortalities in any of the populations.

Size at Collection

Size at collection of parr varied with population (Figure 16). Grande Ronde River parr were collected in September and were the smallest, with a mean fork length of 64.9 mm and weight of 3.55 g. The Catherine Creek and Lostine River parr were each collected in August and were of a similar size. The Lostine River fish had a mean fork length of 76.4 mm and weight of 5.58 g. Mean fork length and weight of the Catherine Creek parr were 76.8 mm and 5.48 g.

Growth

Growth of all populations was similar, as was growth of each treatment within each population (Figure 17).

Catherine Creek

Growth of the Catherine Creek fish was similar between all treatments (Figure 17). Mean fork length and weight of the Freshwater Accelerated growth regime was 182.9 mm and 88.69 g in August 1999, 342.3 mm and 638.96 g in March 2000, 502.5 mm and 1922.81 g in March 2001 and 600.4 mm and 3263 g in March 2002. The Freshwater Natural growth regime was 187.5 mm and 97.87 g in August 1999, 344.4 mm and 635.53 g in March 2000, 504.4 mm and 1886.44 g in March 2001 and 581.7 mm and 3143.64 g in March 2002. The Saltwater Natural growth regime fish grew to 203.7 mm and 126.95 g in August 1999, 359.4 mm and 698.66 g in March 2000, 474.6 mm and 1531.49 mm in March 2001 and 517.4 mm and 1966.68 g in April 2002.

Grande Ronde River

Growth of the Grande Ronde River fish was similar between all treatments (Figure 17). Mean fork length and weight of the Freshwater Accelerated growth regime was 185.1 mm and 96.27 g in August 1999, 363.3 mm and 760.57 g in March 2000, 523.3 mm and 2562.89 g in March 2001 and 543.3 mm and 2612.40 g in March 2002. The Freshwater Natural growth regime was 194.1 mm and 113.57 g in August 1999, 360.8 mm and 734.28 g in March 2000, 543.8 mm 2661.81 g in March 2001 and 577.0 mm and 2979.73 g in March 2002. The Saltwater Natural growth regime fish grew to 208.1 mm and 141.57 in August 1999, 373.1 mm and 837.35 g in March 2000 and 518.2 mm and 2214.90 g in March 2001.

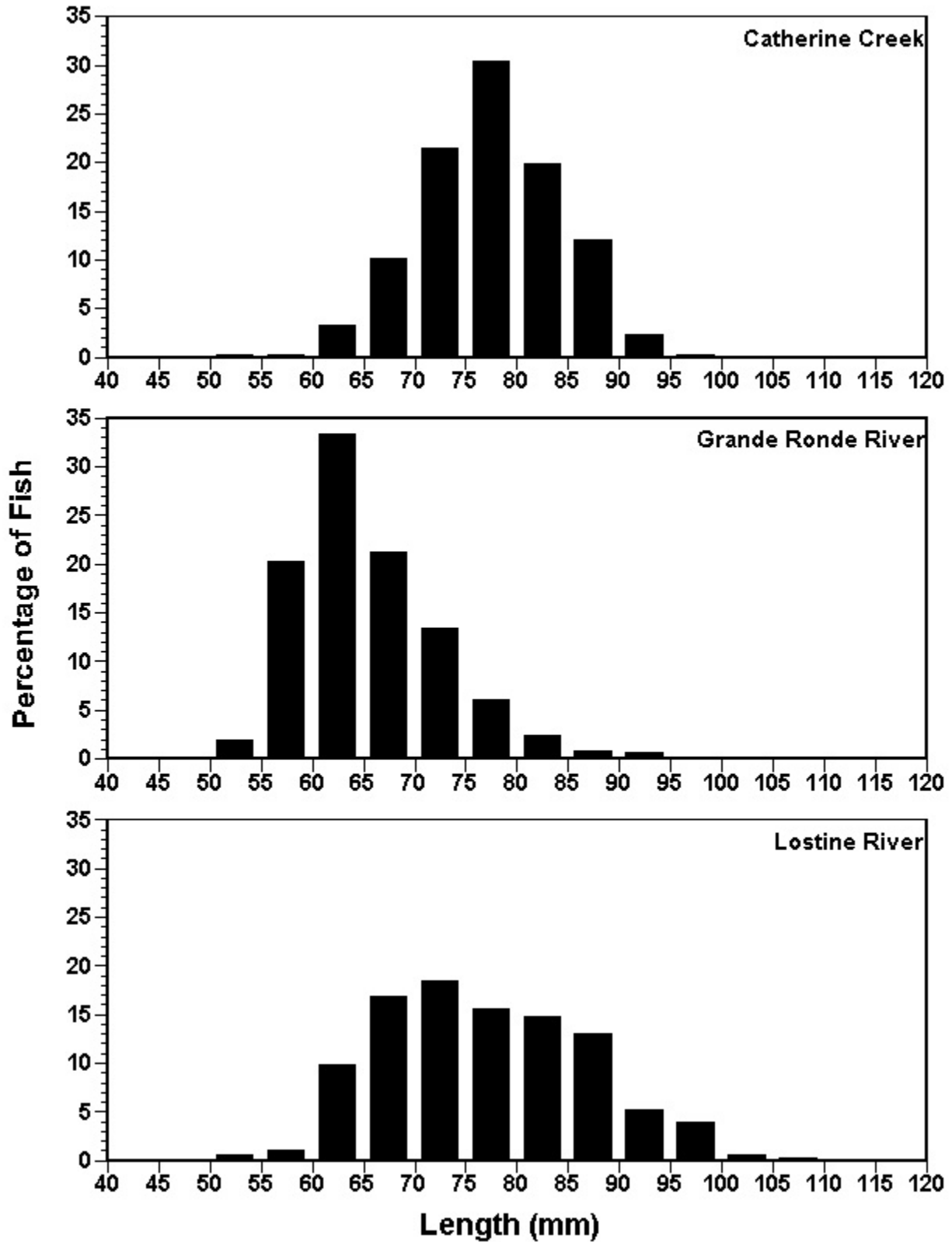


Figure 17 Length frequency distribution for 1997 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1998.

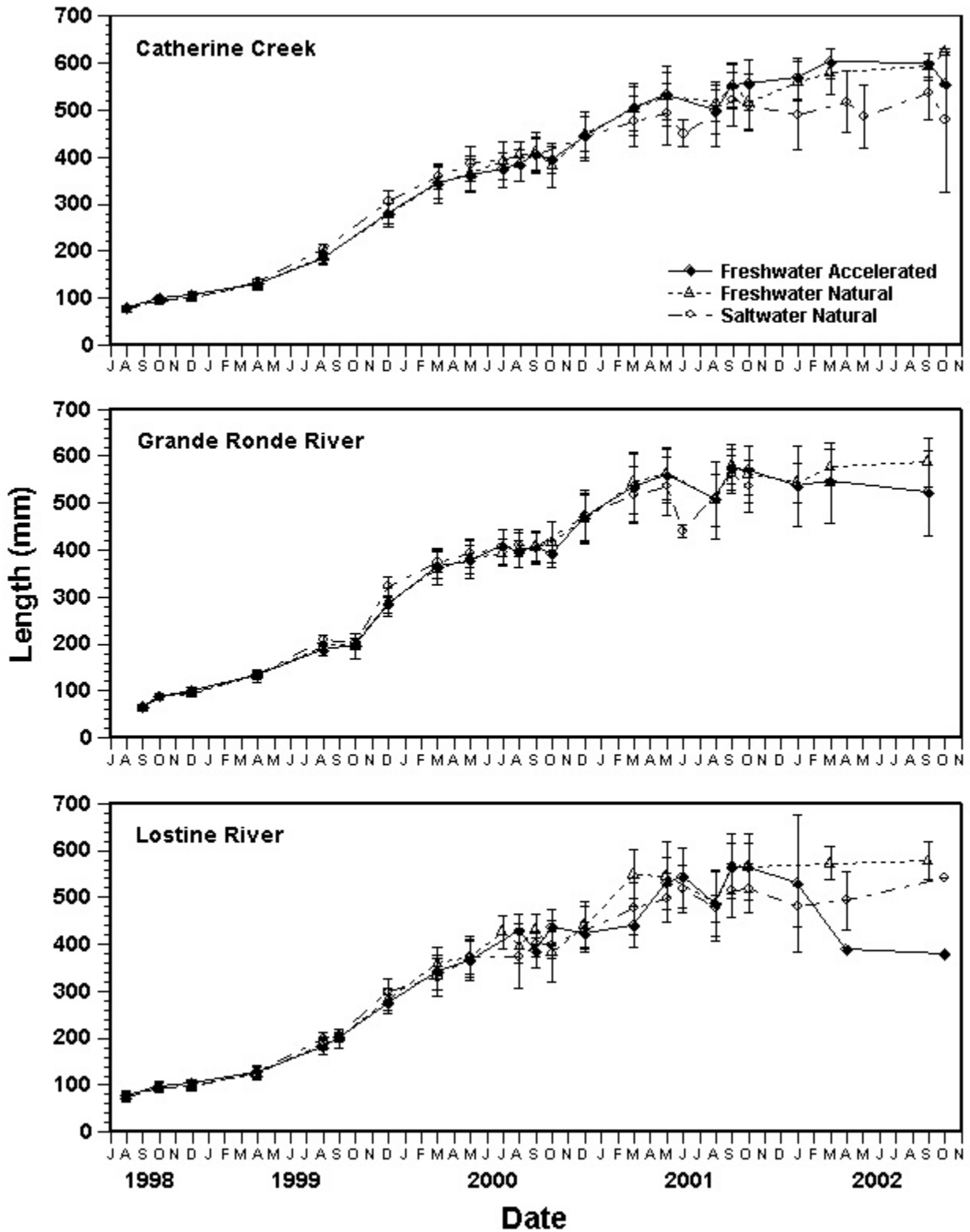


Figure 18 Mean length (± 1 SD) of 1997 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1998-2002.

Lostine River

All treatment groups of Lostine River fish grew similarly until 2002 when small sample sizes showed differences in sizes among treatment groups (Figure 17). Mean fork lengths and weights of Freshwater Accelerated fish were 180.3 mm and 86.30 g in August 1999, 339.8 mm and 584.33 g in March 2000, 529.8 mm and 2245.88 g in March 2001 and 529.8 mm and 2574.27 g in March 2002. The Freshwater Natural fish were 187.8 mm and 98.43 g in August 1999 and 358.9 mm and 680.31 g in March 2000, 549.5 mm and 2527.88 g in March 2001 and 573.3 mm and 2576.60 g in March 2002. The Saltwater Natural fish were 198.5 mm and 120.35 g in August 1999, 329.8 mm and 537.38 g in March 2000, 477.0 mm and 1628.66 g in March 2001 and 494.0 mm and 1443.40 g in April 2002.

Mortality

The entire 1997 cohort has died and causes of mortality varied among populations, treatments and ages (Table 11). Gametes were collected from 72.1% of the fish collected:

Table 11. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SN=Saltwater Natural) and age class of 1997 cohort spring chinook salmon. Note: "unknown" mortalities include 5 Catherine Creek mortalities which have not yet been examined for cause of death.

Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	323	64.9	12	2.4	124	24.9	8	1.6	6	1.2	25	5
GR	417	83.7	7	1.4	40	8.0	6	1.2	3	0.6	25	5.0
LR	342	68.5	4	0.8	96	19.2	17	3.4	8	1.6	32	6.4
<u>Treatment</u>												
FA	371	75.6	8	1.6	83	16.9	10	2.0	5	1.0	14	2.9
FN	379	76.3	7	1.4	75	15.1	13	2.6	2	0.4	21	4.2
SN	328	66.8	8	1.6	96	19.6	7	1.4	10	2.0	47	8.6
<u>Age</u>												
1	0	0.0	3	23.1	3	23.1	0	0.0	2	15.4	5	38.5
2	117	70.1	8	4.8	8	4.8	7	4.2	5	3.0	22	13.2
3	402	70.0	1	0.2	114	19.9	16	2.8	2	0.3	40	6.8
4	510	77.7	10	1.5	112	17.1	6	0.9	7	1.1	11	1.7
5	53	66.3	1	1.3	23	28.7	2	2.5	1	1.3	4	0.0
Total	1082	72.4	23	1.5	260	17.4	31	2.1	17	1.1	82	5.2

64.9% of the Catherine Creek fish, 83.7% from Grande Ronde River and 68.5% from Lostine River. Bacterial kidney disease accounted for 24.9% of the mortalities in the Catherine Creek fish and 19.2% in the Lostine River fish but only 8.0% in the Grande Ronde River fish.

Within treatment groups, 75.6% of the mortalities in the Freshwater Accelerated fish were the result of gamete collection, 76.3% of the Freshwater Natural mortalities and 66.8% of the Saltwater Natural mortalities (Table 11). Bacterial kidney disease was the cause of 19.6% of the Saltwater Natural mortalities, 16.9% of the Freshwater Accelerated and 15.1% in the Freshwater Natural. Unknown causes accounted for 2.9% of the mortalities in the Freshwater Accelerated group, 4.2% of the Freshwater Natural group and 8.6% of the Saltwater Natural fish.

Gamete collection was the cause of 70.1%, 70.0%, 77.7% and 66.3% of the ages 2, 3, 4 and 5 mortalities, respectively (Table 11). Age 1 mortalities were few, but due to unknown (38.5%), operational (23.1%), BKD (23.1%) and other (15.4%) causes. Unknown causes resulted in 13.2% of the age 2 mortalities. Bacterial kidney disease caused 19.9%, 17.1% and 28.7% of the ages 3, 4 and 5 mortalities, respectively.

Survival to Spawning and Age of Maturity

A total of 1082 of 1500 parr collected (72.1%) have contributed gametes through the 2002 spawn. Within treatments, 74.2% of the Freshwater Accelerated fish have matured, 75.4% of the Freshwater Natural fish have matured and 65.6% of the Saltwater Natural fish have matured (Figure 18). Within the Freshwater Accelerated treatment group, 43.9% were females, 50.1% were males that were spawned and 5.9% were males that had their semen cryopreserved. Of the Freshwater Natural group, 42.2% were females, 54.6% males that were spawned and 3.2% males that had semen cryopreserved. In the Saltwater Natural treatment group, 39.3% were females, 55.2% were males that were spawned and 5.5% were males that were cryopreserved.

Sex ratios shifted with age (Figure 19). Of the fish that matured, 10.7% did so at age 2 and all were males: 60.7% were spawned and 39.3% cryopreserved. At age 3, 55.2% of the maturing fish matured: 30.6% were females, 69.0% were spawned males and 0.3% were cryopreserved males. At age 4, 30.2% of the maturing fish matured: 71.7% were females, 26.0% were spawned males and 2.2% were cryopreserved males. At age 5, 3.8% of the maturing fish matured: 65% were females, 35% were spawned males and none were cryopreserved males.

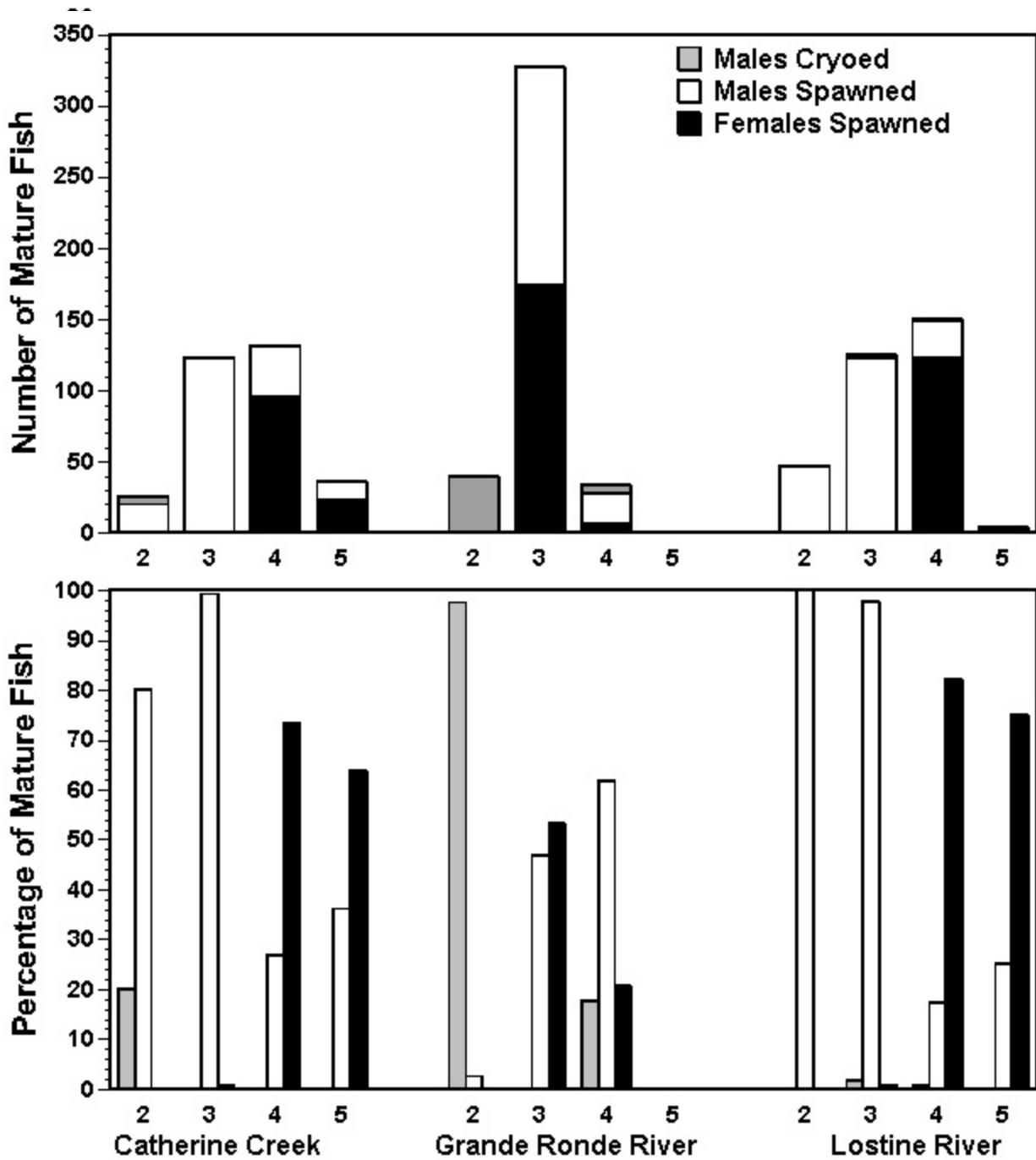
1998 Cohort

Collections

Five hundred 1998 cohort parr were collected from Catherine Creek from 16-18 August 1999 between river kilometers (RK) 29.8-52.0. There were six collection-related mortalities. Five hundred Grande Ronde River parr were collected from 30 August-1 September between RK 296-325. Lostine River parr (498) were collected from 23-25 August between RK 1.3-31.0. There were no collection-related mortalities in Grande Ronde or Lostine river fish.

Size at Collection

Size at collection of chinook salmon parr varied with population (Figure 20). All were collected in August 1999. Catherine Creek parr were the largest and had a mean fork length of 75.1 mm and weight of 6.05 g. Grande Ronde River parr were the smallest, with a mean fork length of 60.0 mm and weight of 3.24 g. Lostine River parr had a mean fork length of 74.6 mm and weight of 6.42 g.



Stock and Treatment

Figure 19 Number and percentage of 1997 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved, at ages 2-5.

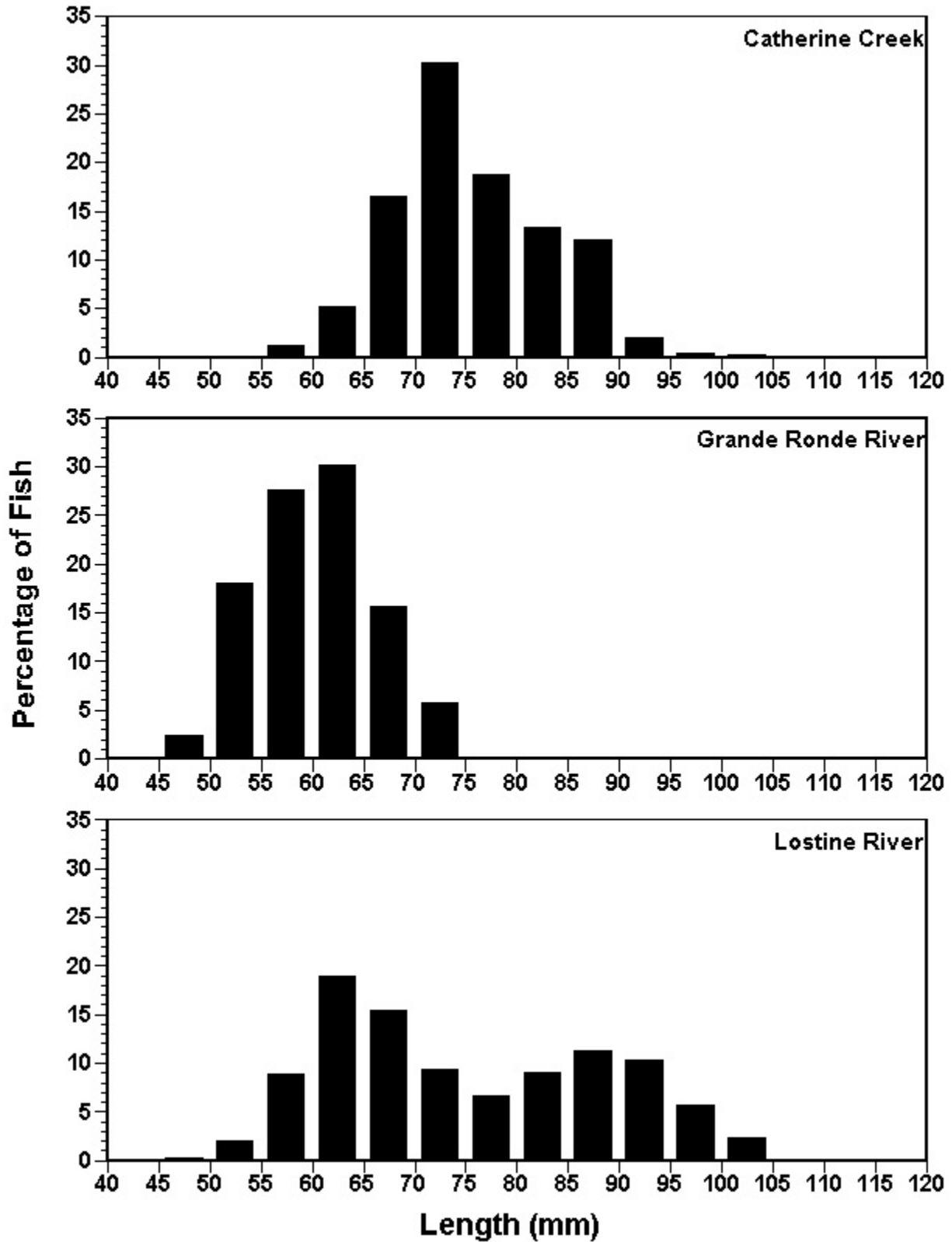


Figure 21 Length frequency distribution for 1998 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1999.

Growth

Due to problems with the water supply, all fish were raised under the same conditions at Lookingglass Fish Hatchery and all groups were transferred to Bonneville Fish Hatchery prior to smoltification, so there are only Freshwater and Saltwater treatment groups for the 1998 cohort. Growth of the 1998 cohort was similar for both treatment groups until the fish began to mature in the 2001 spawning season, when the Freshwater group was larger in all populations (Figure 21).

Catherine Creek

Growth of the Catherine Creek fish was similar among both treatment groups until September 2001 (age 3), when the Freshwater fish began to be consistently larger than the Saltwater group (Figure 21). Mean fork length and weight of the Freshwater group was 360.6 mm and 713.81 g in March 2001 and 513.1 mm and 2020.02 g in March 2002. The Saltwater fish grew to 354.8 mm and 671.51 g in March 2001 and 478.5 mm and 1640.24 g in March 2002.

Grande Ronde River

The Grande Ronde River treatment groups also grew similarly until age 3 (Figure 21). The Freshwater fish were 374.6 mm and 797.67 g in March 2001 and 516.2 mm and 2080.15 g in March 2002. The Saltwater fish grew to 368.3 mm and 728.74 g in March 2001 and 483.5 mm and 1707.28 g in April 2002.

Lostine River

The Lostine River treatment groups also separated at age 3, with the Freshwater-reared group growing faster (Figure 21). Mean fork lengths and weights of Freshwater fish were 363.0 mm and 694.04 g in March 2001 and 533.2 mm and 2258.82 g in March 2002. The Saltwater fish grew to 353.0 mm and 653.13 g in March 2001 and 479.9 mm and 1568.64 g in April 2002.

Mortality

Causes of mortality varied among populations, treatments and ages and 56 mortalities (23 CC; 22 GR; 12 LR) have not yet been examined for cause of death (Table 12). Thirty-three, 12 and 29 fish remain alive for the Catherine Creek, Grande Ronde and Lostine River populations, respectively. Gamete collection was largest cause of mortality for 1998 cohort fish: 69.6% of Catherine Creek mortalities, 45.3% of Grande Ronde River mortalities and 78.5% of Lostine River mortalities. Bacterial kidney disease caused 13.5% of the Catherine Creek mortalities. Bacterial kidney disease hit the Grande Ronde River fish hard in the fall of 2001 and caused 33.8% of the total mortalities in this population and cohort. Another 14.1% died from unknown causes, possibly related to this BKD outbreak. In the Lostine River population, additional sources of mortality were more evenly distributed between BKD (8.7%), unknown causes (4.2%), other causes (3.8%) and other diseases (3.4%).

Within treatment groups, 66.3% of the mortalities in the Freshwater group were due to gamete collection and 21.3% were due to BKD (Table 12). In the Saltwater group 61.7% of the mortalities were due to gamete collection. Unknown causes (18.0%), BKD (14.3%) and other causes (9.4%) were prominent in the Saltwater group.

Age 1 mortalities were low and mostly due to operational (58.8%) and unknown (29.4%)

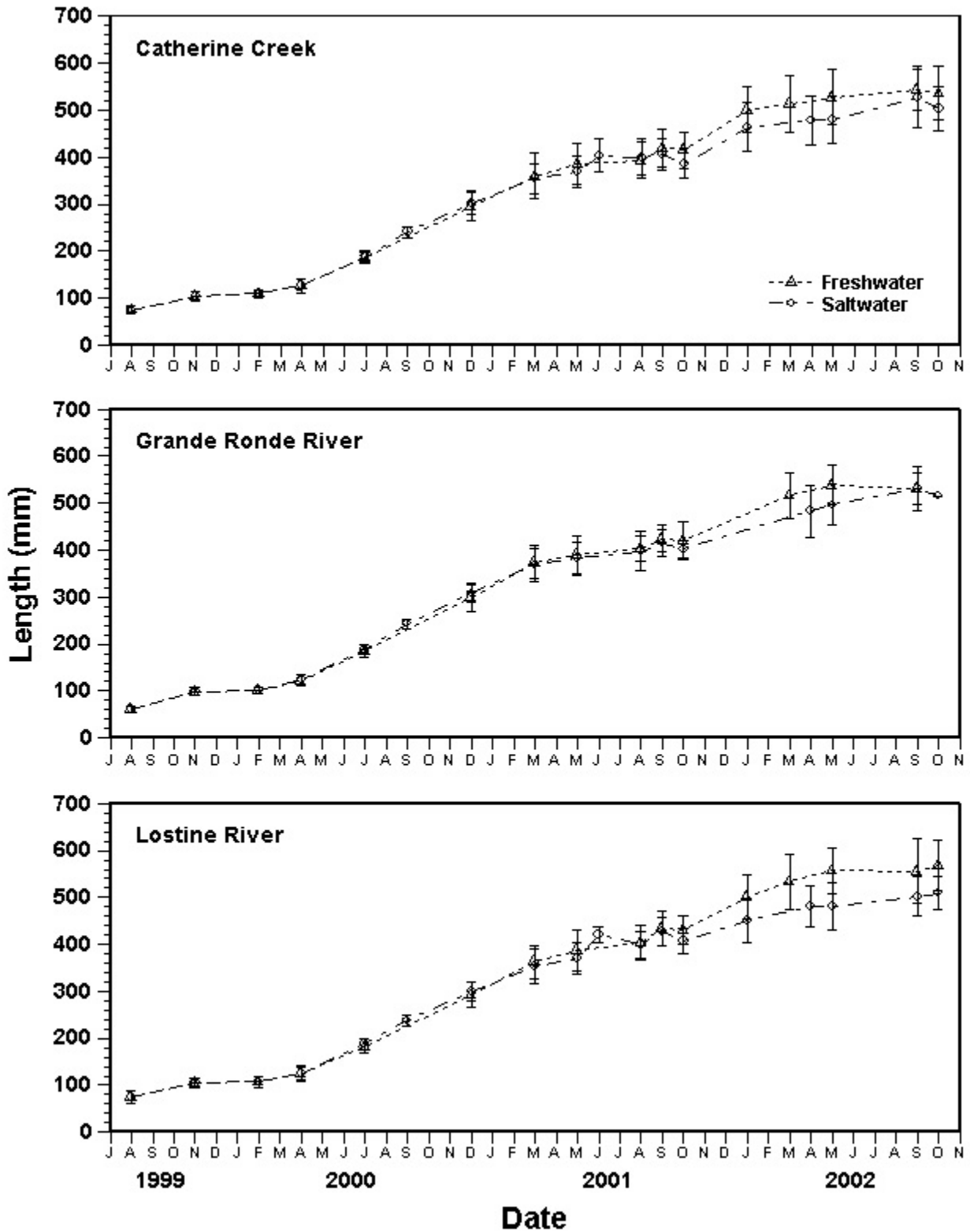


Figure 22 Mean length (± 1 SD) of 1998 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater and Saltwater growth regimes, 1999-2002.

Table 12. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SN=Saltwater Natural) and age class of 1998 cohort spring chinook salmon. Note: "unknown" mortalities include 23 Catherine Creek, 22 Grande Ronde River and 12 Lostine River mortalities which have not yet been examined for cause of death.

Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	325	69.6	11	2.4	63	13.5	24	5.1	6	1.3	38	8.1
GR	221	45.3	4	0.8	165	33.8	9	1.8	20	4.1	69	14.1
LR	368	78.5	7	1.5	41	8.7	16	3.4	18	3.8	20	4.2
<u>Treatment</u>												
FW	626	66.3	8	0.8	201	21.3	45	4.8	27	2.9	38	4
SW	288	61.7	7	1.5	67	14.3	4	0.9	17	3.6	84	18
<u>Age</u>												
1	0	0.0	10	58.8	0	0.0	0	0.0	2	11.8	5	29.4
2	108	65.5	6	3.6	5	3.0	10	6.1	20	12.1	16	9.7
3	429	69.9	0	0.0	108	17.6	10	1.6	18	2.9	49	8.0
4	377	60.0	6	1.0	156	24.8	29	4.6	4	0.6	57	9.1
Total	914	64.2	22	1.5	269	18.9	49	3.4	44	3.1	127	8.9

causes (Table 12). Gamete collection was the cause of 65.5%, 69.9% and 60.0% of the age 2, 3 and 4 mortalities. Other (12.1%) and unknown (9.7%) causes were predominant addition factors in the age 2 fish. At age 3, BKD (17.6% and unknown (8.0%) were additional causes of mortalities. In the age 4 fish, BKD (24.8%) was the largest factor.

Survival to Spawning and Age of Maturity

A total of 914 of 1498 parr collected (61.0%) have contributed gametes through the 2002 spawn. Within treatments, 62.2% of the Freshwater fish have spawned and 58.3% of the Saltwater fish (Figure 22). Within the Freshwater fish, 36.2% were females, 63.6% were males that were spawned and 0.2% were males that had their semen cryopreserved. In the Saltwater group, 33.0% were females, 63.2% were males that were spawned and 3.8% were males that were cryopreserved.

Sex ratios shifted with age (Figure 23). At age 2, 12.4% (of the mature fish) matured and all were males: 93.5% were spawned and 6.5% were cryopreserved. At age 3, 48.0% of the maturing fish matured: 0.2% were females, 98.6% were spawned males and 1.2% were

cryopreserved males. At age 4, 39.6% of the maturing fish matured: 84.4% were females, 15.6% were spawned males and none were cryopreserved males.

1999 Cohort

Collections

The 1999 cohort was collected from Catherine Creek (503 fish) from 14-17 August 2000 between river kilometers (RK) 29.8-52.0. There were three collection-related mortalities. Five hundred Lostine River parr were collected from 21-23 August between RK 1.3-31.0 and water temperature ranged from 11.0-13.0° C. There were no collection-related mortalities in the Lostine River populations. We searched for 1999 cohort Grande Ronde River parr between RK 286-325 from 7-10 August but were unable to capture any Grande Ronde River parr in 2000.

Size at Collection

All chinook salmon parr were collected in August and size at collection varied with population (Figure 24). Lostine River parr were larger, with a mean fork length of 83.2 mm and weight of 6.76 g than the Catherine Creek parr which had a mean fork length of 77.2 mm and weight of 5.26 g.

Growth

The 1999 cohort was the first to completely conform to the three treatments of this study. The problems with the water supply and chilling systems at LFH have been resolved and we successfully treated both simulated natural and accelerated growth groups (Figure 25).

Catherine Creek

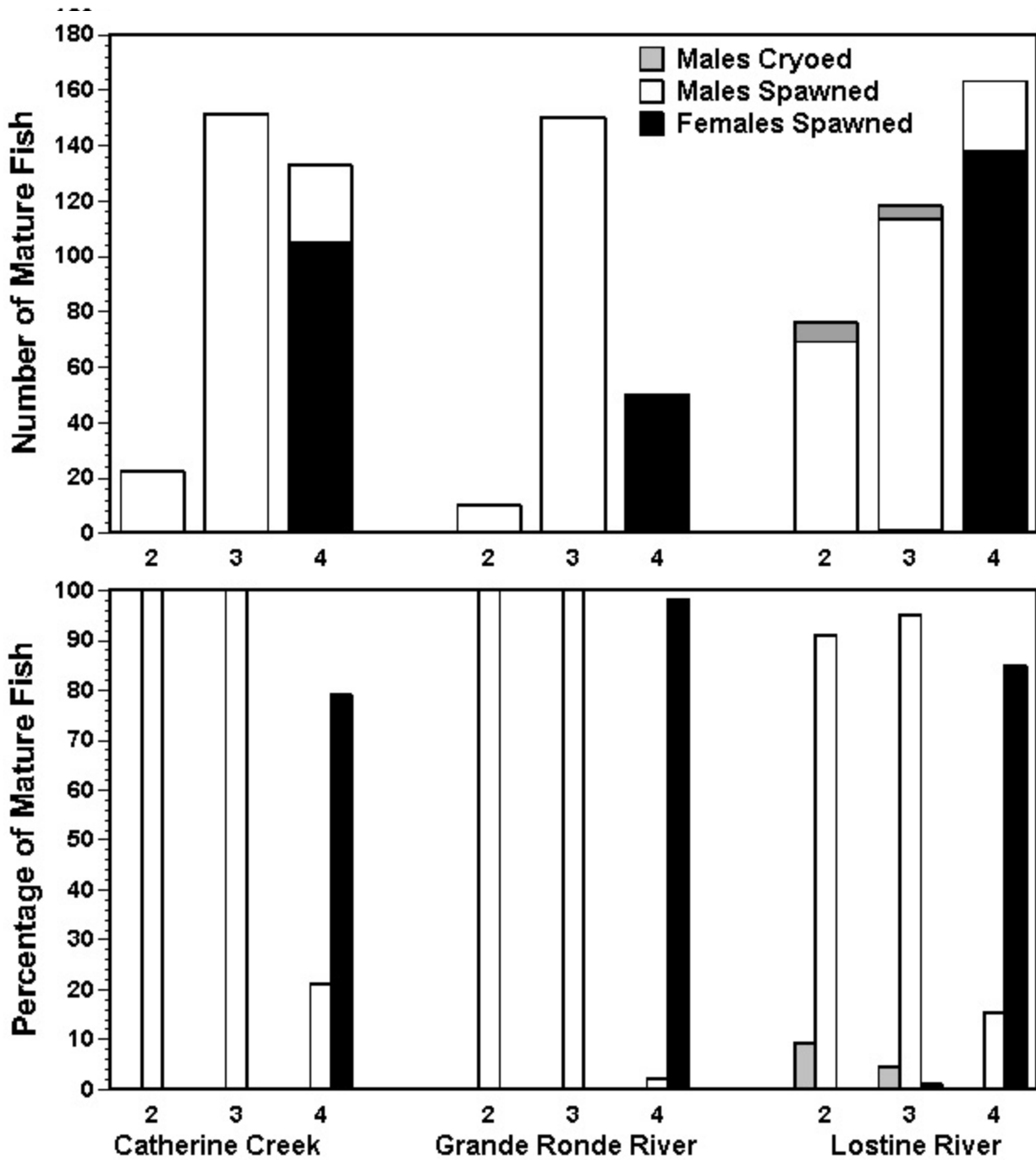
Length of the Catherine Creek chinook salmon varied between the accelerated and natural growth treatment groups (Figure 25). Following smoltification, the freshwater groups grew faster than the Saltwater Natural group, with the Freshwater Natural group growing faster than the Freshwater Accelerated group. Mean fork length and weight of the Freshwater Accelerated group was 77.4 mm and 5.33 g at capture in August 2000 and increased to 103.1 mm and 13.72 g in November 2000 (when the fish were PIT-tagged) and 132.1 mm and 27.48 g at smoltification in April 2001. One year later, at age 3, the Freshwater Accelerated group's mean length and weight were 364.6 mm and 739.16 g in March 2002. Mean fork length and weight of the Freshwater Natural group was 77.3 mm and 5.28 g in August, 99.8 mm and 11.81 g in November, 118.6 mm and 19.76 g at smoltification in April 2001 and 363.4 mm and 731.92 g in March 2002. The Saltwater Natural group fish grew from 76.9 mm and 5.16 g in August to 100.8 mm and 12.51 g in November, 119.3 mm and 21.18 g in April 2001 and 336.6 mm and 584.84 g in April 2002.

Grande Ronde River

No chinook salmon parr were observed or captured in the Grande Ronde River in 2000. So there will be no BY1999 Grande Ronde River fish in the Captive broodstock program.

Lostine River

Size of the Freshwater Accelerated Lostine River salmon had not yet separated from the natural growth groups at the time of PIT-tagging in November but since then the Freshwater



Stock and Treatment

Figure 21 Number and percentage of 1998 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved at age 2.

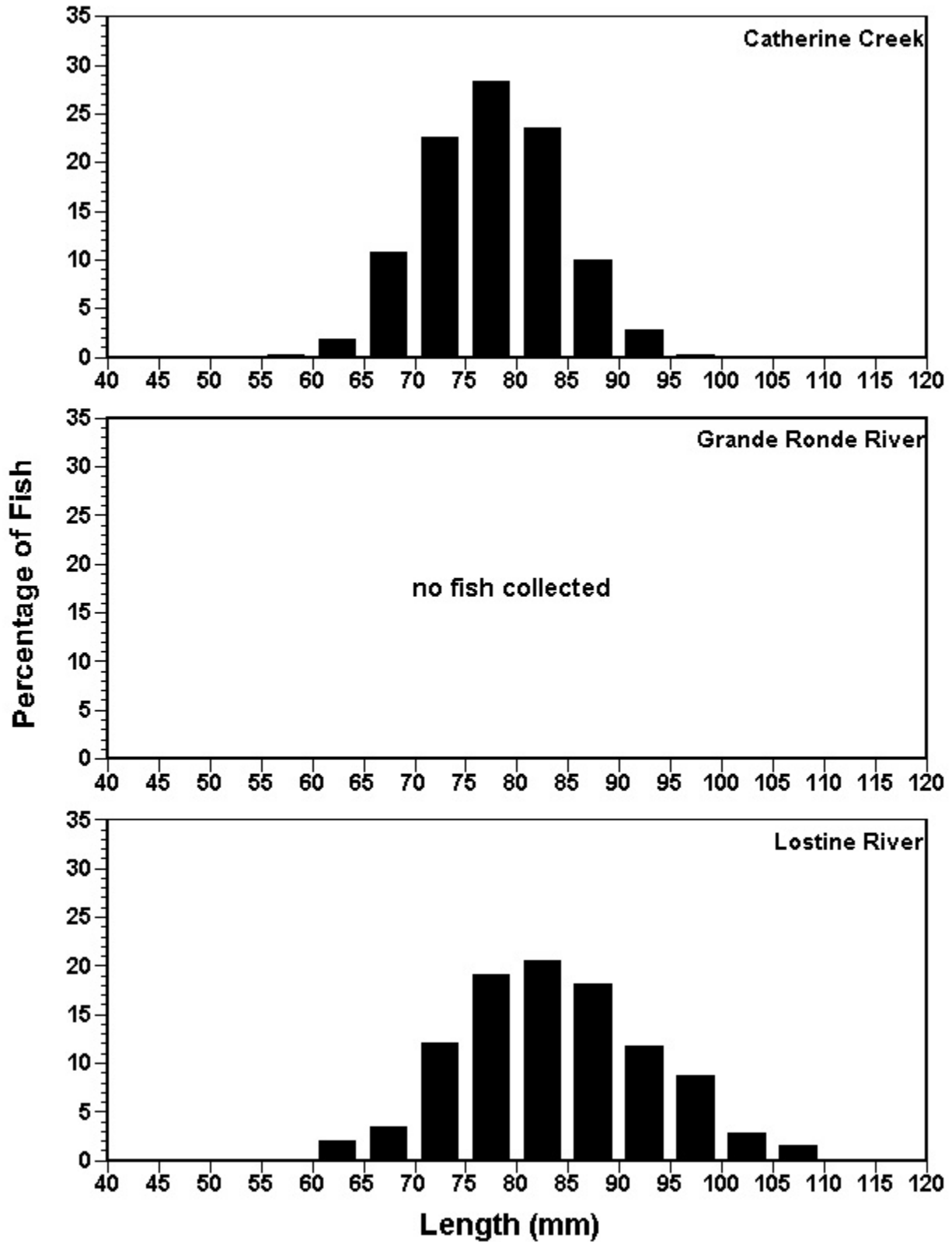


Figure 25 Length frequency distribution for 1999 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 2000.

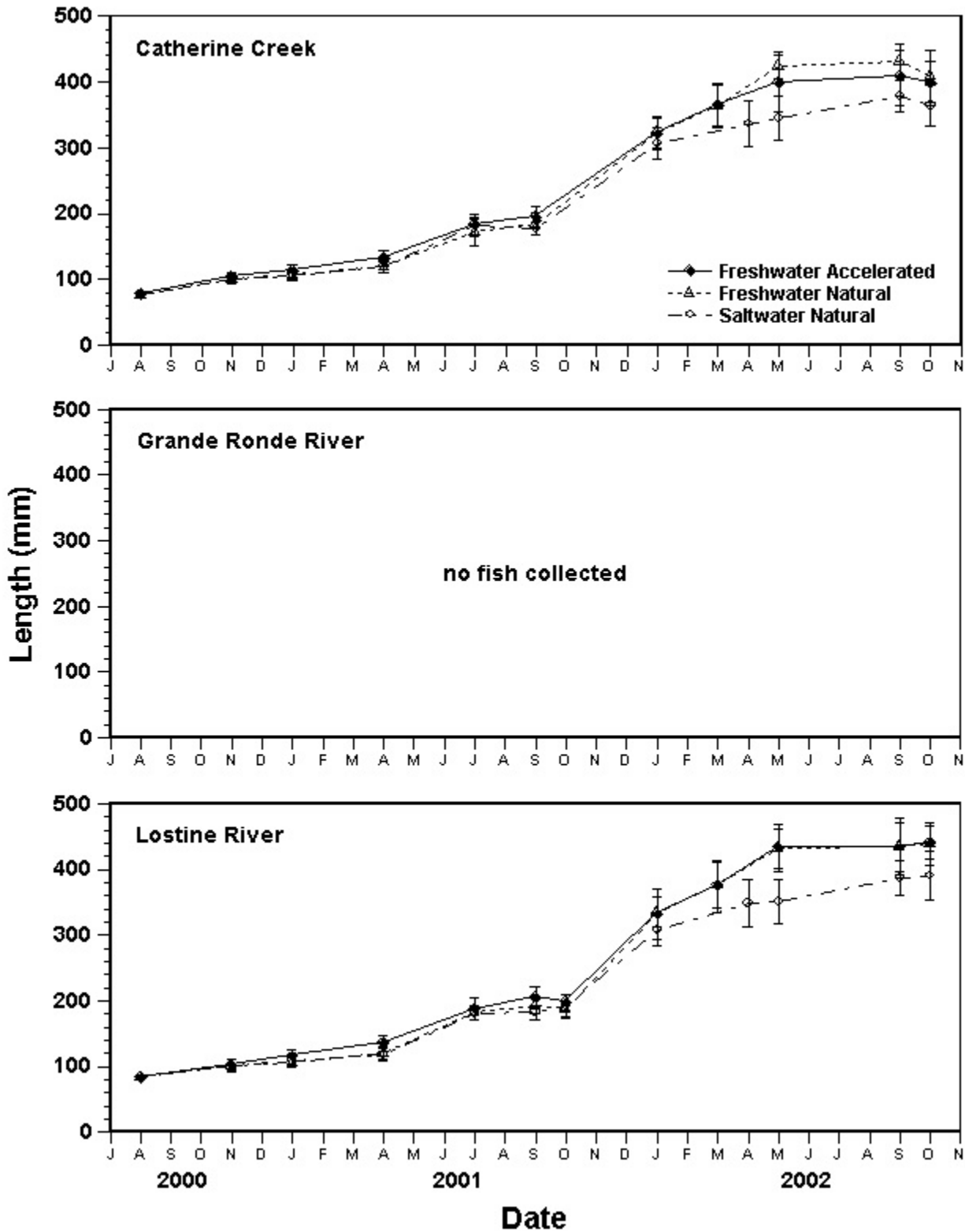


Figure 26 Mean length (± 1 SD) of 1999 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 2000-2002.

Accelerated group grew faster than the natural growth groups (Figure 25). Following transfer to saltwater, the Saltwater Natural group slowed its growth, while size of the two freshwater groups were nearly identical. Mean fork length and weight of the Freshwater Accelerated group was 82.6 mm and 6.56 g at capture in August 2000, increased to 102.1 mm and 13.75 g in November and to 135.1 mm and 29.29 g in April 2001. In March 2002, the Freshwater Accelerated group was a mean of 373.9 mm and 795.27 g. Mean fork length and weight of the Freshwater Natural group was 83.4 mm and 6.78 g in August, 102.4 mm and 12.34 g in November, 119.7 mm and 20.48 g in April 2001 and 376 mm and 802.36 g in March 2002. The fish reared under the Saltwater Natural group grew from 83.7 mm and 6.93 g in August to 101.3 mm and 12.75 g in November, 118.4 mm and 20.40 g in April 2001 and 348.3 mm and 600.96 g in April 2002.

Mortality

Approximately half of the 1999 cohort fish have died, leaving 252 Catherine Creek and 255 Lostine River remaining. Causes of mortality varied among populations, treatments and ages (Table 13). Gamete collection was the largest cause of mortality for 1999 cohort fish: 45.0% of Catherine Creek mortalities and 70.2% of Lostine River mortalities. Other diseases (24.3%) and operational causes (17.9%) caused most of the non-spawning Catherine Creek mortalities. In the Lostine River population, other diseases (13.1%) was the largest factor.

Within treatment groups, 60.4% of the mortalities in the Freshwater Accelerated group were due to gamete collection, 16.6% were due to operational causes and 14.2% were due to other diseases (Table 13). In the Freshwater Natural group, 58.4% of the fish contributed gametes, 16.4% died from other diseases and 14.9% died from operational causes. In the Saltwater Natural group 53.8% of the mortalities were due to gamete collection and 25.4% died from other diseases.

Age 1 mortalities were low and mostly due to operational (44.4%) and unknown (44.4%) causes (Table 13). Gamete collection was the cause of 57.7% and 58.9% of the age 2 and 3 mortalities. Operational causes (30.8%) were the largest additional factor in the age 2 fish. At age 3, other diseases (27.2%) was the largest source of additional mortality.

Survival to Spawning and Age of Maturity

A total of 285 of 1003 parr collected (28.4%) have contributed gametes through the 2002 spawn. Within treatments, 32.0% of the Freshwater Accelerated fish have spawned, 26.9% of the Freshwater Natural fish and 26.6% of the Saltwater Natural fish (Figure 26). Within the Freshwater Accelerated fish, 99.1% were males that were spawned and 0.9% were males that had their semen cryopreserved. In the Freshwater Natural group, 1.1% were females, 98.9% were males that were spawned and none were males that were cryopreserved. In the Saltwater Natural group, 1.1% were females, 97.8% were males that were spawned and 1.1% were males that were cryopreserved.

Sex ratios shifted with age (Figure 27). At age 2, 31.3% of the mature fish matured and all were males: 97.8% were spawned and 2.2% were cryopreserved. At age 3, 68.7% of the maturing fish matured: 1.0% were females, 96.9% were spawned males and 2.1% were cryopreserved males.

Table 13. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SN=Saltwater Natural) and age class of 1999 cohort spring chinook salmon. Note: “unknown” mortalities include 12 Catherine Creek and 7 Lostine River mortalities which have not yet been examined for cause of death.

Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	113	45.0	45	17.9	8	3.2	61	24.3	5	2.0	19	7.6
GR	none collected											
LR	172	70.2	7	2.9	7	2.9	32	13.1	13	5.3	14	5.7
<u>Treatment</u>												
FA	102	60.4	28	16.6	3	1.8	24	14.2	6	3.6	6	3.6
FN	90	58.4	23	14.9	5	3.2	25	16.2	4	2.6	7	4.5
SN	91	53.8	1	0.6	7	4.1	43	25.4	8	4.7	19	11.2
<u>Age</u>												
1	0	0.0	4	44.4	0	0.0	0	0.0	1	11.1	4	44.4
2	90	57.7	48	30.8	1	0.6	3	1.9	4	2.6	10	6.4
3	195	58.9	0	0.0	14	4.2	90	27.2	13	3.9	19	5.7
Total	285	57.5	52	10.5	15	3.0	93	18.8	18	3.6	33	6.7

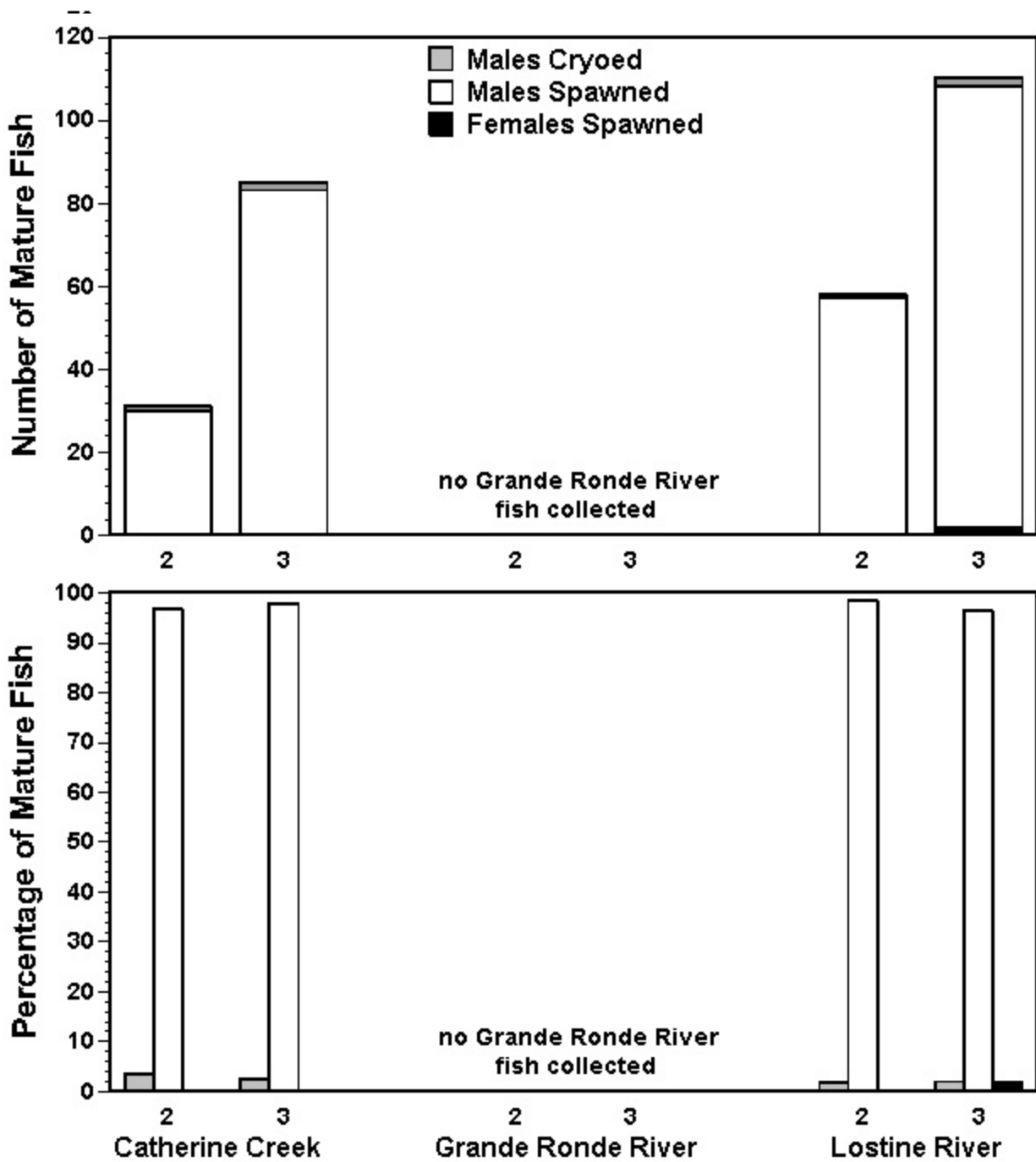
2000 Cohort

Collections

The 2000 cohort (503 parr) was collected from Catherine Creek from 6-9 August 2001 from RK 32.2-52.0. Water temperature ranged from 12.0-17.0° C. Grande Ronde River fish (502) were collected from 20-22 August between RK 296-325 (no fish were found above RK 325, although we searched up to RK 337). Water temperature ranged from 9.7-14.2° C. The Lostine River parr (503) were collected from 13-16 August between RK 1.3-31.0 and water temperature ranged from 9.5-17.0° C. There was one collection-related mortality in the Lostine River.

Size at Collection

Size at collection varied with population (Figure 28). Lostine River parr were largest, with a mean fork length of 77.6 mm and weight of 6.17 g. Catherine Creek parr had a mean fork length of 70.5 mm and weight of 4.42 g. Grande Ronde River parr were the smallest, at 62.8 mm and 2.84 g.



Stock and Treatment

Figure 28. Number and percentage of 1999 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

River parr but none in the Catherine Creek or Grande Ronde River populations.

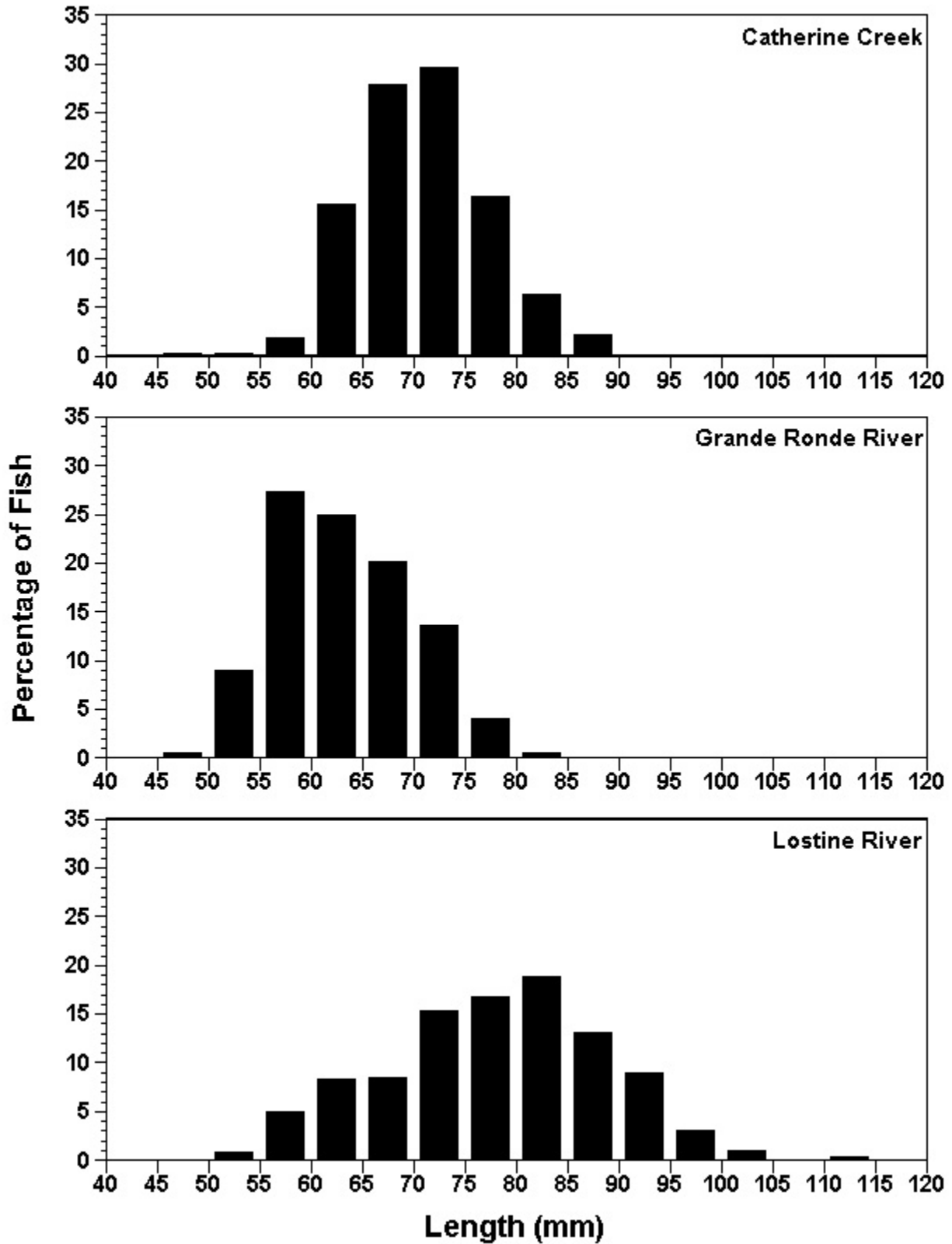


Figure 29 Length frequency distribution for 2000 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 2001.

Growth

The 2000 cohort (and later cohorts) was divided into four treatment groups: two pre-smolt treatments and two post-smolt treatments. One half of the fish from each population was reared under a simulated natural pre-smolt growth regime and the other half under an accelerated growth regime. One half of each of the pre-smolt treatment groups will be reared in freshwater and the other half reared in saltwater. This creates Saltwater Natural and Saltwater Accelerated groups which will be reared to maturity at Manchester Marine Lab and Freshwater Natural and Freshwater Accelerated groups which will be reared Bonneville Fish Hatchery. The 2000 cohort has been in captivity for 17 months and all populations are showing similar growth patterns (Figure 29). The accelerated pre-smolt groups grew faster to smoltification, as expected, However, increased post-smolt growth in the natural growth groups and removal (due to maturation) of the larger fish from the accelerated groups, resulted in the fish from each of the treatment groups being of a similar size in July 2002.

Catherine Creek

Length of the Catherine Creek salmon varied between the accelerated and natural growth groups up to smoltification but became similar afterwards (Figure 29). Mean fork length and weight of the Natural growth group was 69.8 mm and 4.30 g at capture in August 2001 and grew to 95.5 mm and 10.84 g in November 2001 (when they were PIT-tagged) and 123.7 mm and 22.58 g in April 2002, approximately one month before transfer to BOH or MML. The Accelerated growth group was 71.1 mm and 4.55 g in August 2001 and increased to 106.2 mm and 15.75 g in November. They were substantially larger than the Natural growth group, at 143.2 mm and 35.43 g, in April 2002. However, by July 2002 (approximately two months following transfer) the Natural growth groups had caught up with the Accelerated fish: 179.3 mm and 77.98 g and 185.4 mm and 84.22 g for the Freshwater Natural and Freshwater Accelerated fish, respectively, and 188.1 mm and 91.13 g and 191.6 mm and 94.54 g for the Saltwater Natural and Saltwater Accelerated fish, respectively. Some of the larger Freshwater Accelerated (2), Saltwater Accelerated (10) and Saltwater Natural (4) males matured in 2002.

Grande Ronde River

Length of the Grande Ronde River salmon varied between the accelerated and natural growth groups up to smoltification but became similar afterwards (Figure 29). Mean fork length and weight of the Natural growth group was 63.0 mm and 2.89 g at capture in August 2001 and grew to 88.8 mm and 8.73 g in November 2001 (when they were PIT-tagged) and 121.0 mm and 21.80 g in April 2002, approximately one month before transfer to BOH or MML. The Accelerated growth group was 62.5 mm and 2.79 g in August 2001 and increased to 96.6 mm Saltwater Natural and Saltwater Accelerated fish, respectively. Three of the larger Saltwater Accelerated males matured in 2002.

Lostine River

Length of the Lostine River salmon varied between the accelerated and natural growth groups up to smoltification but became similar afterwards (Figure 29). Mean fork length and weight of the Natural growth group was 77.1 mm and 6.01 g at capture in August 2001 and grew to 99.0 mm and 12.10 g in November 2001 (when they were PIT-tagged) and 126.7 mm and 24.46 g in April 2002, approximately one month before transfer to BOH or MML. The

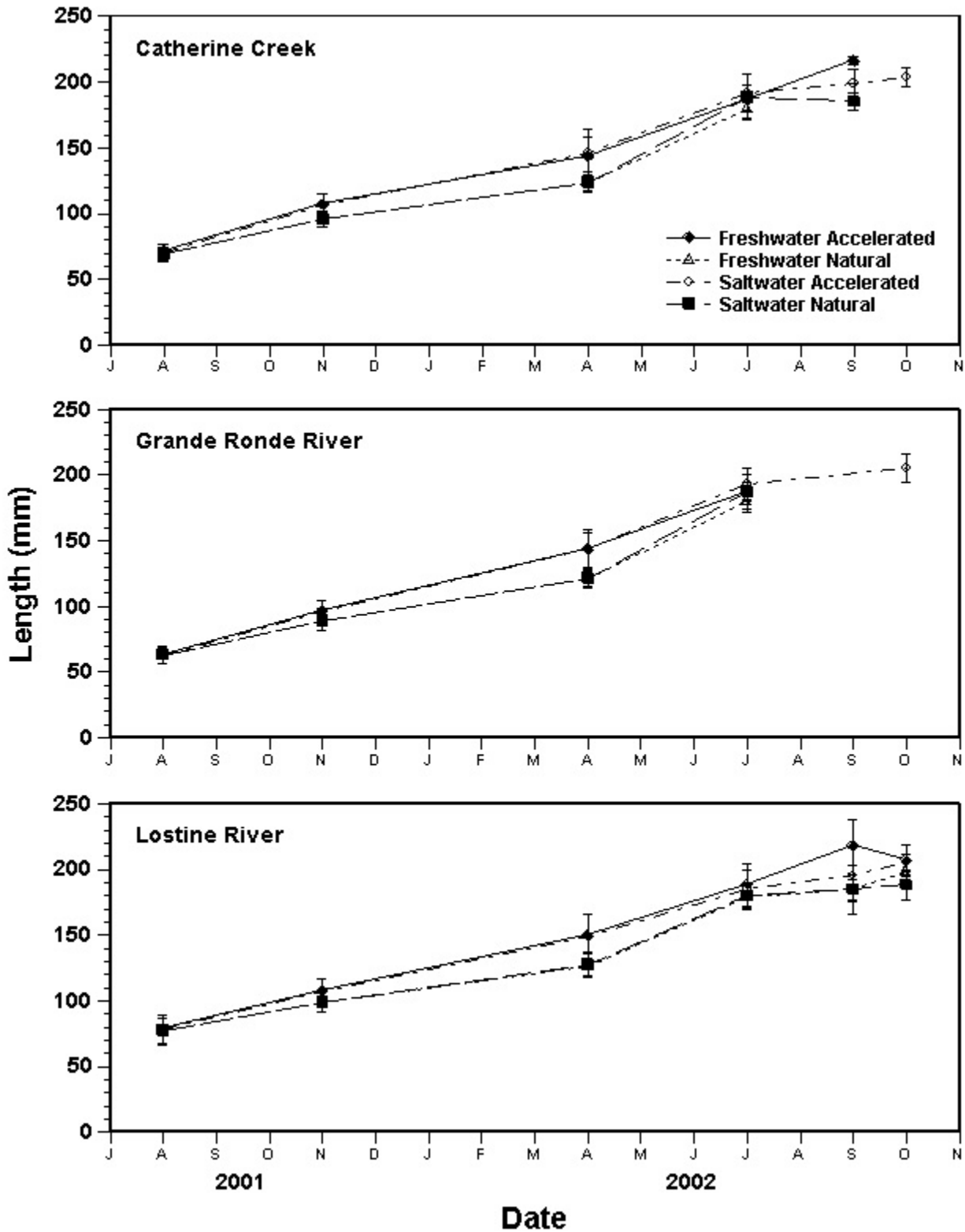


Figure 30 Mean length (± 1 SD) of 2000 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural, Saltwater Accelerated and Saltwater Natural growth regimes, 2001-2002.

Accelerated growth group was 78.0 mm and 6.34 g in August 2001 and increased to 107.4 mm and 16.80 g in November. They were substantially larger than the Natural growth group, at 149.0 mm and 40.79 g, in April 2002. However, by July 2002 (approximately two months following transfer) the Natural growth groups had caught up with the Accelerated fish: 178.8 mm and 77.81 g and 188.1 mm and 89.35 g for the Freshwater Natural and Freshwater Accelerated fish, respectively, and 179.8 mm and 79.06 g and 185.1 mm and 84.14 g for the Saltwater Natural and Saltwater Accelerated fish, respectively. Some of the larger males (17 FA, 8 FN, 6 SA and 9 SN) matured in 2002.

Mortality

A total of 197 of the 2000 cohort fish have died: 44 Catherine Creek, 39 Grande Ronde River and 114 Lostine River (Table 14). Gamete collection comprised 38.6%, 15.4% and 36.8% of the Catherine Creek, Grande Ronde River and Lostine River population mortality, respectively. Non-spawning mortality in the Catherine Creek was caused mostly by unknown causes (43.2%). Grande Ronde River fish died mostly from unknown (56.4%) and operational (12.8%) causes. populations, with only 10 and 11 fish, respectively, having died. In Lostine River fish, 28.9 % were due to unknown causes and BKD caused 14.0% of the mortalities. Note that “unknown” mortalities include 17 Catherine Creek, 22 Grande Ronde River and 26 Lostine River mortalities which have not yet been examined for cause of death.

Within treatment groups, other causes (31.8%), unknown causes and other diseases (27.3%, each) and operational causes (13.6%) were the causes of pre-smolt mortality in the Accelerated growth group (Table 14). In the Natural growth group, operational causes (52.6%) and BKD (21.1%) were the primary causes of mortality. 50.0% of the mortalities in the Freshwater Accelerated group were due to gamete collection, 33.3% were due to unknown causes and 9.5% were due to BKD. In the Freshwater Natural group, 30.8% of the dead fish contributed gametes and 38.5% died from BKD. In the Saltwater Accelerated group, 42.3% contributed gametes and only one other fish died (1.9% due to other causes). In the Saltwater Natural group 40.0% of the mortalities were due to gamete collection and 8.6% from other causes.

Age 1 mortalities were mostly due to operational (43.3%) and unknown (20.0%) causes (Table 14). Gamete collection was the cause of 64.4% of the age 2 mortalities. Bacterial kidney disease (15.8%) and other causes (12.9%) were the largest additional mortality factors in the age 2 fish.

Survival to Spawning and Age of Maturity

A total of 65 of 1508 parr collected (4.3%) have contributed gametes through the 2002 spawn. Within treatments, 5.6% of the Freshwater Accelerated fish have spawned, 4.5% of the Freshwater Natural fish, 5.3% of the Saltwater Accelerated fish and 6.1% of the Saltwater Natural fish. All of the mature fish were males that were spawned, except 30.4% of the Saltwater Natural group, which were cryopreserved.

Only age 2 fish could have spawned for this cohort and all were males. Of those that matured, 96.4% were spawned and 3.6% had their semen cryopreserved.

Table 14. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (FA=Freshwater Accelerated; FN=Freshwater Natural; SA=Saltwater Accelerated; SN=Saltwater Natural; XA= pre-smolt Accelerated; XN= pre-smolt Natural) and age class of 2000 cohort spring chinook salmon. Note: “unknown” mortalities include 17 Catherine Creek, 22 Grande Ronde River and 26 Lostine River mortalities not yet examined for cause of death.

Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	17	38.6	3	6.8	2	4.5	0	0.0	3	6.8	19	43.2
GR	6	15.4	5	12.8	0	0.0	2	5.1	4	10.3	22	56.4
LR	42	36.8	5	4.4	16	14.0	8	7.0	10	8.8	32	28.9
<u>Treatment</u>												
FA	21	50.0	0	0.0	4	9.5	1	2.4	2	4.8	13	33.3
FN	8	30.8	0	0.0	10	38.5	2	7.7	2	7.7	4	15.4
SA	22	42.3	0	0.0	0	0.0	0	0.0	1	1.9	29	55.8
SN	14	40.0	0	0.0	0	0.0	0	0.0	3	8.6	18	51.4
XA	0	0.0	3	13.6	0	0.0	6	27.3	7	31.8	6	27.3
XN	0	0.0	10	52.6	4	21.1	1	5.3	2	10.5	2	10.5
<u>Age</u>												
1	0	0.0	13	43.3	2	6.7	5	16.7	4	13.3	6	20.0
2	65	39.2	0	0.0	16	9.6	5	3	13	7.8	67	40.4
Total	65	33.2	13	6.6	18	9.1	10	5.1	17	8.6	73	37.2

2001 Cohort

Collections

Five hundred parr from the 2001 cohort were collected from Catherine Creek from 5-8 August 2002 between river kilometers (RK) 32.2-52.0. Water temperature ranged from 11.0-15.0° C. Grande Ronde River fish (461) were collected from 12-15, 23, 26 and 27 August and on 5-6 September between RK 288-326.4 (only two fish were found above RK 320, and no fish were found below RK 296). Water temperature ranged from 9.0-15.0° C. Five hundred Lostine River parr were collected from 19-21 August between RK 1.3-31.0 and water temperature ranged from 8.0-13.0° C. There were no collection-related mortalities in any population.

Size at Collection

Size at collection varied with population (Figure 30). Lostine River parr had a mean fork

length of 70.5 mm and weight of 4.54 g. Catherine Creek parr had mean fork lengths and weights of 70.9 mm and 4.38 g, respectively. Grande Ronde River parr were the smallest, with a mean fork length of 67.9 mm and mean weight of 3.78 g.

Growth

The 2001 cohort will be divided into four treatment groups - combinations of two pre-smolt growth regimes (accelerated and natural) and two post-smolt rearing environments (freshwater and saltwater). One half of the fish from each population is being reared under a simulated natural pre-smolt growth regime and the other half under an accelerated growth regime. The fish have been in captivity for four months and size separation is apparent between the natural and accelerated growth groups in all populations (Figure 31).

Catherine Creek

Length of the Catherine Creek salmon varied between the accelerated and natural growth groups (Figure 31). Mean fork length and weight of the Accelerated growth group was 71.2 mm and 4.44 g at capture in August 2002 and increased to 104.6 mm and 15.75 g in November 2002 (when they were PIT-tagged). Mean fork length and weight of the Natural growth regime was 70.6mm and 4.32 g in August and 96.9 mm and 11.62 g in November.

Grande Ronde River

Length of the Grande Ronde River salmon varied between the accelerated and natural growth groups (Figure 31). We had difficulty finding and collecting fish from the Grande Ronde River in 2002 so collections were made during two periods in August and September. Mean fork length and weight of the Accelerated growth group was 67.7 mm and 3.43 g at in August and 75.5 mm and 5.21 g in September 2002. In November 2002 (when they were PIT-tagged), these fish had increased to 100.7 mm and 12.97 g. Mean fork length and weight of the Natural growth regime was 66.3 mm and 3.47 g in August and 75.1 mm and 5.05 g in September. The fish had reached 92.4 mm and 10.02 g in November.

Lostine River

Size of the Lostine River salmon varied between the accelerated and natural growth groups (Figure 31). Mean fork length and weight of the Accelerated growth group was 70.6 mm and 4.59 g at capture in August 2002 and increased to 100.0 mm and 13.68 g in November. Mean fork length and weight of the Natural growth regime was 70.3 mm and 4.50 g in August and 92.7 mm and 10.43 g in November.

Mortality

Thirteen of the 2001 cohort fish have died: two Catherine Creek, one Grande Ronde River and ten Lostine River (Table 15). Six of the mortalities (all Lostine River) were from other diseases, six (4 LR; 1 GR; 1 CC) from unknown causes and one (CC) from operational causes.

Within treatments, five Accelerated growth fish died, four from unknown causes and one from other diseases (Table 15). Five natural growth fish died from other diseases, two from unknown causes and one from operational causes.

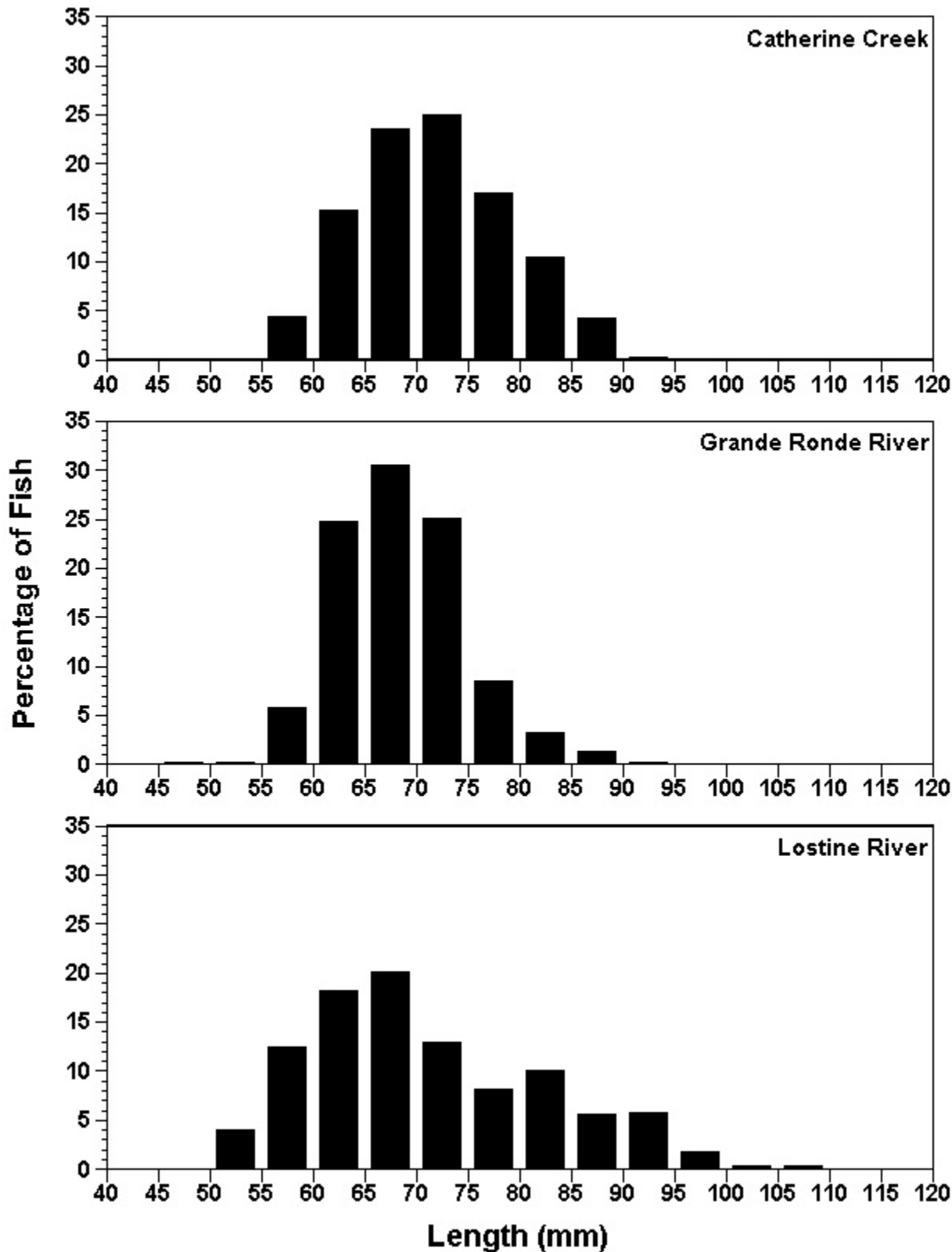


Figure 31 Length frequency distribution for 2001 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 2002.

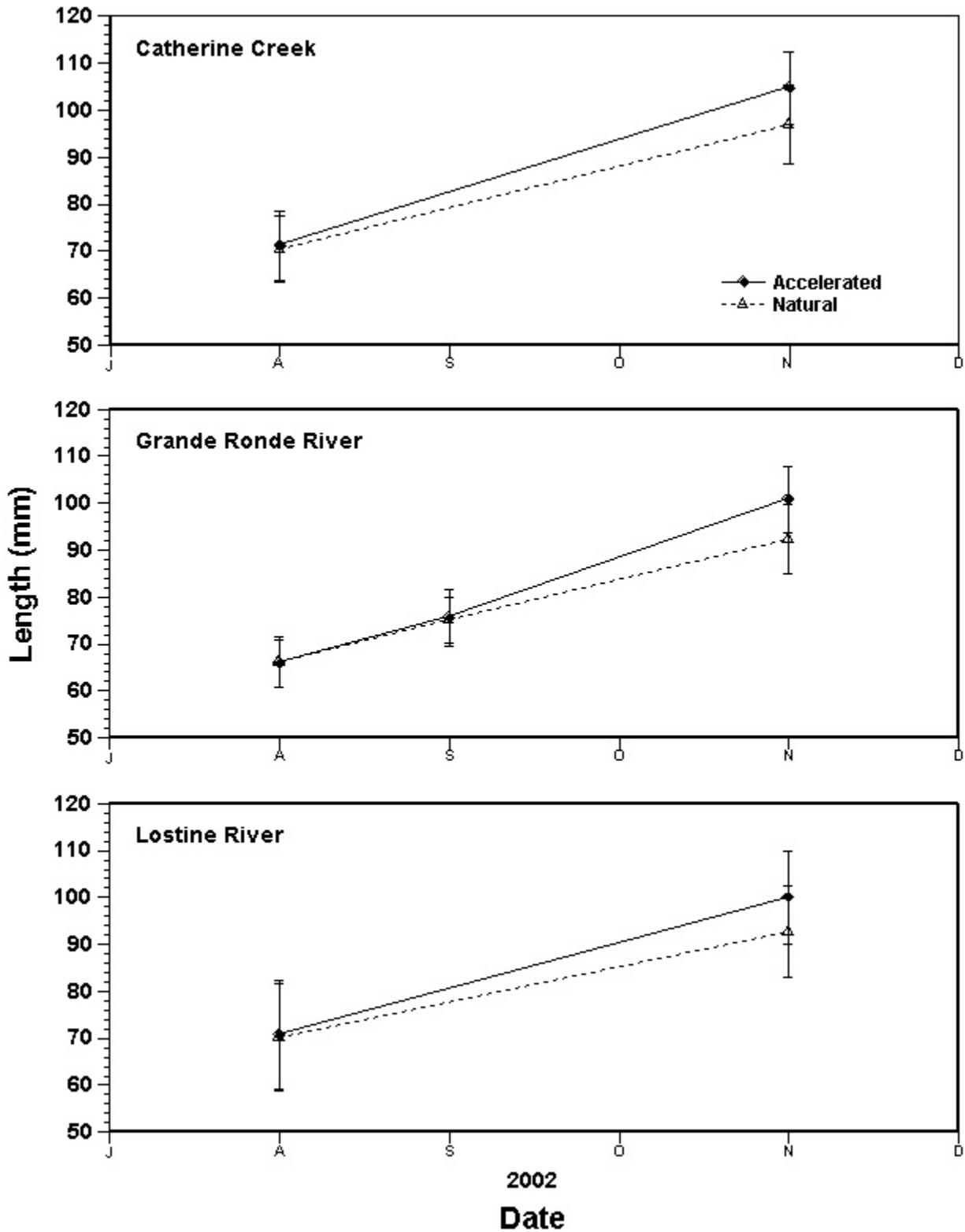


Figure 32 Mean length (± 1 SD) of 2001 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Accelerated and Natural pre-smolt growth regimes, 2002.

Table 15. Number and percentage of fish from which gametes were collected and mortalities due to operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each population (CC=Catherine Creek; GR=Grande Ronde River; LR=Lostine River), treatment (XA= pre-smolt Accelerated; XN= pre-smolt Natural) and age class of 2001 cohort spring chinook salmon. Note: “unknown” mortalities include 1 Catherine Creek, 1 Grande Ronde River and 3 Lostine River mortalities which have not yet been examined for cause of death.

Variable	Gametes		Operational		BKD		Other diseases		Other		Unknown	
	N	%	N	%	N	%	N	%	N	%	N	%
<u>Stock</u>												
CC	0	0.0	1	25	0	0.0	0	0.0	0	0.0	3	75
GR	0	0.0	0	0.0	0	0.0	4	80	0	0.0	1	20
LR	0	0.0	0	0.0	0	0.0	6	46.2	2	15.4	5	38.5
<u>Treatment</u>												
XA	0	0.0	0	0.0	0	0.0	5	41.7	1	8.3	6	50
XN	0	0.0	1	12.5	0	0.0	5	55.6	1	11.1	3	33.3
<u>Age</u>												
1	0	0.0	1	7.7	0	0.0	10	47.6	2	9.5	9	42.9
Total	0	0.0	1	7.7	0	0.0	10	47.6	2	9.5	9	42.9

Spawning

1998

Number of spawners

A total of 428 fish matured and contributed gametes (were spawned or had semen cryopreserved) in 1998: 198 (62.3%) males and 120 (37.7%) females. Within the treatment groups, only five (1.6%) were from the Freshwater Accelerated groups and all were males (Figure 32). One hundred seventy-two (54.1%) were from the Freshwater Natural group: 118 (68.6%) males and 54 (31.4%) females. One hundred forty-one (44.3%) Saltwater Natural fish were also spawned: 75 (53.2%) males and 66 (46.8%) females.

The age distribution of spawners in 1998 included ages 2–4 and males matured at an earlier age than females (Figure 32). Of the males, 13.5% were age 2, 65.5% were age 3 and 21.0% were age 4. Of the females, 0.8% were age 3 and 99.2% age 4.

Fecundity

Fecundity varied among treatments and age classes of spawning females. Mean fecundity of Freshwater Natural females was 1401 eggs, ranging from 141-2318 eggs (Figure 33). Mean Fecundity of Saltwater Natural females was 1314 eggs (range: 57-2341 eggs). Within age classes, fecundity was measured for only one 3-year old female: 1111 eggs (Figure 33). Four-year old females had a mean fecundity of 1355 eggs (range: 57-2341 eggs).

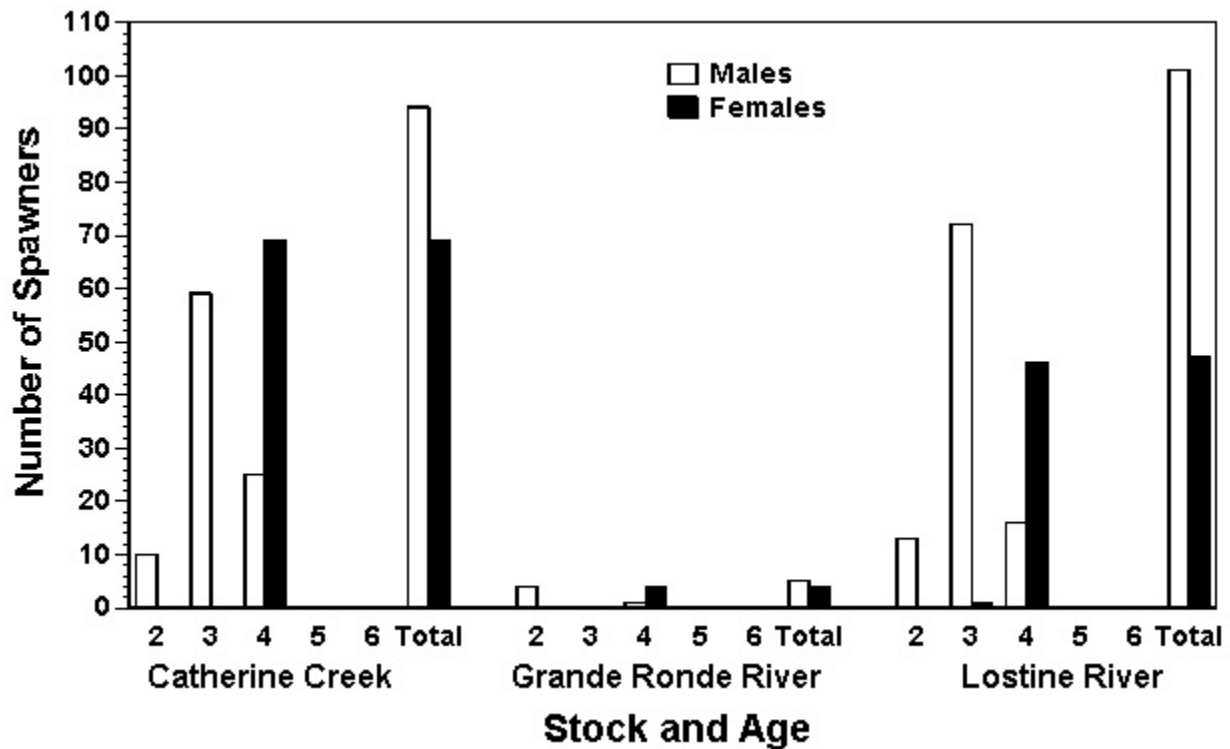
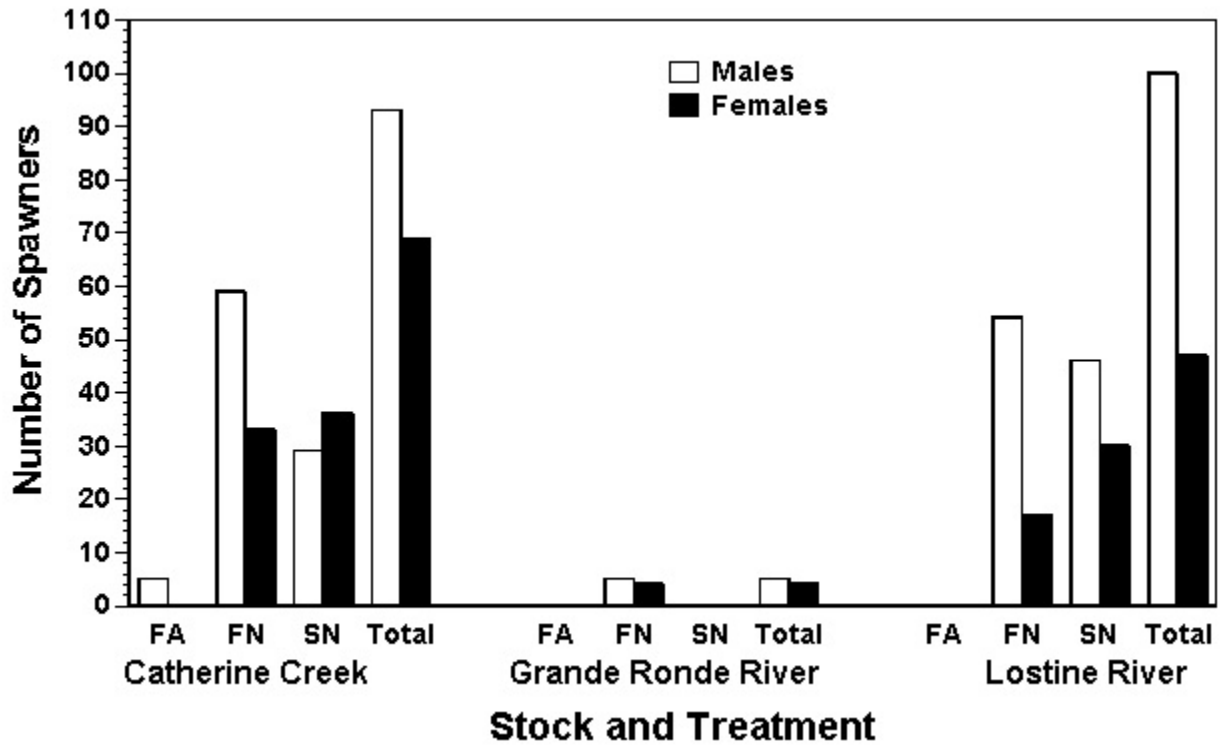


Figure 33 Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3 and 4 (bottom) of Catherine Creek, Grande Ronde River and Lostine River chinook salmon populations spawned in 1998.

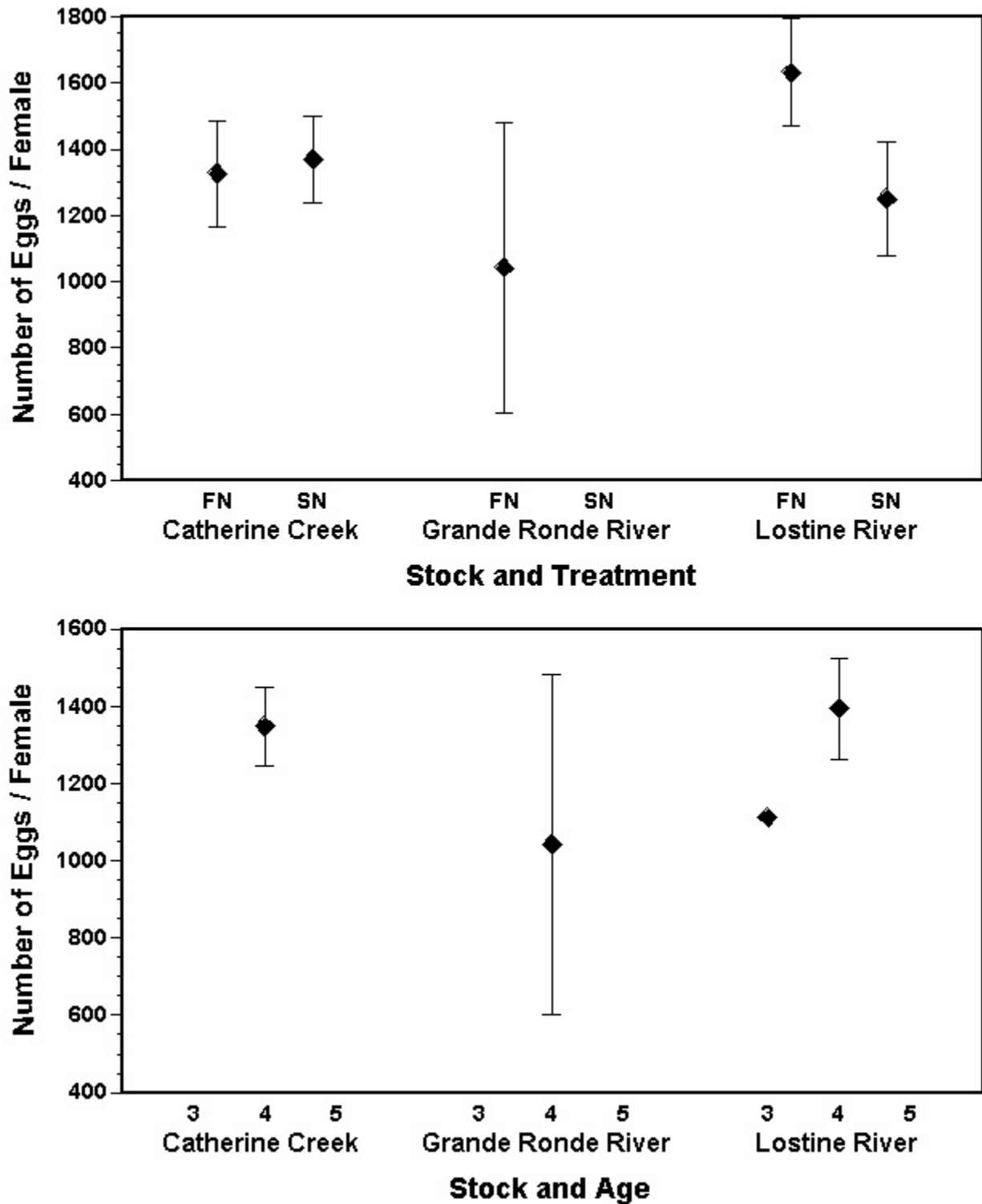


Figure 34 Mean ($\pm 95\%$ CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3 and 4 (bottom) in 1998.

Egg weight

Mean egg weight varied among treatments and age of spawning females. Mean egg weight of Freshwater Natural females was 0.200 g and ranged from 0.127-0.318 g (Figure 34). Mean weight of eggs from Saltwater Natural females was 0.169 g and ranged from 0.070-0.248 g.

Eggs were weighed from only one age 3 female: mean egg weight was 0.116 g (Figure 34). Mean egg weight of age 4 females was 0.183 g and ranged from 0.070-0.318 g.

Fertility

Mean percentage of eggs that reached the eyed stage (an estimate of fertilization rate) varied with the treatment group and age of the male or female. The mean percentage of eyed eggs from females in the Freshwater Natural treatment group was 41.4% and ranged from 0-97% (Figure 35). In females from the Saltwater Natural treatment, 66.5% reached the eyed stage and ranged from 0-98%.

Fertility was measured for only one 3-year old female: 75% (Figure 36). In age 4 females, 55.0% of the eggs reached the eyed stage and ranged from 0-98%.

Mean fertilization rate of eggs by males in the Freshwater Accelerated group was 73.9% and ranged from 49-84% (Figure 36). In the Freshwater Natural group, mean fertilization rate was 45.4%, ranging from 0-97%. In the Saltwater Natural group, mean fertilization rate was 71.7% and ranged from 0-97%.

Fertilization rate varied little with male age (Figure 36). Mean fertilization rate for age 3 males was 53.5% and ranged from 0-97%. Mean fertilization rate for age 4 males was 57.7% (range: 0-97%). Age 5 males had a mean fertilization rate of 52.4%, ranging from 0-95%.

No cryopreserved semen was used to fertilize eggs in 1998.

1999*Number of spawners*

A total of 686 fish matured and contributed gametes (were spawned or had semen cryopreserved) in 1999: 382 (55.7%) males and 307 (44.3%) females. Within the treatment groups, 117 (17.1%) were from the Freshwater Accelerated groups: 81 (69.2%) were males and 36 (30.8%) were females (Figure 37). Three hundred seventy-one (54.1%) were from the Freshwater Natural group: 186 (50.1%) males and 185 (49.9%) females. In the Saltwater Natural fish, 198 (28.9%) were spawned: 115 (58.1%) males and 83 (41.9%) females.

Spawners in 1999 included ages 2-5 and males matured at an earlier age than females (Figure 37). Of the males, 17.8% were age 2, 64.6% were age 3, 11.8% were age 4 and 5.8% were age 5. Of the females, 0.7% were age 3, 49.3% age 4 and 50.0% were age 5.

Fecundity

Fecundity varied among treatments and age classes of spawning females. Mean fecundity of Freshwater Accelerated females was 1503 eggs and ranged from 312-2274 eggs (Figure 38). Mean fecundity of Freshwater Natural females was 1781 eggs and ranged from 65-3365 eggs. Mean Fecundity of Saltwater Natural females was 1477 eggs (50-2802 eggs). Within age classes, mean fecundity was 1566.5 eggs for 3-year old females and ranged from 1105-2028 eggs (Figure 38). For 4-year old females, mean fecundity was 1592 eggs and ranged from 307-2796 eggs. Five-year old females had a mean fecundity of 1744 eggs, ranging from 65-3365 eggs.

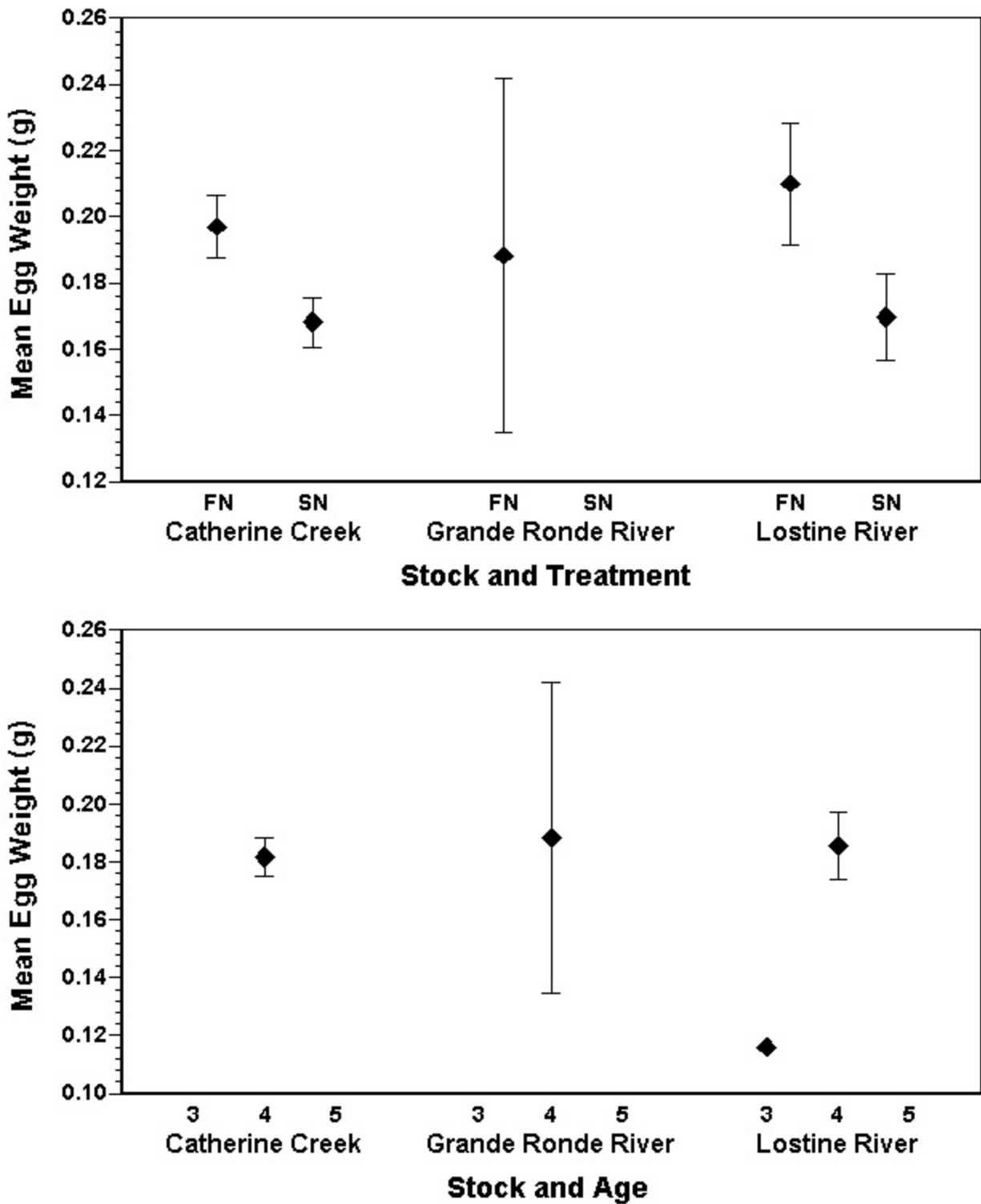


Figure 35 Mean ($\pm 95\%$ CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3 and 4 (bottom) in 1998.

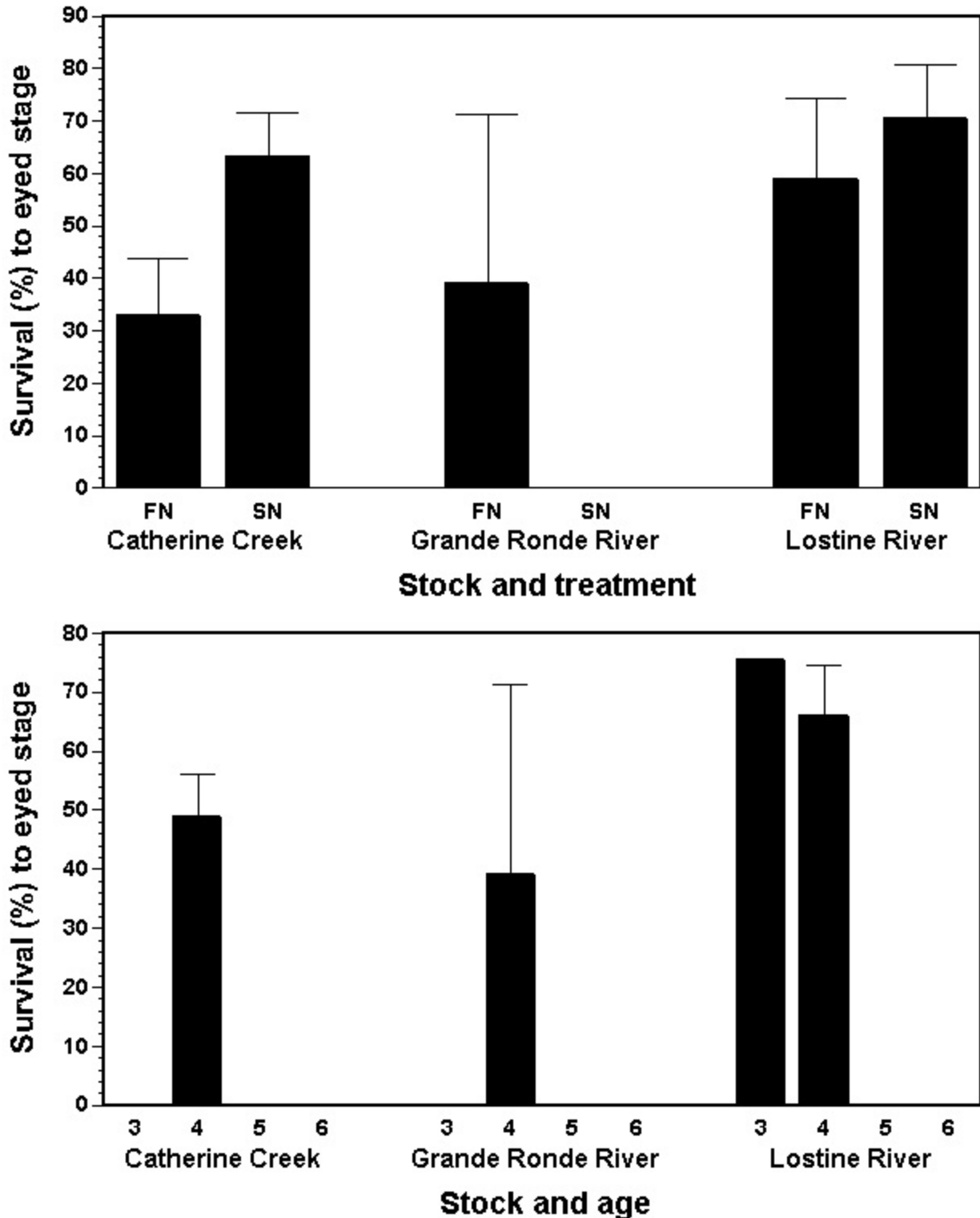


Figure 36 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon females raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and spawned at ages 3 and 4 (bottom) in 1998.

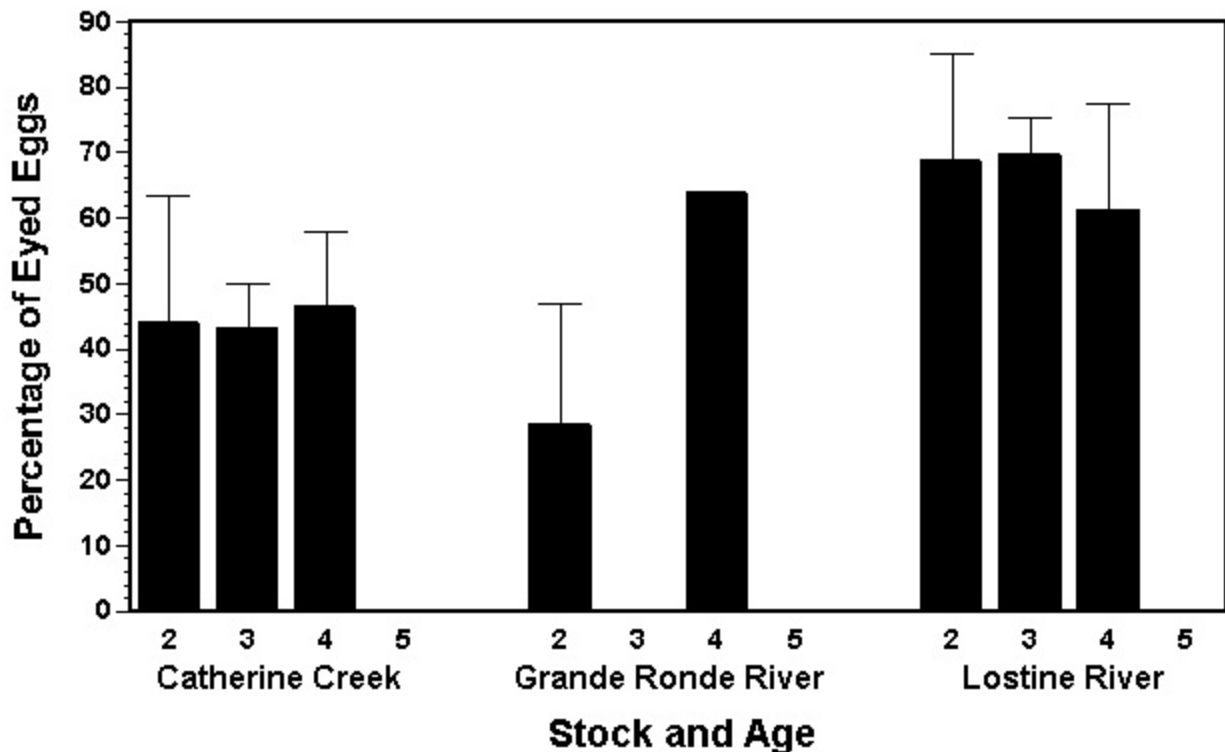
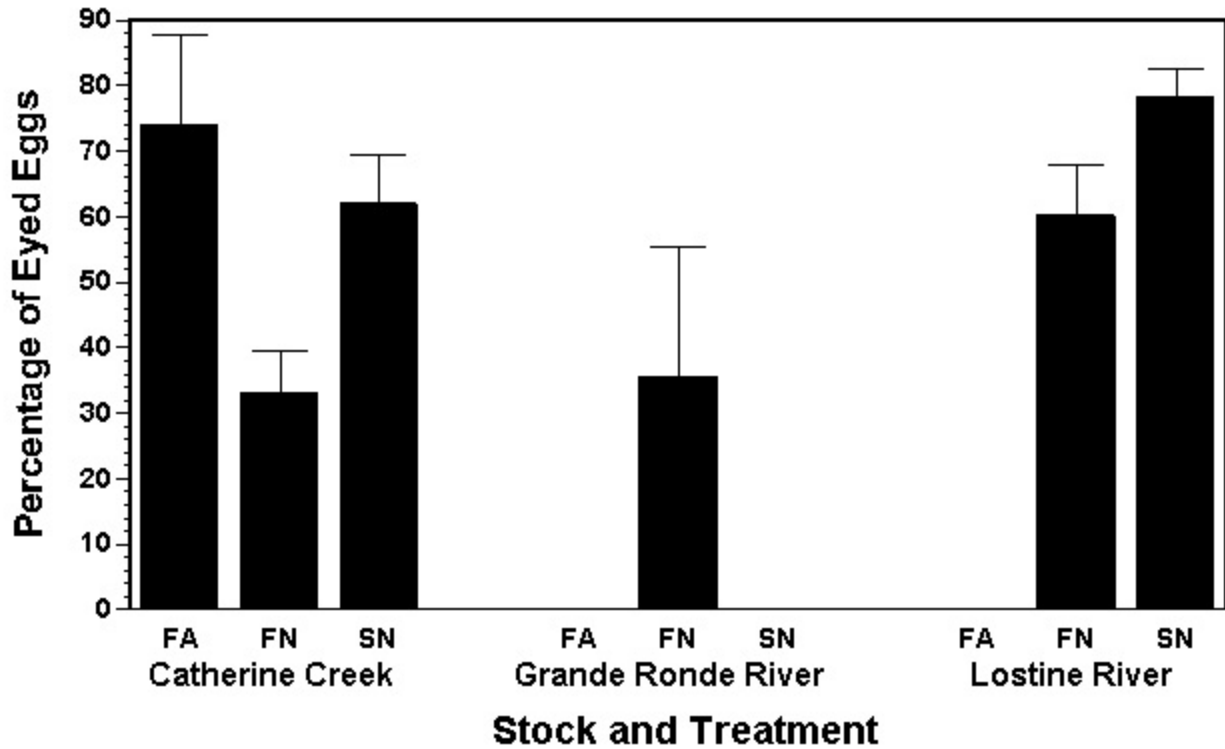


Figure 37 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and spawned at ages 2, 3 and 4 (bottom) in 1998.

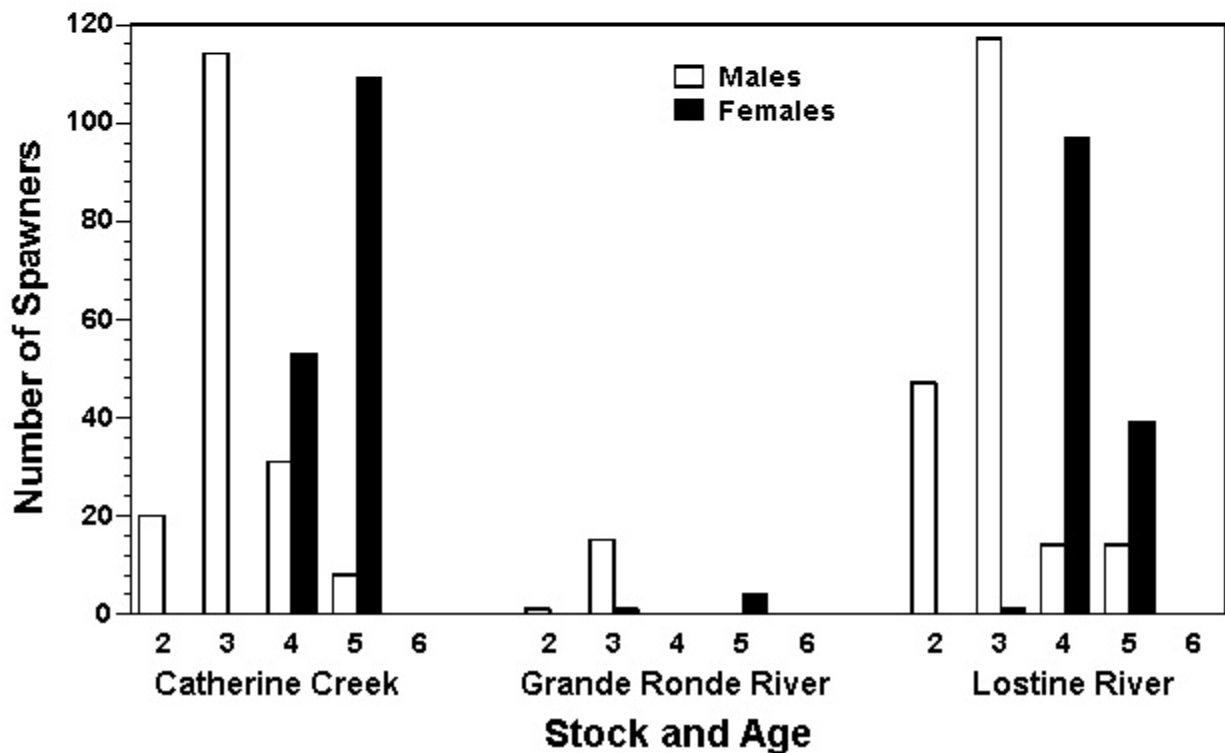
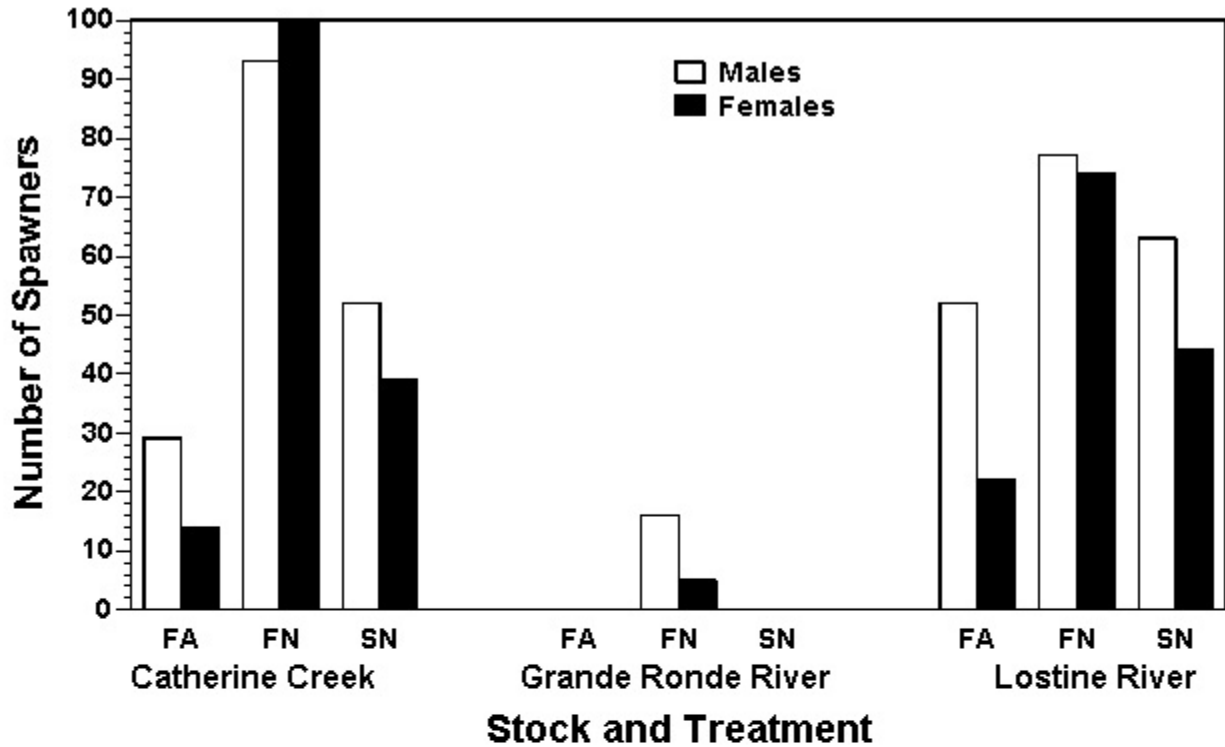


Figure 38 Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4 and 5 (bottom) of Catherine Creek, Grande Ronde River and Lostine River chinook salmon populations spawned in 1999.

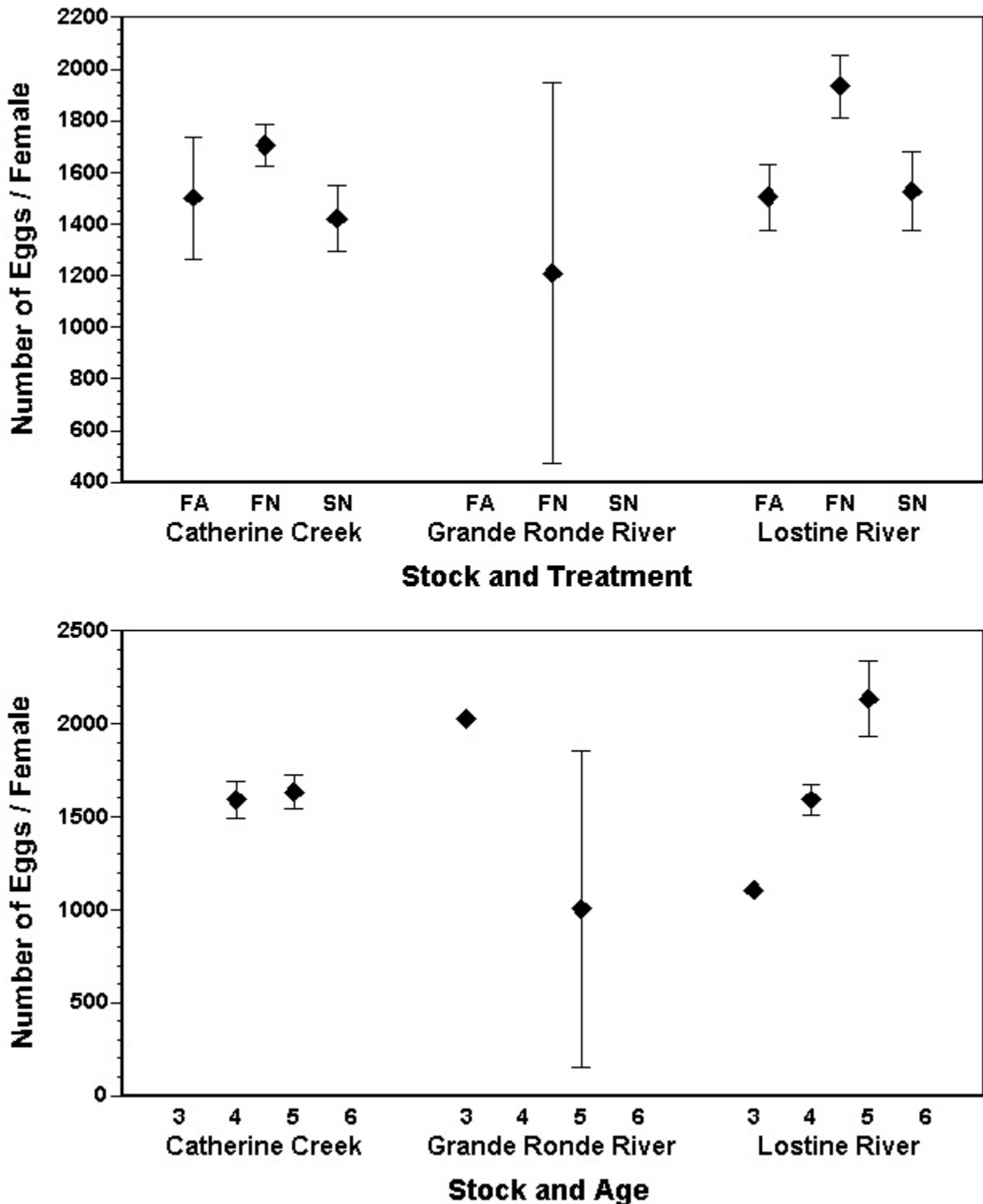


Figure 39 Mean ($\pm 95\%$ CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3, 4 and 5 (bottom) in 1999.

Egg weight

Mean egg weight varied among treatments and age of spawning females. Mean egg weight of Freshwater Accelerated females was 0.202 g and ranged from 0.136-0.271 g (Figure 39). Mean egg weight of Freshwater Natural females was 0.232 g and ranged from 0.142-0.345 g. Mean weight of eggs from Saltwater Natural females was 0.204 g, ranging from 0.117-0.289 g. Mean egg weight age 4 females was 0.207 g, ranging from 0.136-0.288 (Figure 39). Mean egg weight of age 5 females was 0.230 g and ranged from 0.117-0.345 g.

Fertility

Mean percentage of eggs that reached the eyed stage (an estimate of fertilization rate) varied with the treatment group and age of the male and/or female and between the use of fresh vs. cryopreserved semen (Figure 40). The mean percent eyed eggs from females in the Freshwater Accelerated treatment group was 57.7% and ranged from 0-99%. The mean percent eyed eggs from females in the Freshwater Natural treatment group was 74.7%, ranging from 0-100%. In Saltwater Natural females, 67.7% reached the eyed stage, ranging from 0-99%.

Fertility increased with age of females (Figure 40). Mean fertility of age 3 females was 53.1% and ranged from 8-98%. Age 4 females had a mean fertilization rate of 68.3%, ranging from 0-100%. In age 5 females, 73.6% of the eggs reached the eyed stage and ranged from 0-100%. Mean fertilization rate of eggs by males in the Freshwater Accelerated group was 68.5% and ranged from 8-100% (Figure 41). In the Freshwater Natural group, mean fertilization rate was 79.7%, ranging from 0-99%. In the Saltwater Natural group, mean fertilization rate was 74.0% and ranged from 0-100%. Fertilization rate varied little among age classes of males (Figure 41). Mean fertilization rate for age 2 males was 81.0% and ranged from 11-99%. Mean fertilization rate for age 3 males was 73.9% and ranged from 0-100%. Mean fertilization rate for age 4 males was 79.6% and ranged from 0-100%. Age 5 males had a mean fertilization rate of 61.5%, ranging from 25-89%.

Fertilization rate was much better with fresh semen than with cryopreserved semen (Figure 42). Mean fertilization rate using fresh semen was 75.6% and ranged from 0-100%. Using cryopreserved semen, mean fertilization rate was only 25.9%, ranging from 0-90%.

2000*Number of spawners*

A total of 1097 fish matured and contributed gametes (were spawned or had semen cryopreserved) in 2000: 641 (58.4%) males and 458 (41.6%) females. Within the treatment groups, 368 (33.5%) were from the Freshwater Accelerated groups: 205 (55.7%) were males and 163 (44.3%) were females (Figure 43). Four hundred seventeen (38.0%) were from the Freshwater Natural group: 243 (58.3%) males and 174 (41.7%) females. In the Saltwater Natural fish, 312 (28.4%) were spawned: 193 (61.9%) males and 119 (38.1%) females.

Spawning age distribution in 2000 included ages 2-6 and males matured at an earlier age than females (Figure 43). Of the males, 19.3% were age 2, 67.7% were age 3, 12.9% were age 4 and 0.2% were age 5. Of the females, 86.2% were age 4, 12.3% age 5 and 1.5% were age 6.

Fecundity

Fecundity varied among treatments and age classes of spawning females. Mean fecundity of Freshwater Accelerated females was 1838.9 eggs and ranged from 431-3728 eggs (Figure 44).

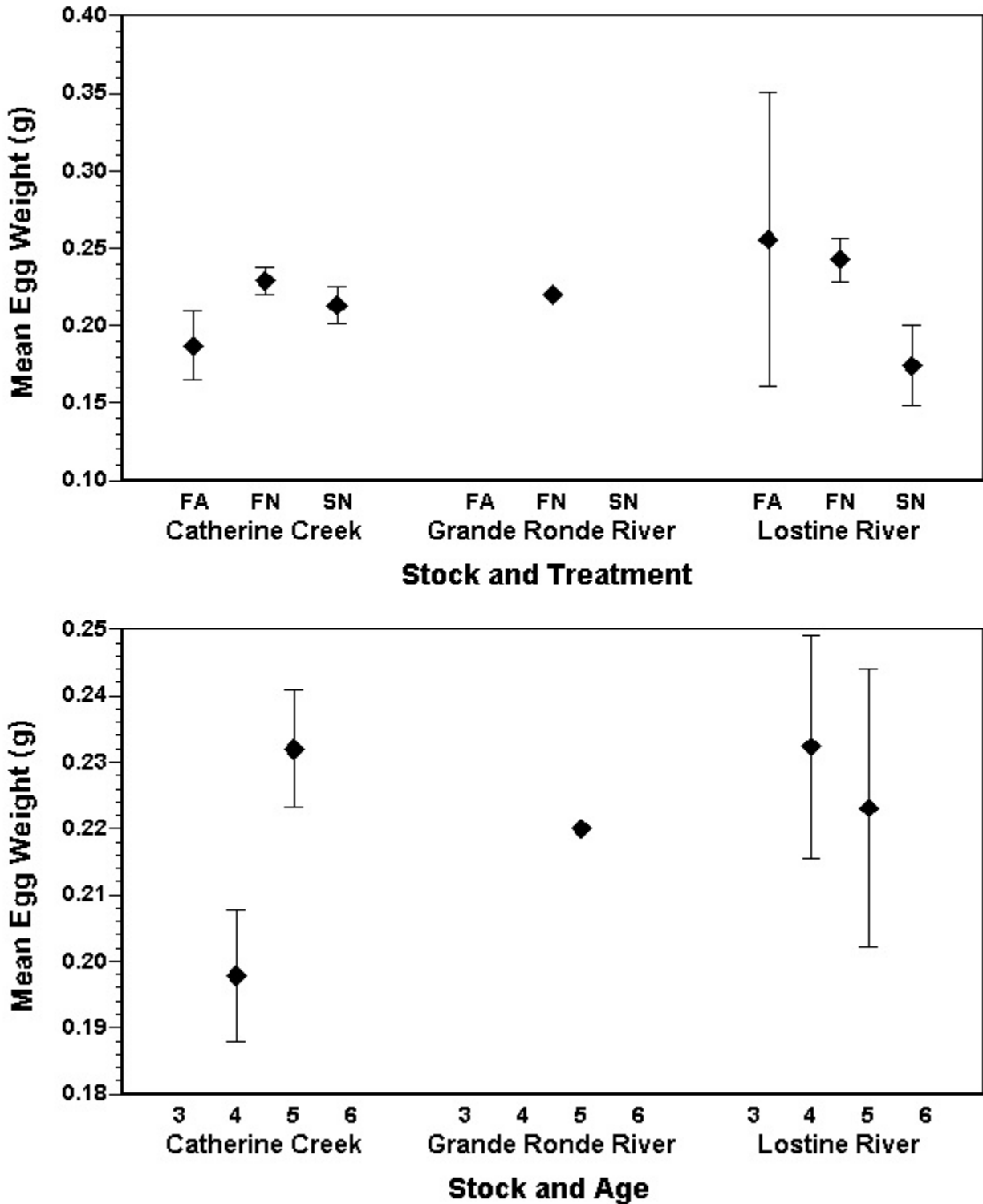


Figure 40 Mean ($\pm 95\%$ CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3, 4 and 5 (bottom) in 1999.

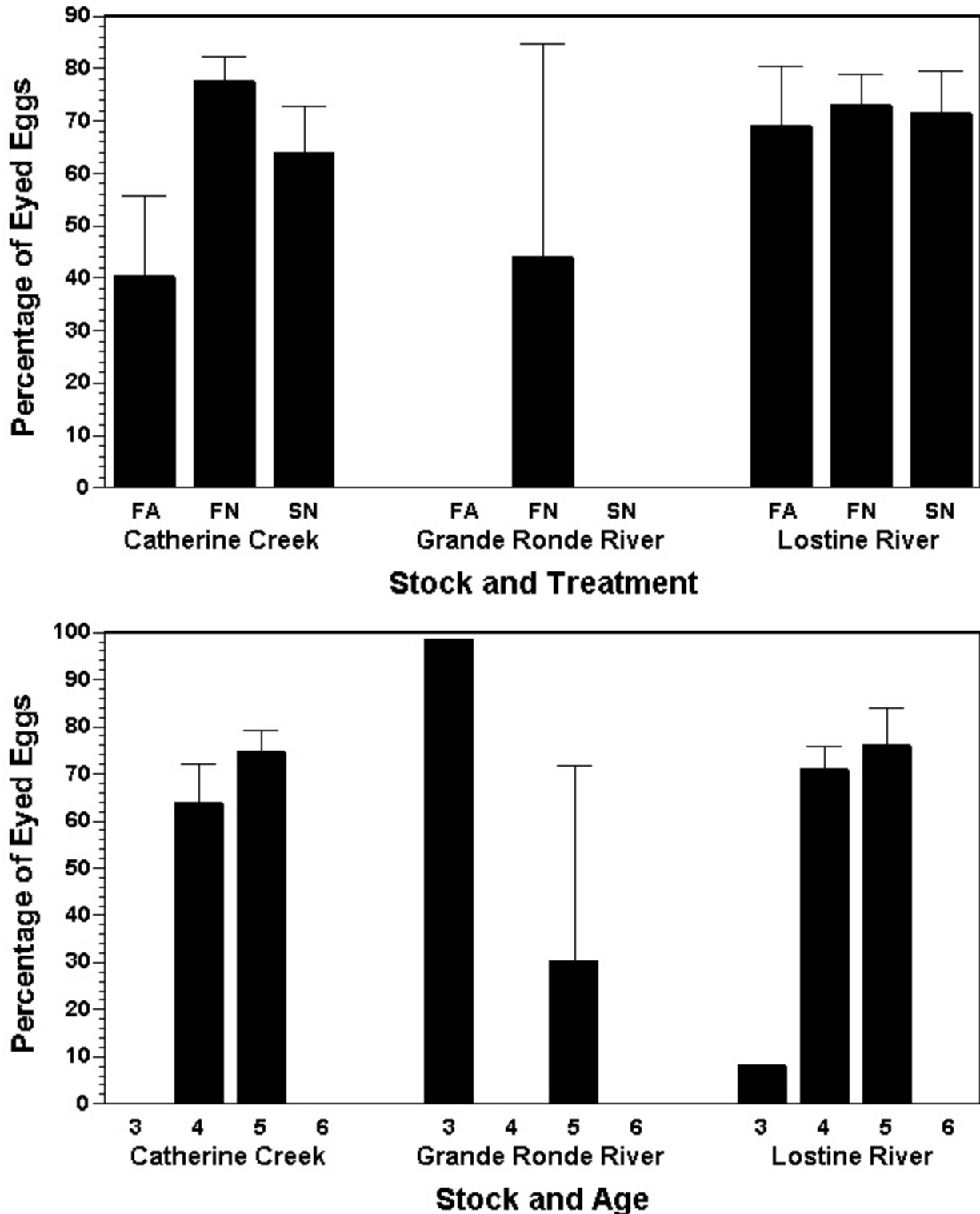


Figure 41 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) from spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4 and 5 year old (bottom) Catherine Creek, Grande Ronde River and Lostine River spawned in 1999.

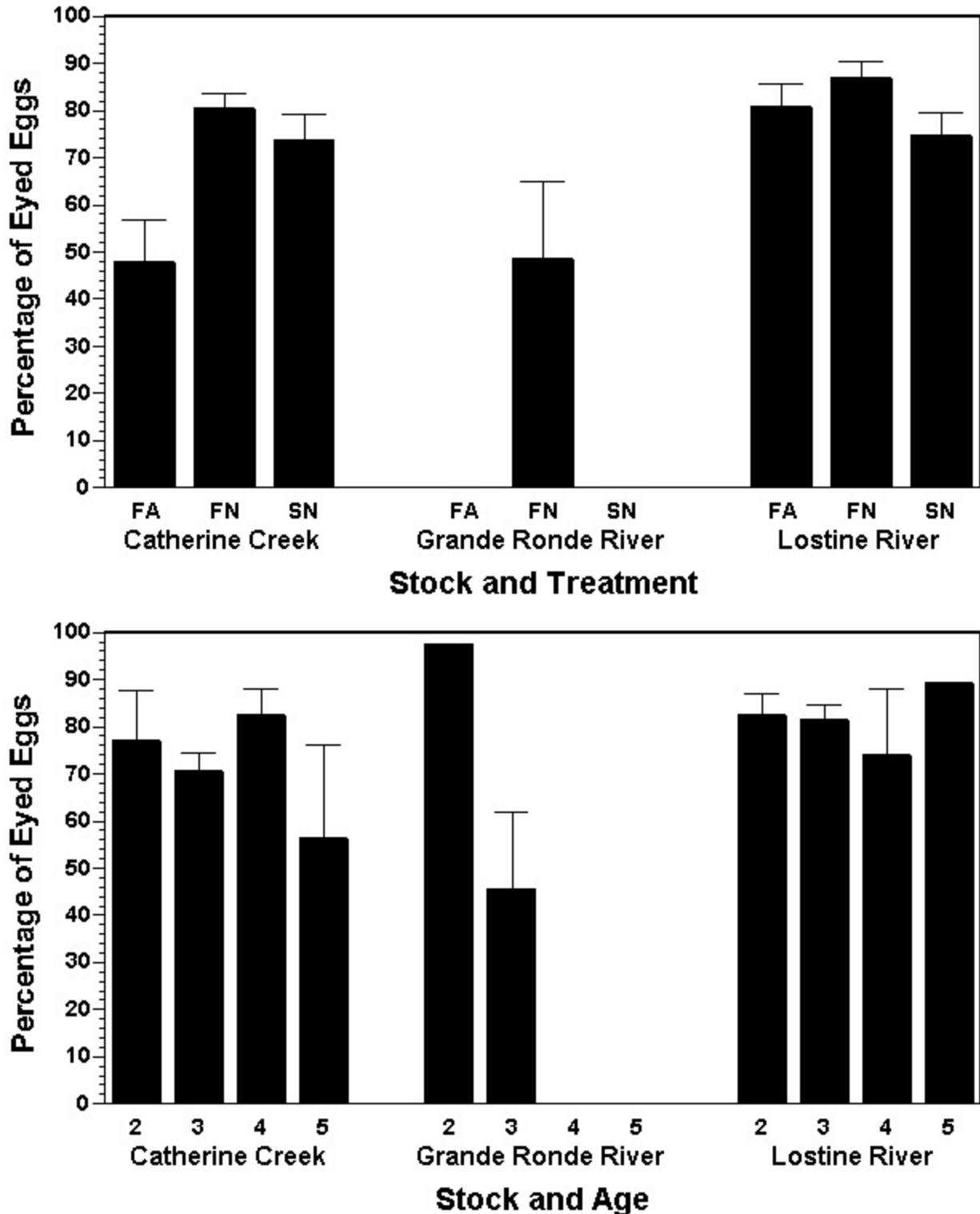


Figure 42 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 2, 3, 4 and 5 year old (bottom) in 1999.

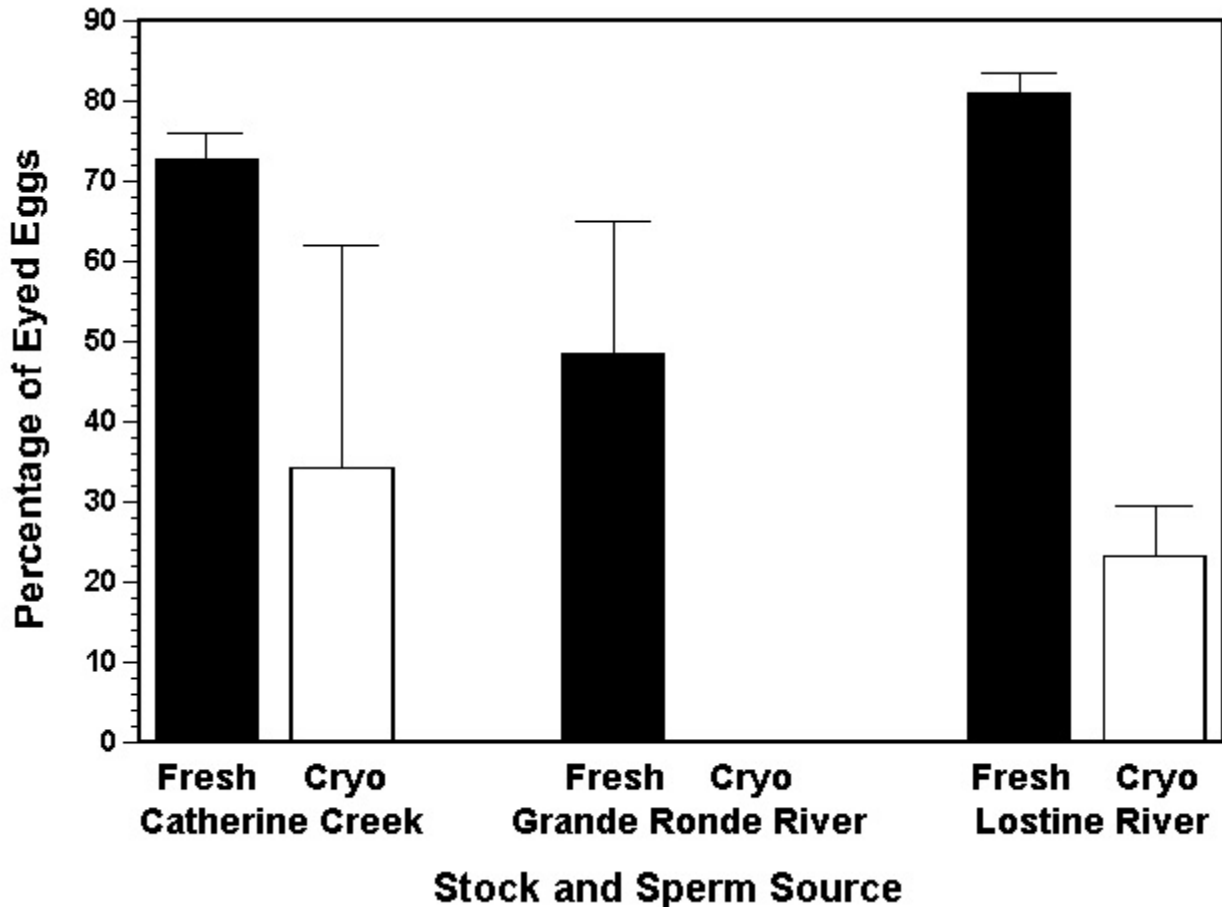


Figure 43 Mean ($\pm 95\%$ CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males, 1999.

Mean fecundity of Freshwater Natural females was 2003.5 eggs and ranged from 524-3712 eggs. Mean fecundity of Saltwater Natural females was 1396.5 eggs (386-3439 eggs). Within age classes, mean fecundity was 1816.4 eggs for 4-year old females and ranged from 386-3728 eggs (Figure 44). For 5-year old females, mean fecundity was 1593.1 eggs and ranged from 431-3297 eggs. Six-year old females had a mean fecundity of 1507.8 eggs, ranging from 462-2477 eggs.

Egg weight

Mean egg weight varied among treatments and age of spawning females. Mean egg weight of Freshwater Accelerated females was 0.213 g and ranged from 0.130-0.393 g (Figure 45). Mean egg weight of Freshwater Natural females was 0.212 g, ranging from 0.124-0.359 g. Mean weight of eggs from Saltwater Natural females was 0.202 g, ranging from 0.121-0.379 g. Mean egg weight age 4 females was 0.208 g, ranging from 0.124-0.351 (Figure 45). Mean egg weight of age 5 females was 0.216 g and ranged from 0.121-0.393 g. Mean egg weight of age 6 females was 0.271 g and ranged from 0.164-0.359 g.

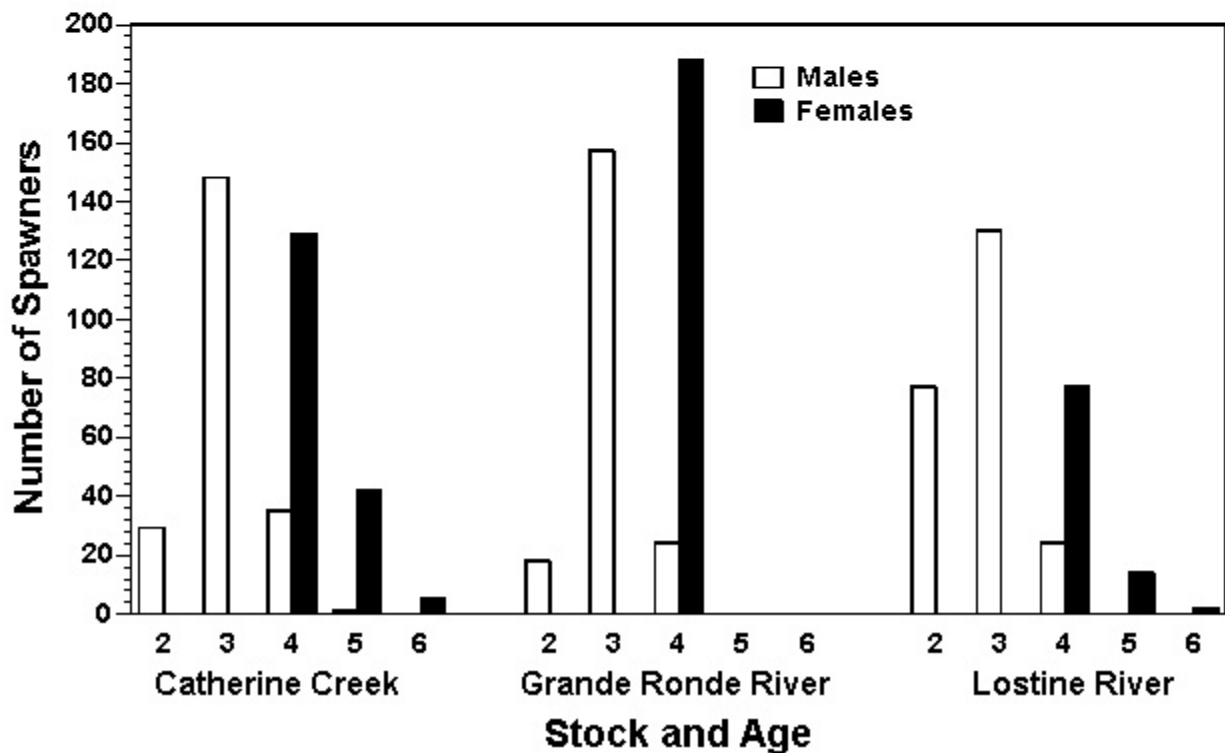
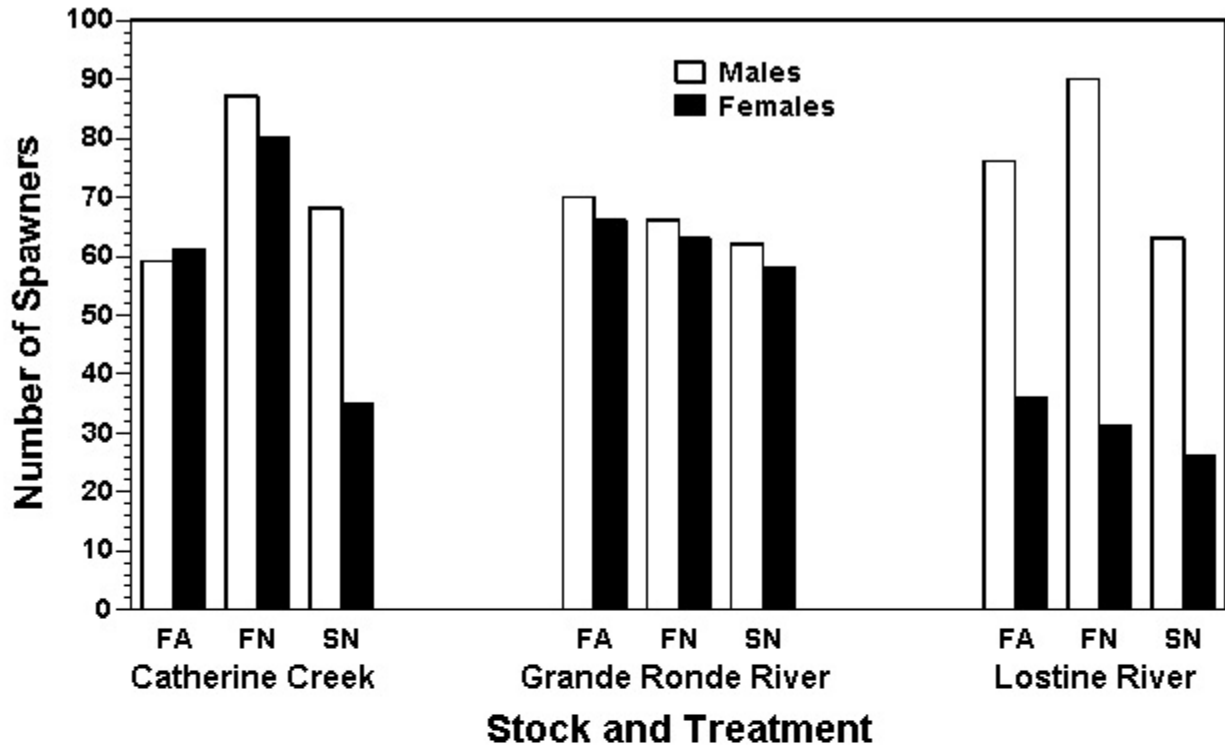


Figure 44 Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River chinook salmon populations spawned in 2000.

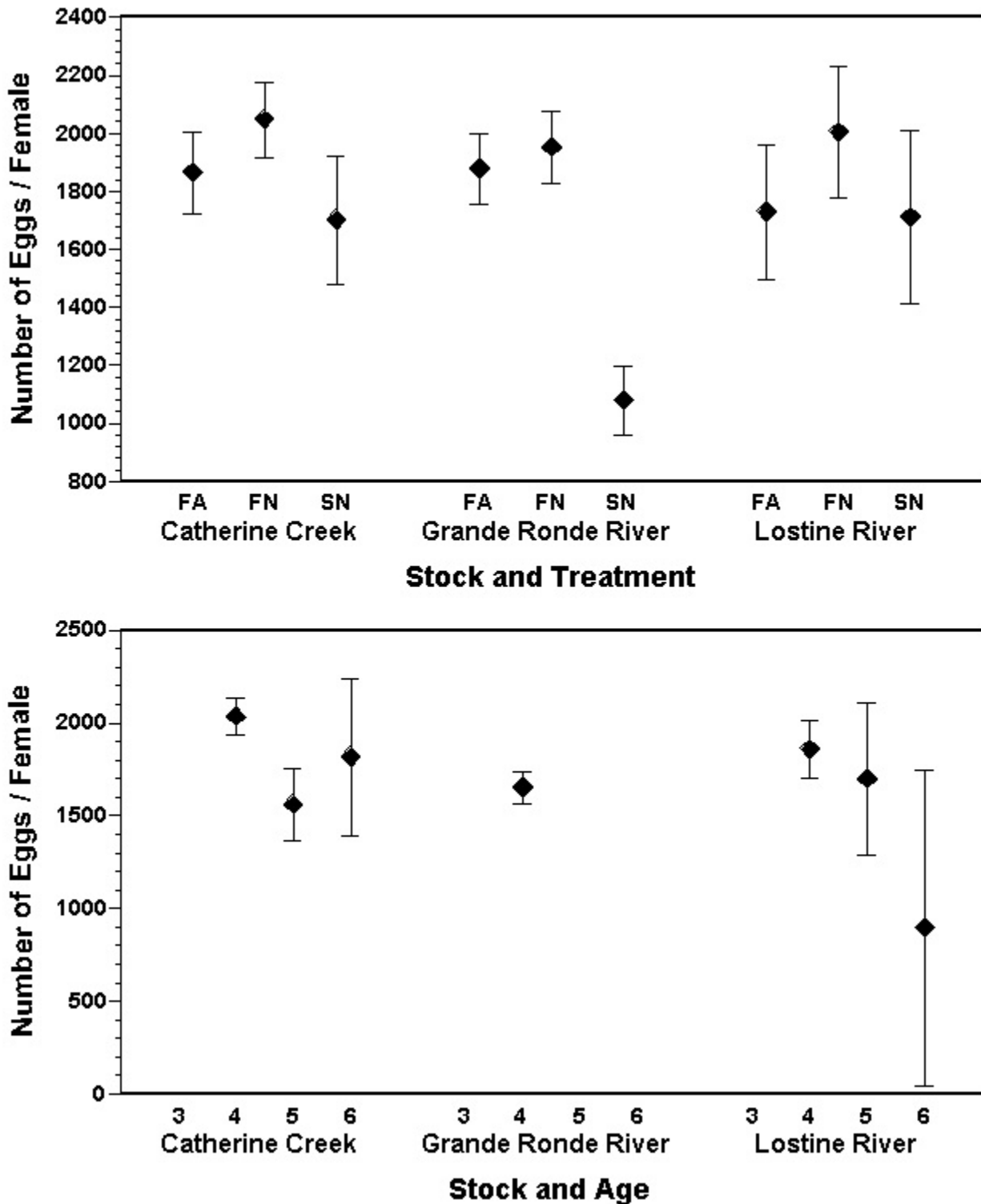


Figure 45 Mean ($\pm 95\%$ CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3, 4, 5 and 6 (bottom) in 2000.

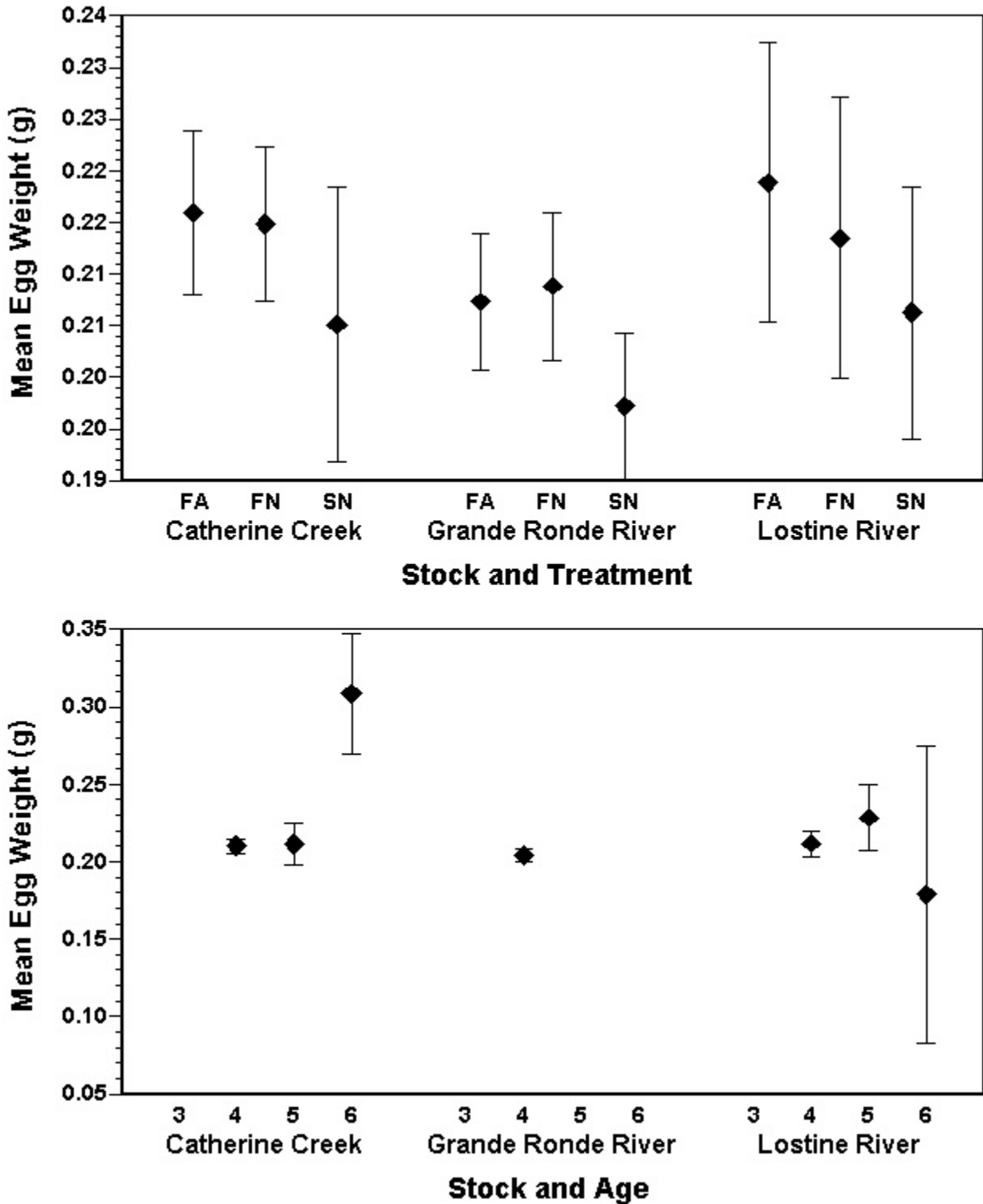


Figure 46 Mean ($\pm 95\%$ CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3, 4, 5 and 6 (bottom) in 2000.

Fertility

Mean percentage of eggs that reached the eyed stage (an estimate of fertilization rate) varied with the treatment group and age of the male and/or female and between the use of fresh vs. cryopreserved semen. The mean percent eyed eggs from females in the Freshwater Accelerated treatment group was 84.8% and ranged from 0-99% (Figure 46). The mean percent eyed eggs from Freshwater Natural females was 79.2%, ranging from 0-100%. In Saltwater Natural females, 78.5% reached the eyed stage, ranging from 0-100%.

Fertility decreased with age of females (Figure 46). Mean fertility of age 4 females was 84.0% and ranged from 0-100%. Age 5 females had a mean fertilization rate of 63.1%, ranging from 0-99%. In age 6 females, fertilization rate was 54.4% (3- 88%). Mean fertilization rate of eggs by males in the Freshwater Accelerated group was 83.3% and ranged from 2-99% (Figure 47). In the Freshwater Natural group, mean fertilization rate was 84.5%, ranging from 0-100%. In the Saltwater Natural group, mean fertilization rate was 76.7% and ranged from 2-99%. Fertilization rate varied little among age classes of males (Figure 47). Mean fertilization rate for age 2 males was 75.6% and ranged from 0-99%. Mean fertilization rate for age 3 males was 84.4% and ranged from 0-100%. Mean fertilization rate for age 4 males was 76.3% and ranged from 0-99%. Fertilization rate for only one age 5 male was measured: 32.7%.

Fertilization rate was much better with fresh semen than with cryopreserved semen (Figure 48). Mean fertilization rate using fresh semen was 81.7% and ranged from 0-100%. Using cryopreserved semen, mean fertilization rate was only 37.3%, ranging from 0-99%.

2001*Number of spawners*

A total of 1098 fish matured and contributed gametes (were spawned or had semen cryopreserved) in 2001: 631 (58.2%) males and 454 (41.8%) females (Figure 49). Within the treatment groups, 365 (32.9%) were from the Freshwater Accelerated groups: 190 (52.1%) were males and 163 (47.9%) were females. Three hundred ninety-four (35.5%) were from the Freshwater Natural group: 220 (55.8%) males and 174 (44.2%) females. In the Saltwater Natural fish, 351 (31.6%) were spawned: 221 (63.0%) males and 130 (37.0%) females.

Age distribution of spawners in 1999 ranged from 2-6 with males maturing earlier than females (Figure 49). Of the males, 14.3% were age 2, 68.0% age 3, 15.4% age 4, 2.1% age 5 and 0.3% age 6. Of the females, 0.2% were age 3, 86.6% age 4, 12.1% age 5 and 1.0% were age 6.

Fecundity

Fecundity varied among treatments and age of spawning females. Mean fecundity of Freshwater Accelerated females was 2043.3 eggs and ranged from 762-4378 eggs (Figure 50). Mean fecundity of Freshwater Natural females was 2221.7 eggs and ranged from 276-4270 eggs. Mean Fecundity of Saltwater Natural females was 1985.1 eggs and ranged from 122-4251 eggs. Within age classes, fecundity was 1068 eggs for the one 3-year old female spawned in 2001 (Figure 50). For 4-year old females mean fecundity was 2126.2 eggs and ranged from 122-4378 eggs. For 5-year old females, mean fecundity was 1942.6 eggs and ranged from 342-3351 eggs. Six-year old females had a mean fecundity of 1159.8 eggs, ranging from 276-1940 eggs.

Egg weight

Mean egg weight varied among treatments and age of spawning females. Mean egg

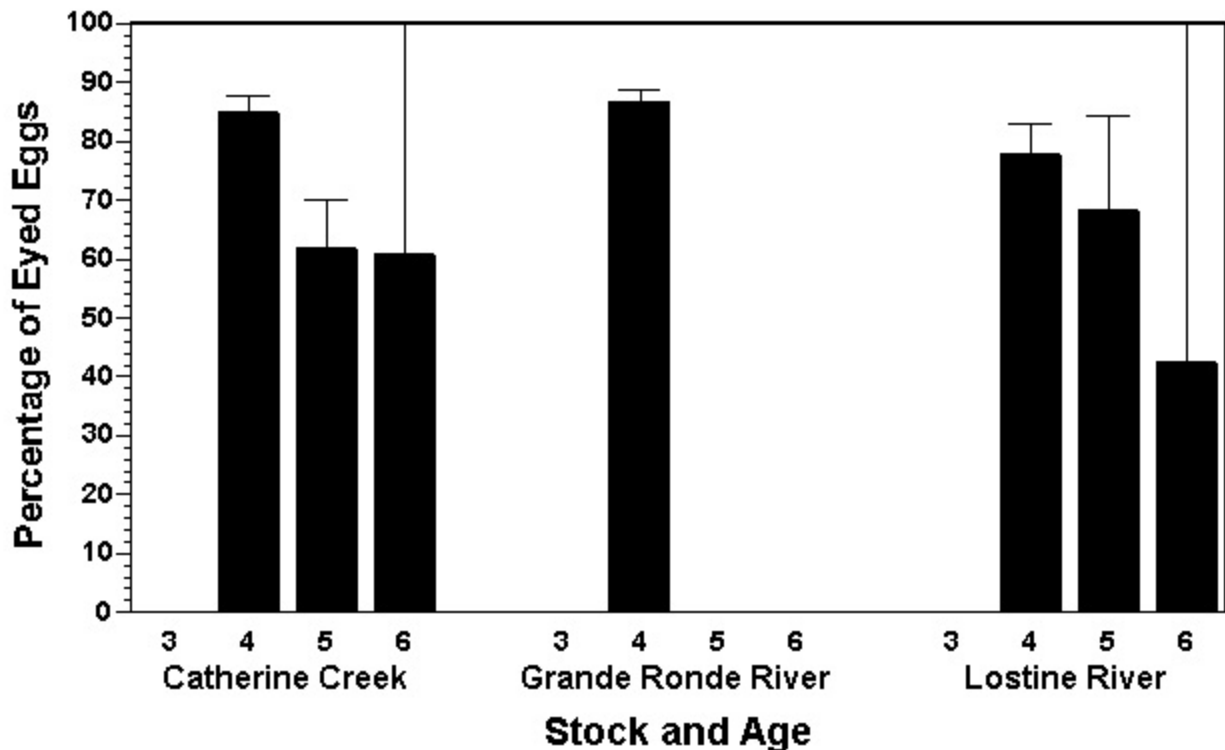
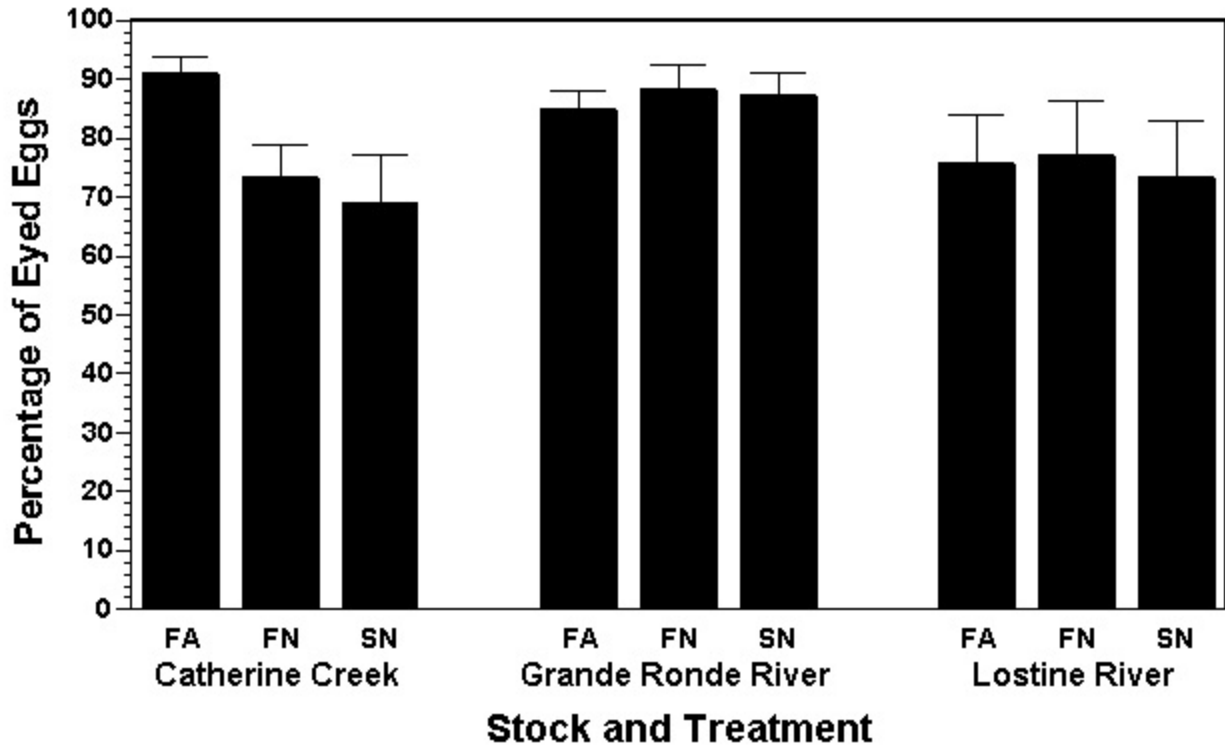


Figure 47 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) from spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) Catherine Creek, Grande Ronde River and Lostine River spawned in 2000.

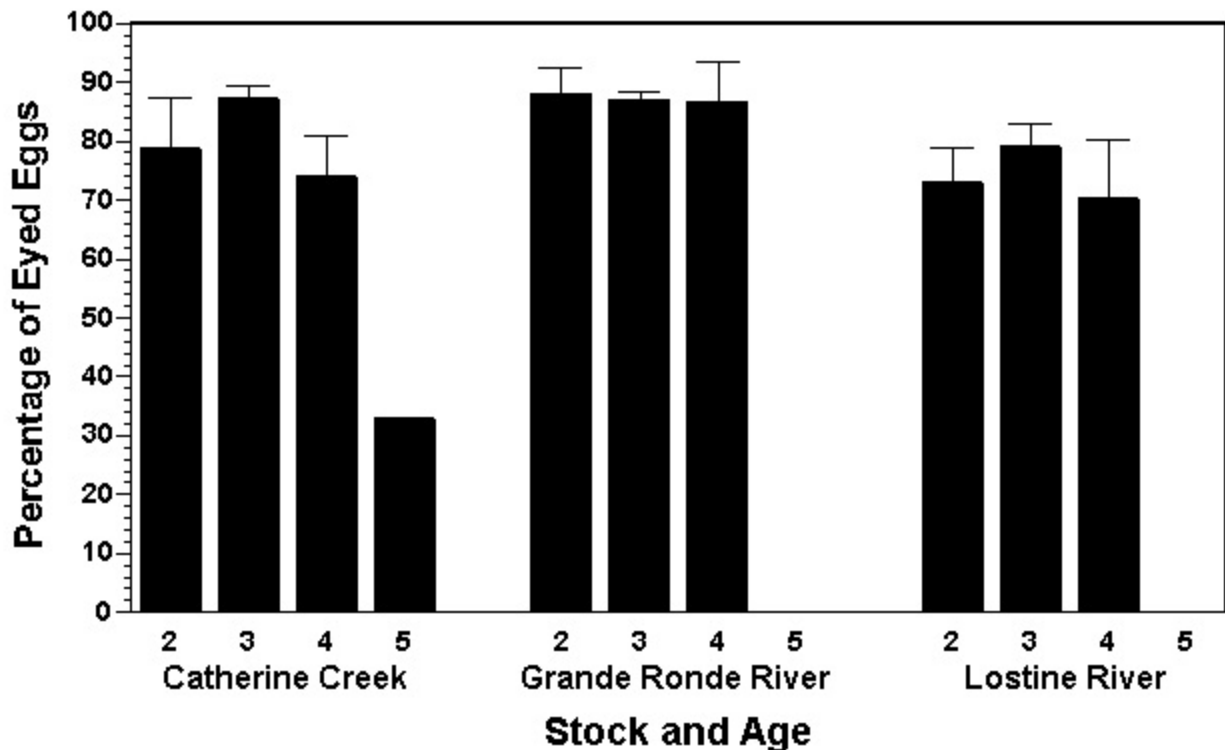
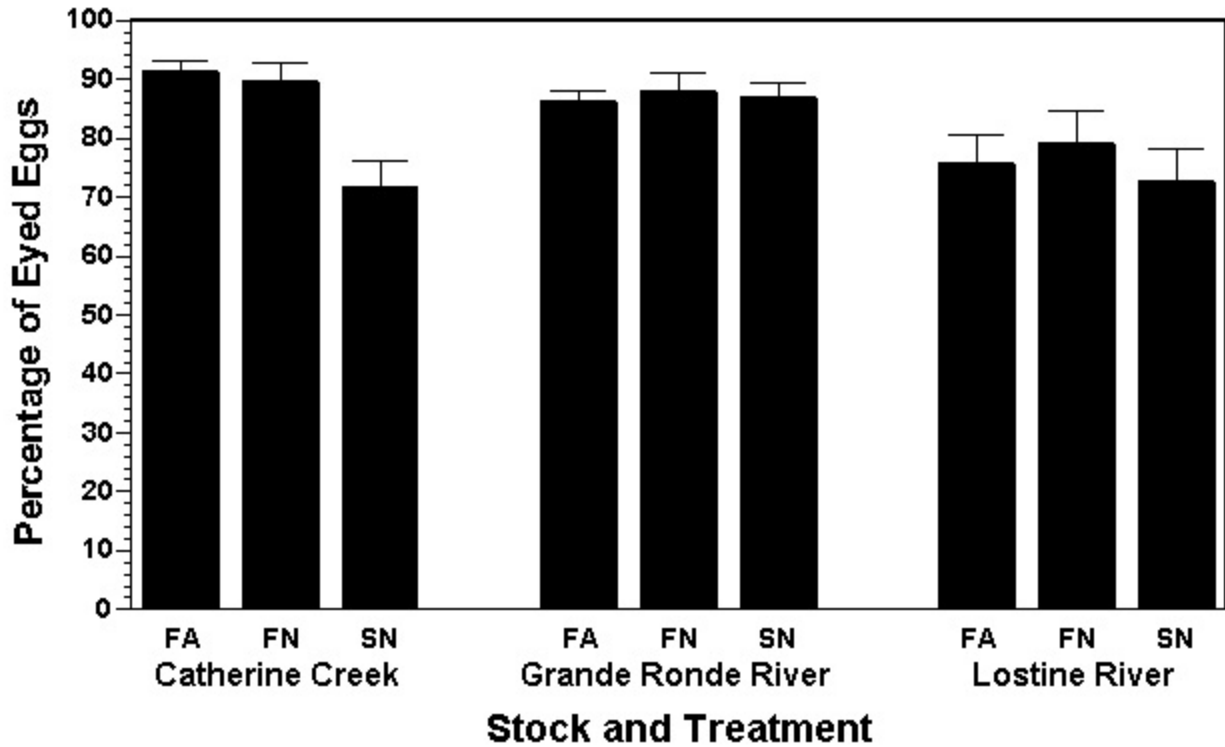


Figure 48 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) in 2000.

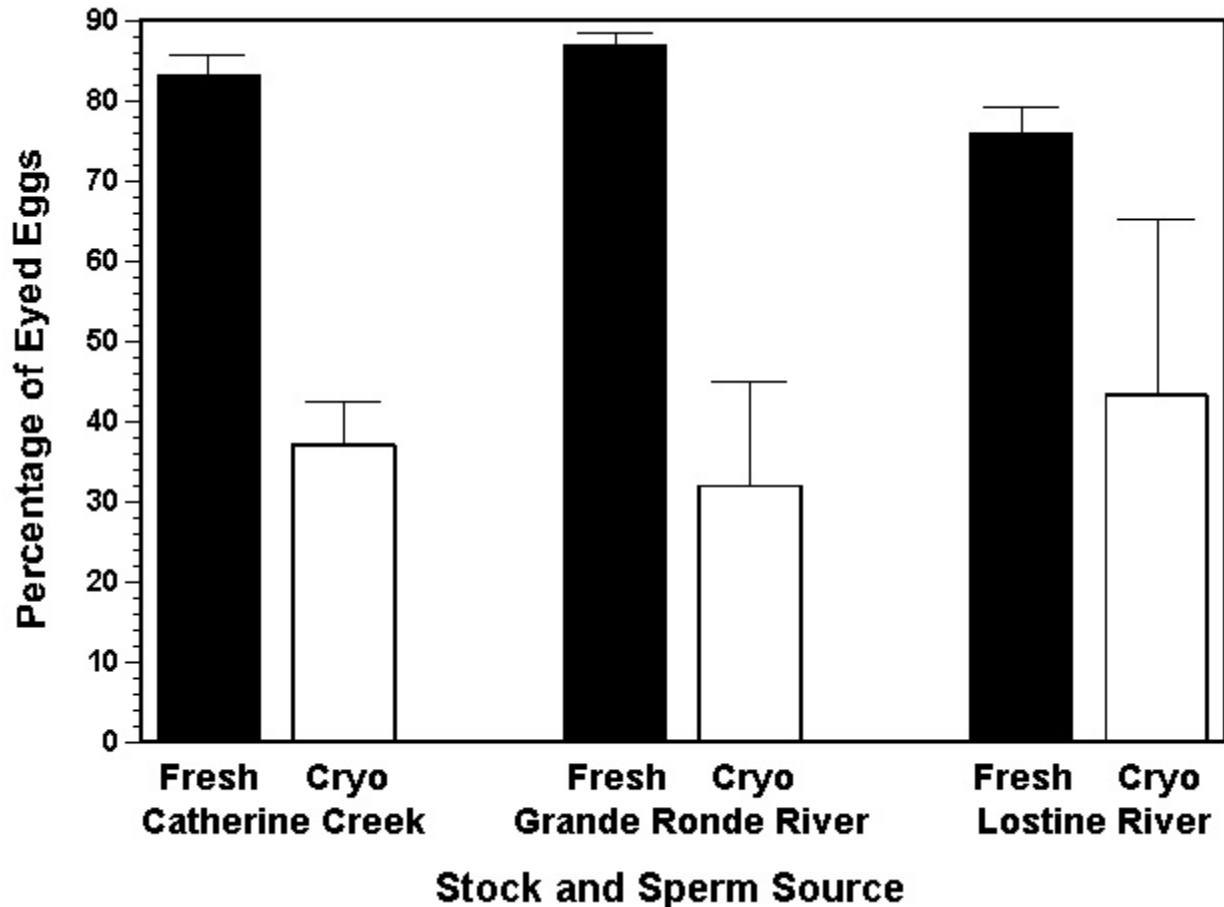


Figure 49 Mean ($\pm 95\%$ CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males, 2000.

weight of Freshwater Accelerated females was 0.218 g and ranged from 0.06-0.32 g (Figure 51). Mean egg weight of Freshwater Natural females was 0.214 g and ranged from 0.14-0.31 g. Mean weight of eggs from Saltwater Natural females was 0.202 g, ranging from 0.11-0.32 g. Mean egg weight of age 4 females was 0.211 g, ranging from 0.06-0.32 (Figure 51). Mean egg weight of age 5 females was 0.220 g and ranged from 0.13-0.32 g. Mean egg weight of age 6 females was 0.240 g and ranged from 0.18-0.31 g.

Fertility

Mean percentage of eggs that reached the eyed stage (an estimate of fertilization rate) varied with the treatment group and age of the male and/or female. The mean percent eyed eggs from females in the Freshwater Accelerated treatment group was 85.9% and ranged from 3.0-99.5% (Figure 52). The mean percent eyed eggs from females in the Freshwater Natural treatment group was 87.7%, ranging from 0-99.1%. In Saltwater Natural females, 84.6% reached the eyed stage, ranging from 0-99.6%.

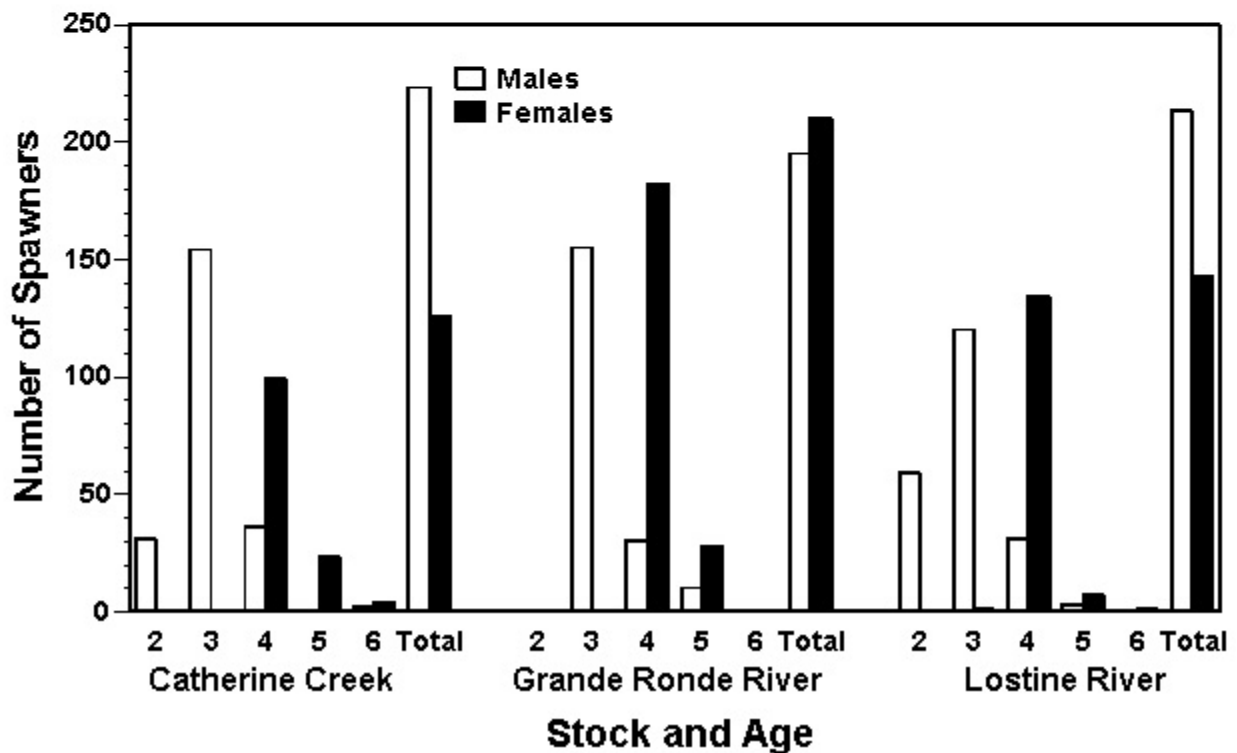
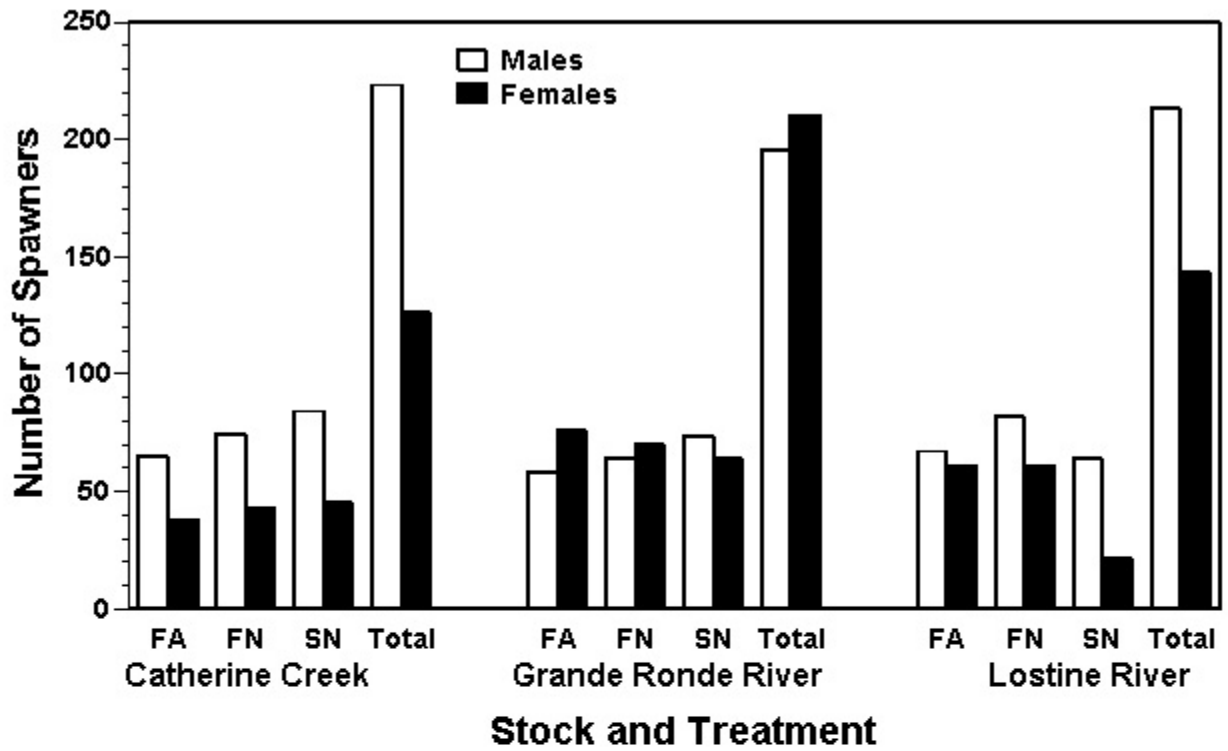


Figure 50 Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River chinook salmon populations spawned in 2001.

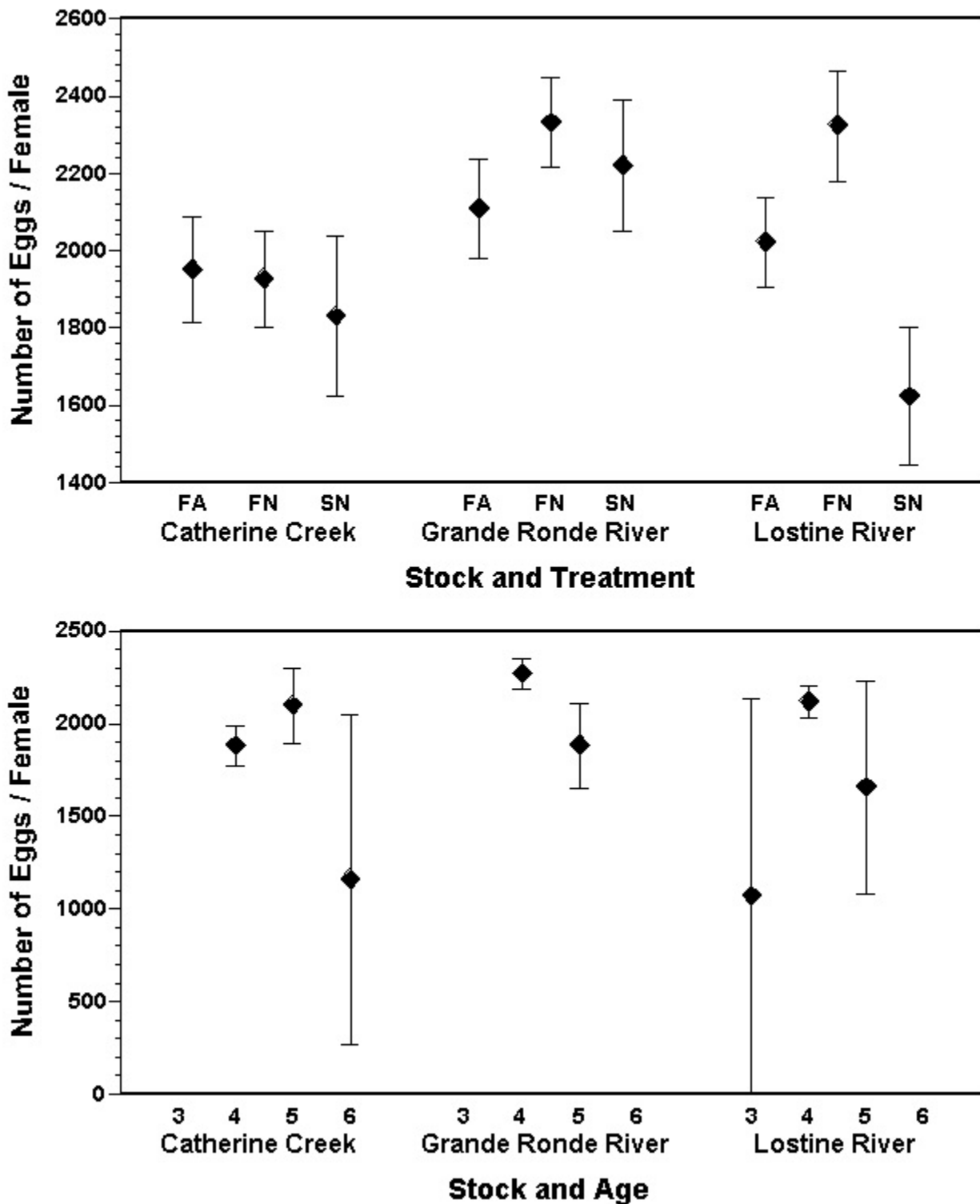


Figure 51 Mean ($\pm 95\%$ CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3, 4, 5 and 6 (bottom) in 2001.

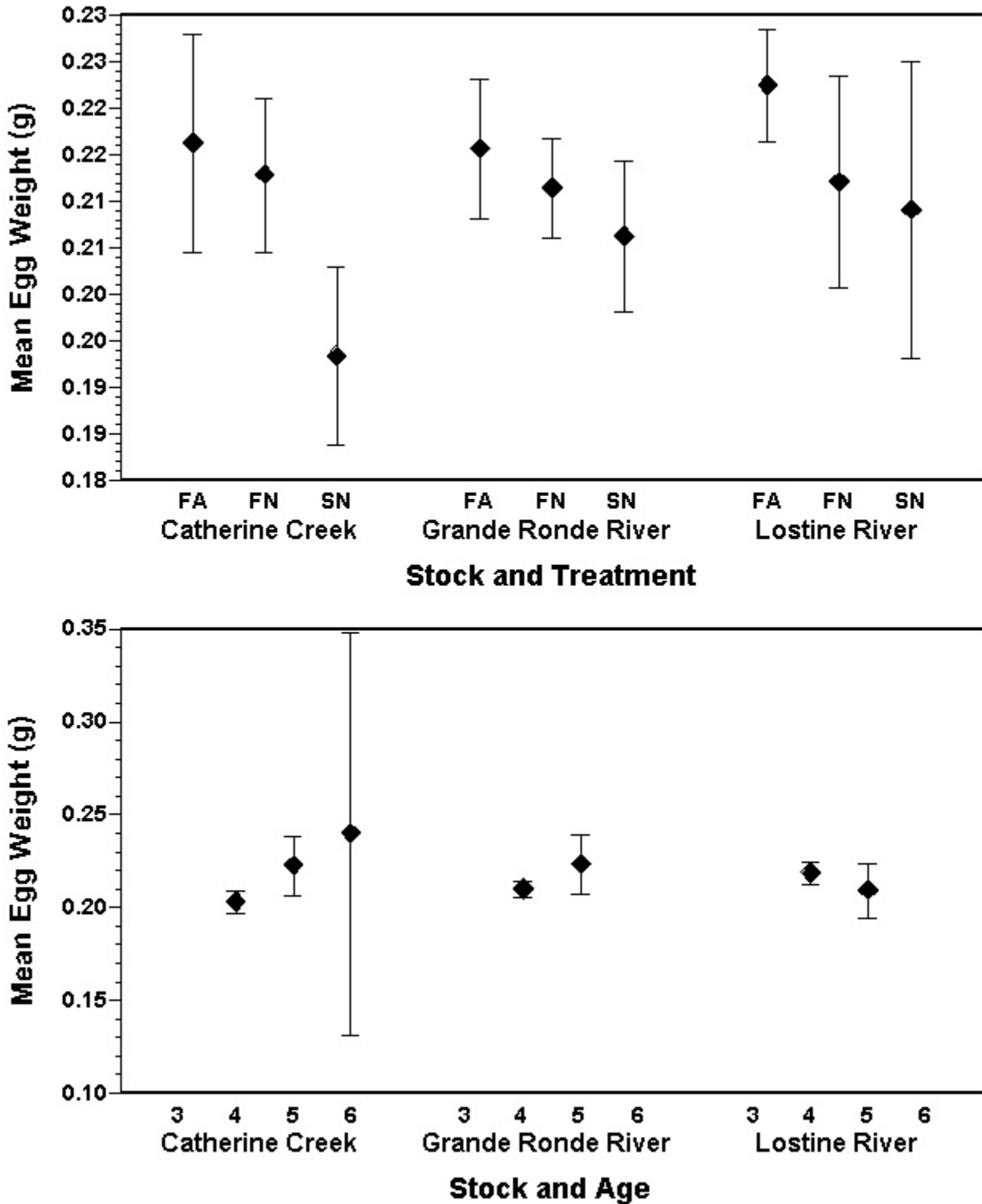


Figure 52 Mean ($\pm 95\%$ CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon that were raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and matured at ages 3, 4, 5 and 6 (bottom) in 2001.

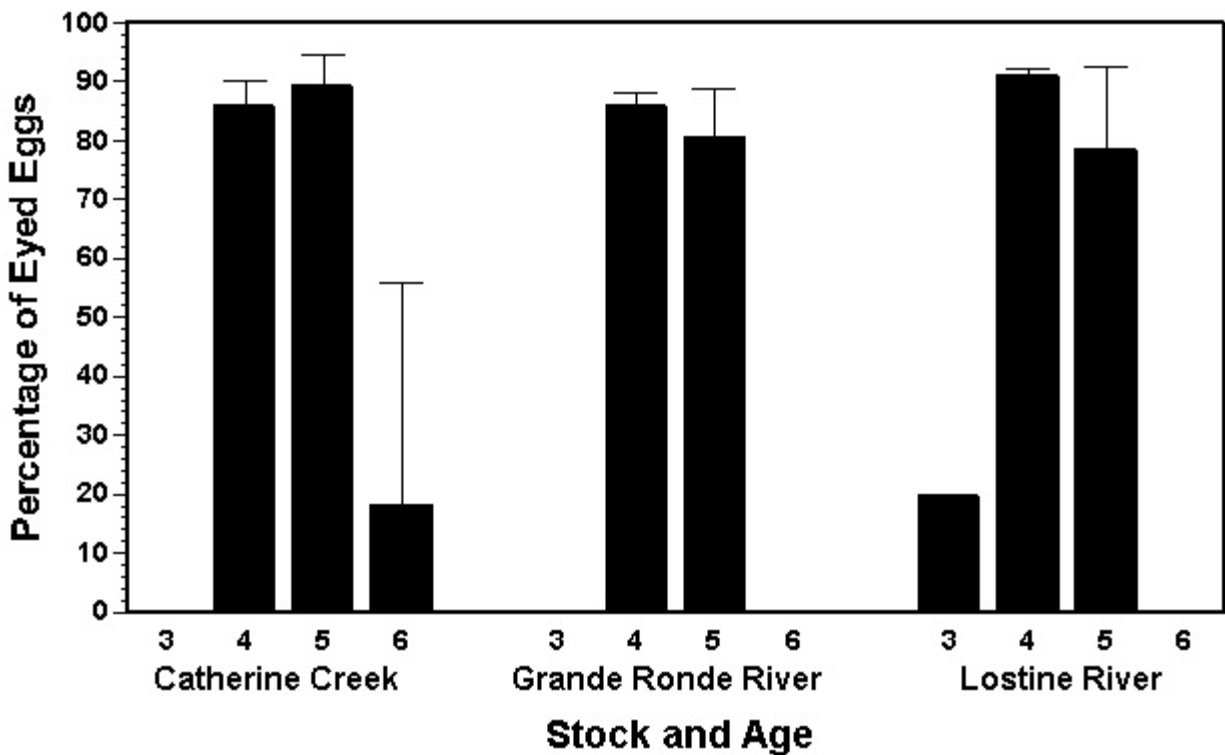
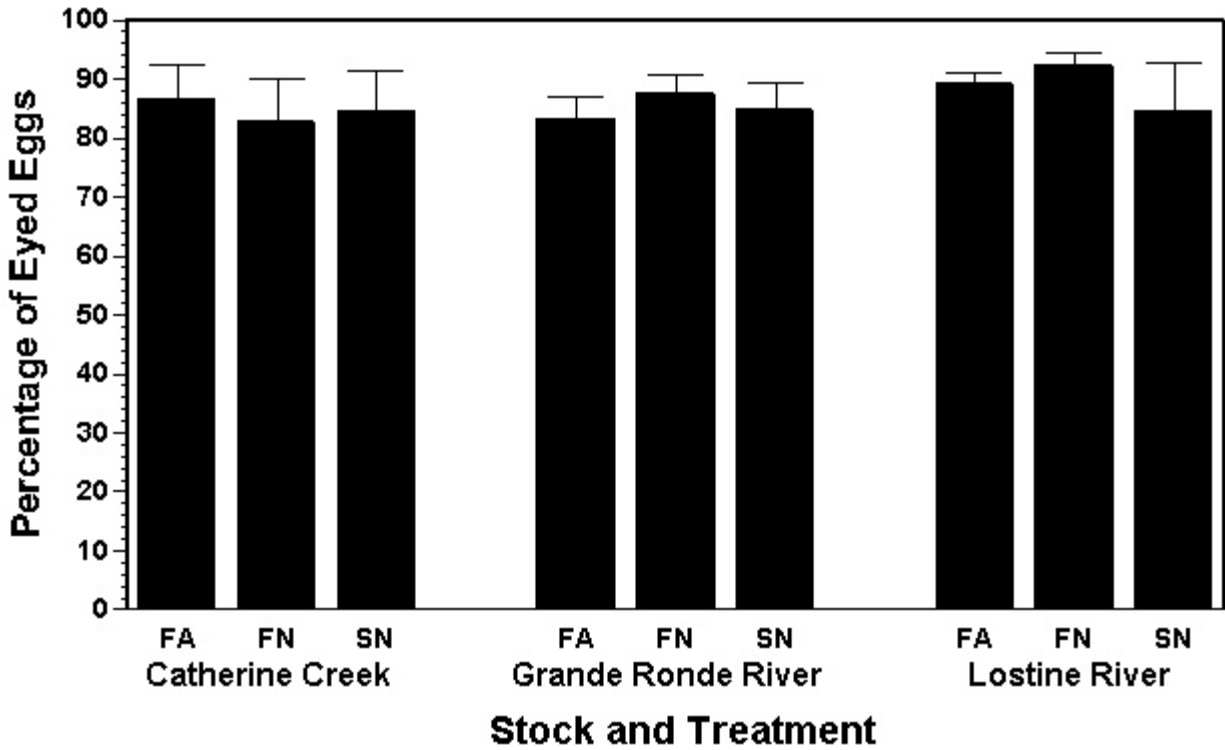


Figure 53 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year olds (bottom) spawned in 2001.

Fertility decreased with age of females (Figure 52). Fertility of the one age 3 female was 19.5%. Mean fertility of age 4 females was 87.4% and ranged from 0-99.6%. Age 5 females had a mean fertilization rate of 83.9%, ranging from 10.4-99.5%. In age 6 females, 18.1% (range: 0-66.3%) of the eggs reached the eyed stage. Mean fertilization rate of eggs by males in the Freshwater Accelerated group was 89.8% and ranged from 54-99% (Figure 53). In the Freshwater Natural group, mean fertilization rate was 87.6%, ranging from 2-99%. In Freshwater males (1998 cohort fish, for which pre-smolt treatments were indistinguishable), mean fertilization rate was 87.8% and ranged from 0-99%. In the Saltwater Natural group, mean fertilization rate was 83.9% and ranged from 8-100%. And in the Saltwater males (1998 cohort), mean fertilization rate was 87.2% and ranged from 8-99%. Fertilization rate varied little among age classes of males (Figure 53). Mean fertilization rate for age 2 males was 90.6% and ranged from 2-99%. Mean fertilization rate for age 3 males was 87.6% and ranged from 0-99%. Mean fertilization rate for age 4 males was 84.3% and ranged from 2-100%. Age 5 mean fertilization rate was 79.2%. Mean fertilization rate for the two age 6 males was 76.2% and ranged from 54-98%.

No cryopreserved semen was used for spawning in 2001. Fertilization rates for males from Catherine Creek ranged from 0-100% with a mean of 86.6% (Figure 54). Mean fertilization rate for Grande Ronde River males was 86.6% and ranged from 50-98%. In the Lostine River males, mean fertilization rate was 89.2%, ranging from 8-99%.

2002

The 2002 spawn varied from previous years in that we spawned only within Saltwater and Freshwater treatment groups and did not consider pre-smolt rearing as a treatment for spawning. This was to reduce the complication caused by introducing a fourth treatment (the Saltwater Accelerated group that was added to the 2000 cohort) to the spawning routine.

Additionally, we conducted our first maturity sort in late March/early April (instead of May) using ultrasound to determine maturity and sex. This was done to transfer maturing fish from saltwater to freshwater at a time more similar to when wild fish of these populations would be entering freshwater. We also ceased feeding the maturing fish earlier (after sorting for maturity). Our expectation was that this would induce the fish to spawn earlier - closer to the time that wild fish of these populations spawn. However, these efforts caused no change in the time of spawning and resulted in 40 fish being determined to be maturing at the earlier maturation sort that did not mature (all were from Catherine Creek and freshwater treatments). Nine of these fish died and the remainder were returned to the appropriate immature tanks as soon as they were determined to be immature.

Lastly, we had an unusually high number of fish (74 fish: 22 females; 54 males) died prior to spawning (due to fungal infection). Twenty-six fish (22 females; 4 males) died prior to 1 September and 48 (all males) died on or after 1 September but did not ripen. At the first maturity sort, we intentionally held one quarter of the saltwater-reared fish in saltwater that were determined to be maturing so that we could use those fish as a control in the event that the fish spawned earlier this year. These fish and those that were identified as maturing in the second maturity sort (May) were used to test whether the fish that died prior to spawning were more likely to have been from the early transfer groups. There was no significant difference ($P>0.1$) in mortality between those fish that were transferred to mature tanks earlier versus those that were transferred later.

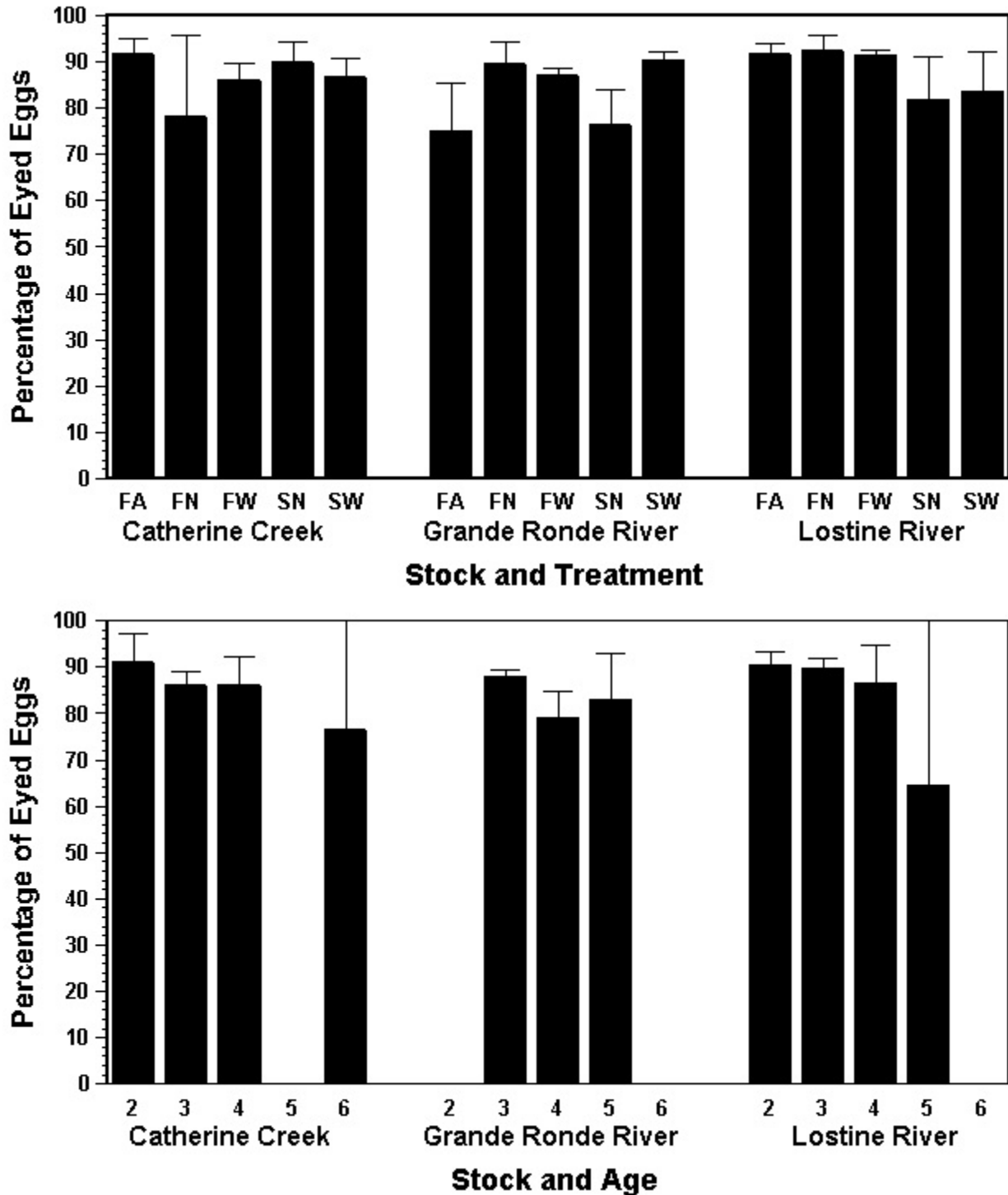


Figure 54 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN), Freshwater (FW; 1998 cohort, in which the pre-smolt treatments were indistinguishable), Saltwater Natural (SN) and Saltwater (SW; 1998 cohort) treatments (top) and from 2, 3, 4, 5 and 6 year olds (bottom) spawned in 2001.

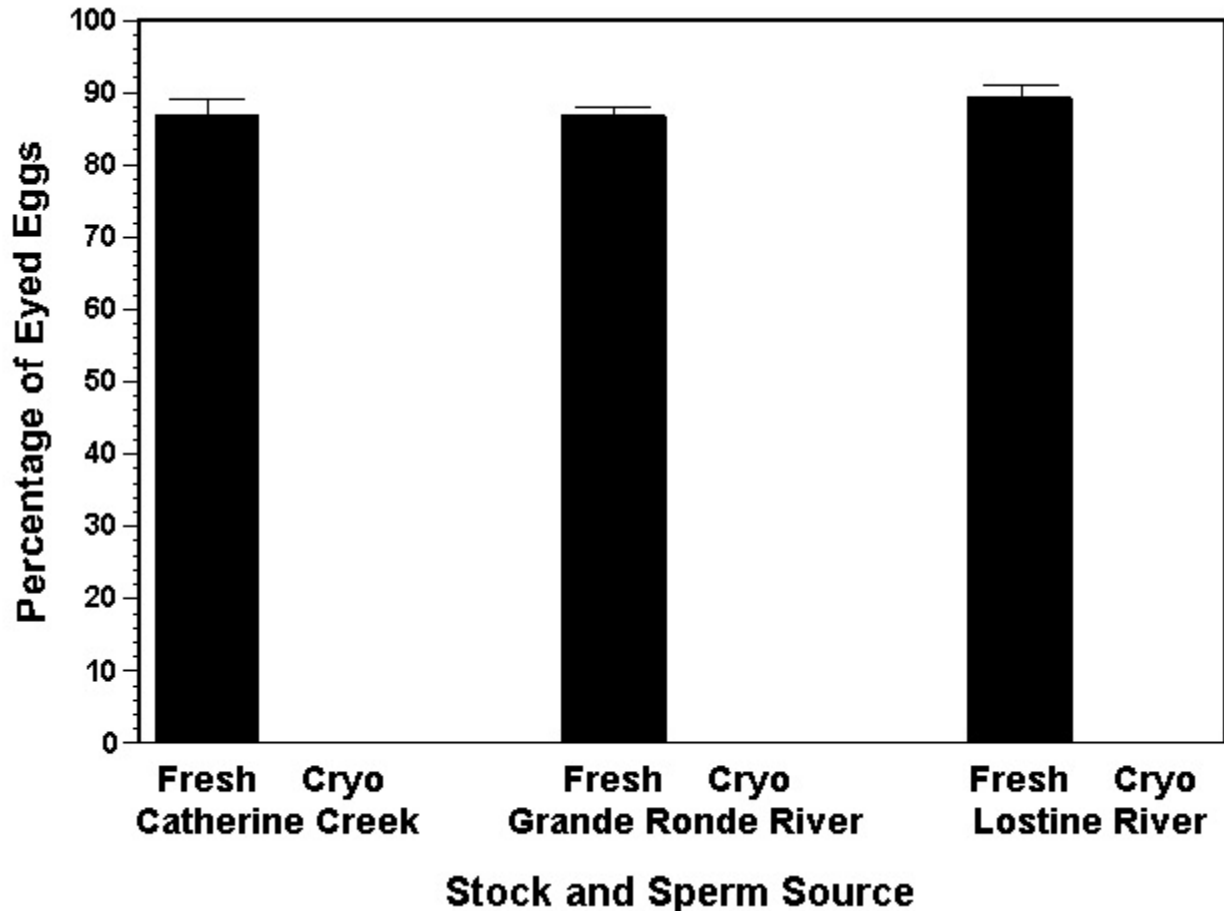


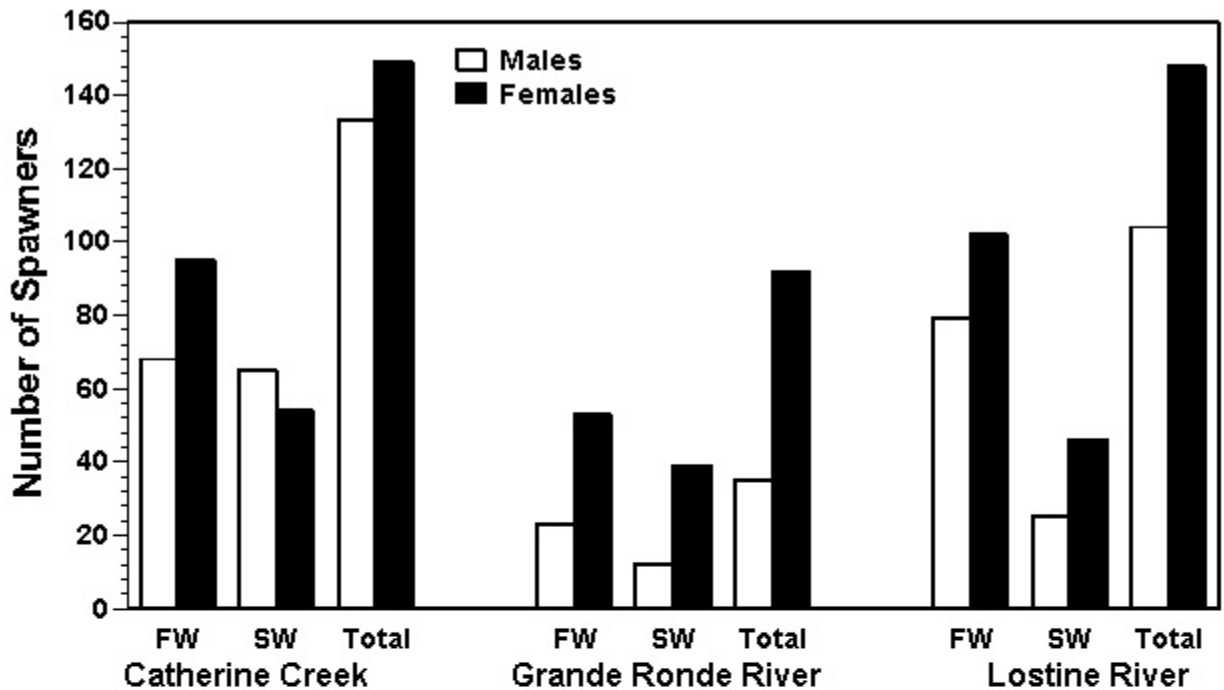
Figure 55 Mean ($\pm 95\%$ CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males in 2001. Note: no cryopreserved semen was used for spawning in 2001.

Number of spawners

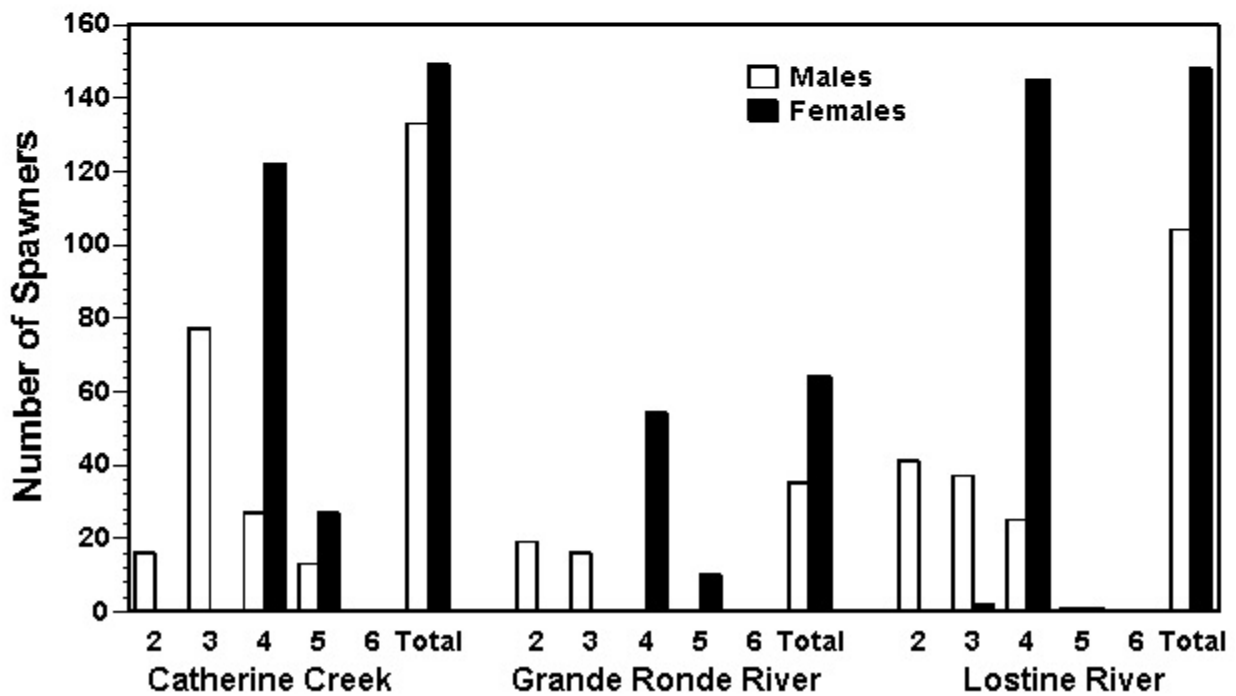
A total of 661 fish matured and contributed gametes (were spawned or had semen cryopreserved) in 2001: 272 (41.1%) males and 389 (58.9%) females (Figure 55). Within the treatment groups, 420 (63.5%) were from the Freshwater groups: 170 (40.0%) were males and 250 (60.0%) were females. Two hundred forty-one (36.5%) were from the Saltwater groups: 102 (42.3%) males and 139 (57.7%) females. Age distribution of spawners in 2002 included ages 2-5 and males matured at an earlier age than females (Figure 55). Of the males, 27.9% were age 2, 47.8% age 3, 19.1% age 4 and 5.1% age 5. Of the females, 0.6% were age 3, 88.9% age 4 and 10.5% age 5.

Fecundity

Fecundity varied among treatments and age classes of spawning females. Mean fecundity of Freshwater females was 1921.6 eggs and ranged from 168-3692 eggs (Figure 56). For



Stock and Treatment



Stock and Age

Figure 56 Number of males and females from Freshwater (FW) and Saltwater Natural (SW) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River chinook salmon populations spawned in 2002.

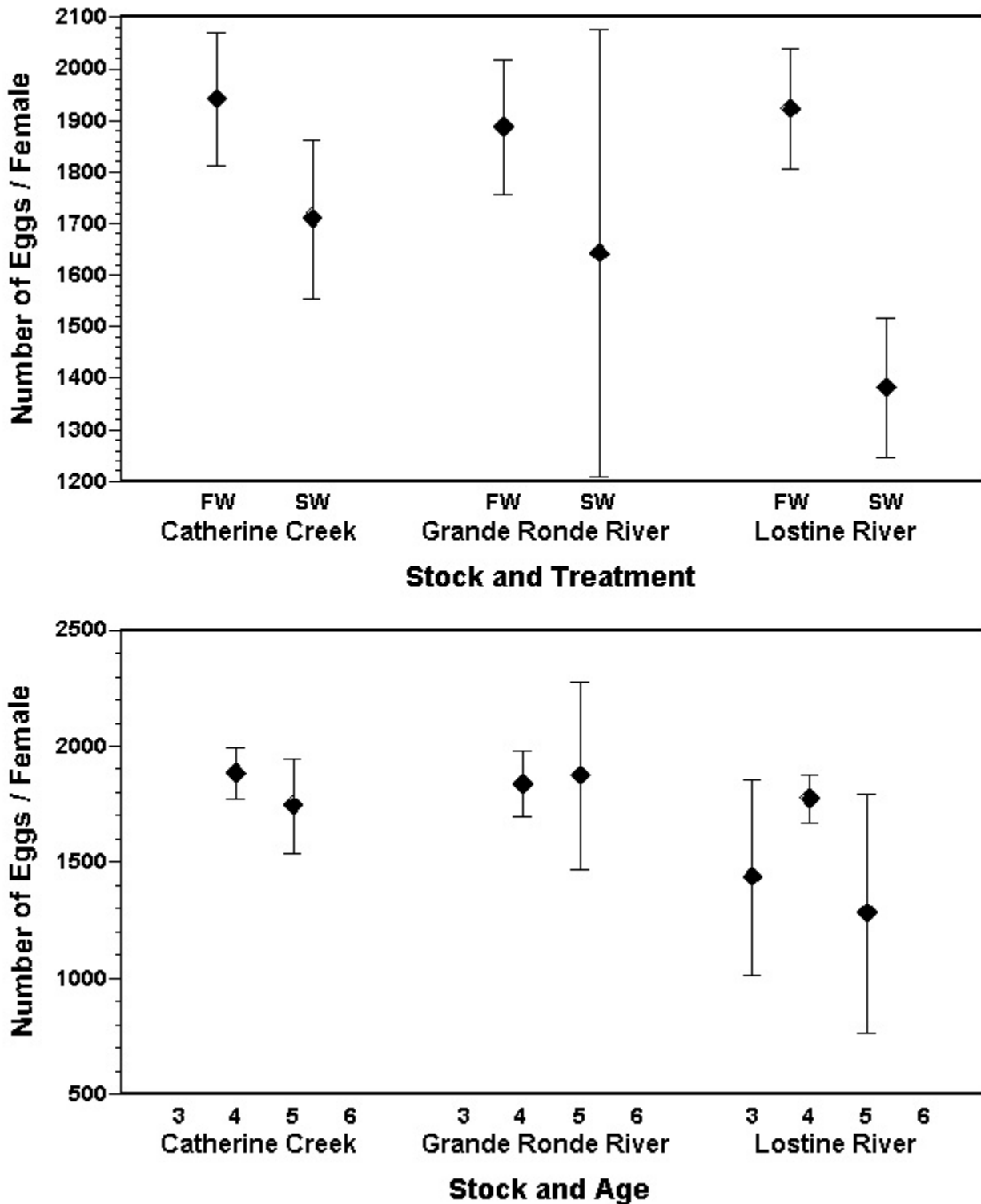


Figure 57 Mean ($\pm 95\%$ CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River that were raised under Freshwater (FW) and Saltwater (SW) treatments (top) and matured at ages 3, 4, 5 and 6 (bottom) in 2002.

Saltwater-reared females, mean fecundity was 1566.5 eggs and ranged from 102-3280 eggs. Within age classes, mean fecundity was 1435.2 eggs for the two 3-year old females spawned in 2002 (Figure 56). For 4-year old females mean fecundity was 1824.1 eggs and ranged from 102-3692 eggs. For 5-year old females, mean fecundity was 1728.6 eggs and ranged from 768-2956 eggs. No age 6 females were spawned in 2002.

Egg weight

Mean egg weight varied among treatments and age of spawning females. Mean egg weight of Freshwater females was 0.187 g and ranged from 0.10-0.33 g (Figure 57). For the females in the Saltwater groups, mean weight of eggs was 0.194 g, ranging from 0.08-0.36 g. Mean egg weight of the two age 3 females was 0.141 g, ranging from 0.10-0.19. Mean egg weight age 4 females was 0.186 g, ranging from 0.08-0.36. Mean egg weight of age 5 females was 0.221 g and ranged from 0.10-0.34 g.

Fertility

Mean percentage of eggs that reached the eyed stage (an estimate of fertilization rate) varied with the treatment group and age of the male or female and with the use of fresh vs. cryopreserved semen. The mean percent eyed eggs from females in the Freshwater Accelerated treatment group was 85.9% and ranged from 3-99.5% (Figure 58). The mean percent eyed eggs from females in the Freshwater Natural treatment group was 87.7%, ranging from 0-99.1%. In Saltwater Natural females, 84.6% reached the eyed stage, ranging from 0-99.6%.

Fertility decreased with age of females (Figure 58). Fertility of the one age 3 female was 19.5%. Mean fertility of age 4 females was 87.4% and ranged from 0-99.6%. Age 5 females had a mean fertilization rate of 83.9%, ranging from 10.4-99.5%. In age 6 females, 18.1% (range: 0-66.3%) of the eggs reached the eyed stage.

Mean fertilization rate of eggs by males in the Freshwater Accelerated group was 83.3% and ranged from 2-99% (Figure 59). In the Freshwater Natural group, mean fertilization rate was 84.5%, ranging from 0-100%. In the Saltwater Natural group, mean fertilization rate was 76.7% and ranged from 2-99%.

Fertilization rate varied little among age classes of males (Figure 59). Mean fertilization rate for age 2 males was 75.6% and ranged from 0-99%. Mean fertilization rate for 3-year old males was 84.4% and ranged from 0-100%. Mean fertilization rate for age 4 males was 76.3% and ranged from 0-99%. Fertilization rate was 32.7% for the one age 5 male measured.

Fertilization rate was much better with fresh semen than cryopreserved semen (Figure 60). Mean fertilization rate using fresh semen was 81.7% and ranged from 0-100%. Using cryopreserved semen resulted in a mean fertilization rate of only 37.3% (0-99%).

Analyses

Here, we analyze the available data to evaluate the benchmarks for success developed at the beginning of this program, as well as the effect of our treatments on specific indices pertaining to production (e.g., mortality, survival, size of spawners, fertility, egg weight) of chinook salmon in the captive broodstock program. It should be understood that these analyses are preliminary and cursory. For some of these benchmarks, we have sufficient data to begin to evaluate our effort. For the F_1 benchmarks, we have only begun to collect those data. More comprehensive analyses will be conducted when the data are more complete. However, these

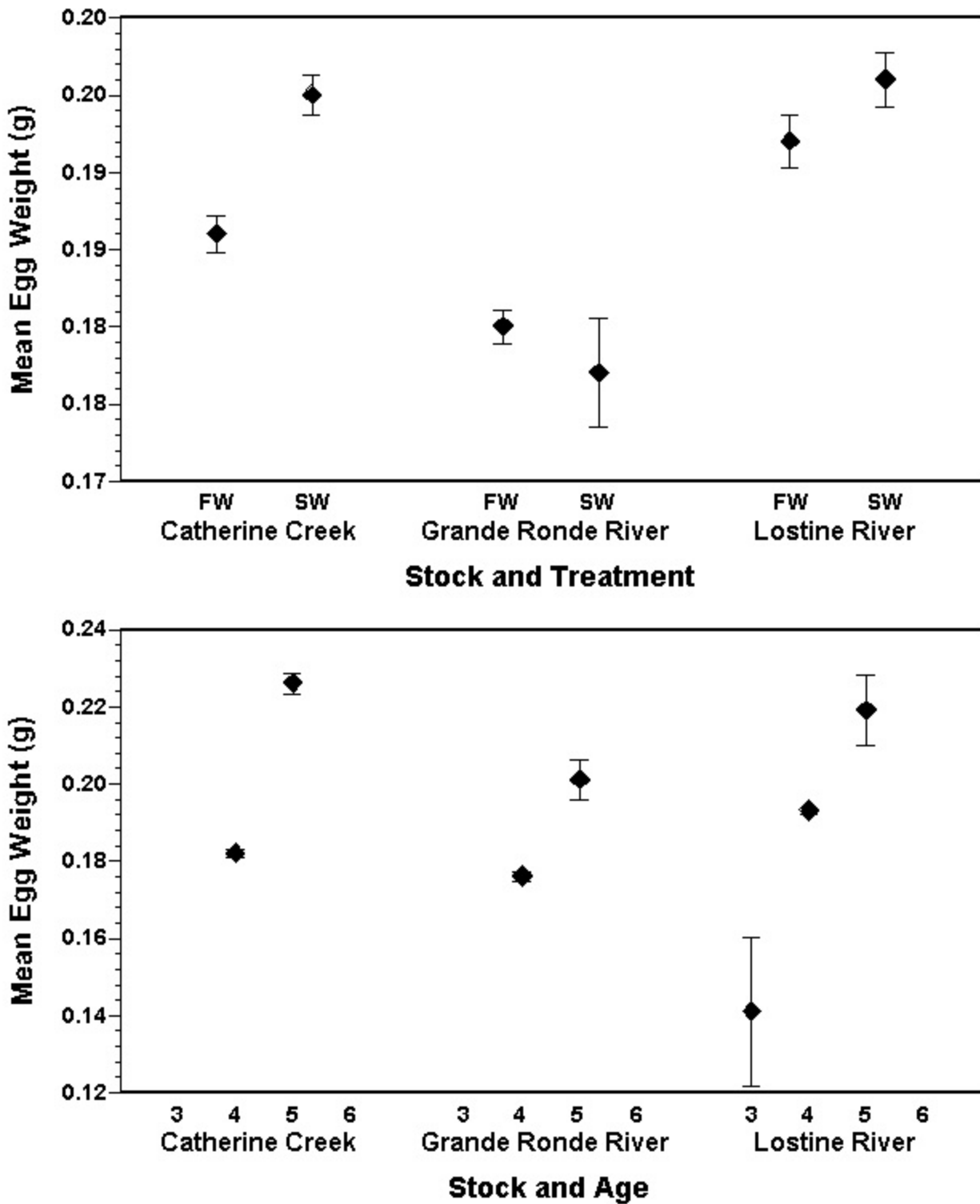


Figure 58 Mean ($\pm 95\%$ CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon that were raised in freshwater (FW) or saltwater (SW) for post-smolt growth (top) and matured at ages 3, 4, 5 and 6 (bottom) in 2002.

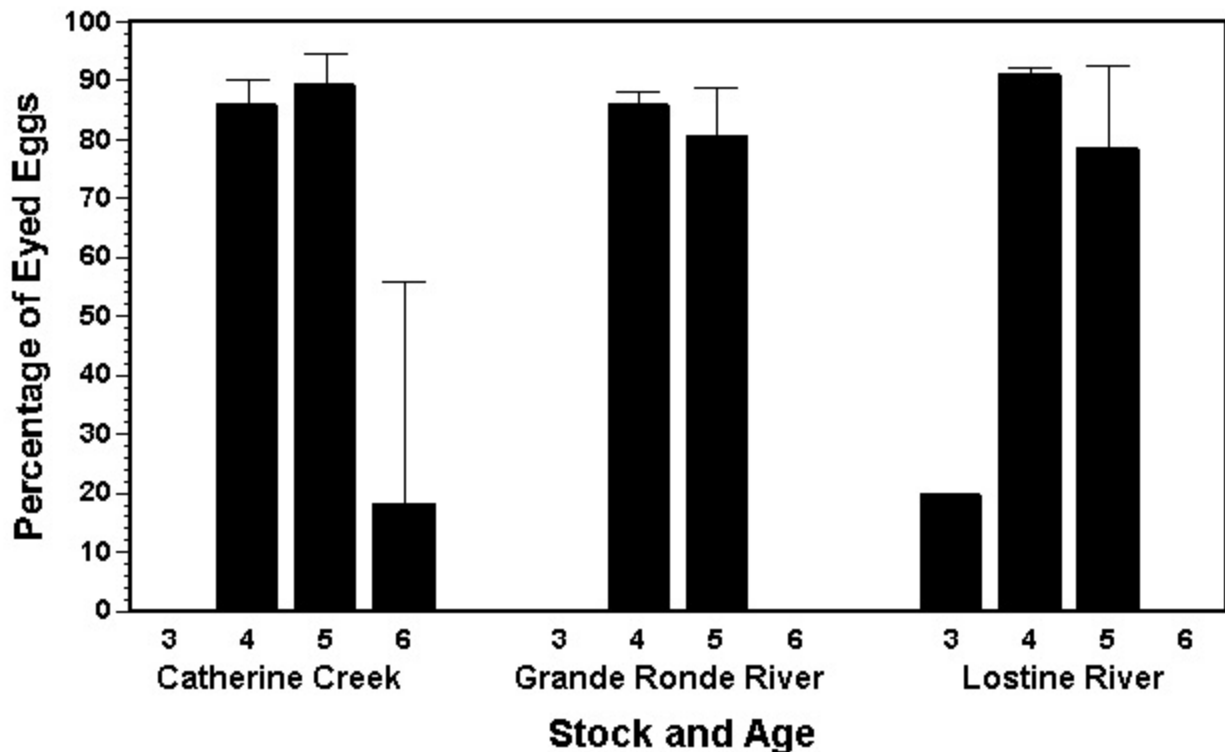
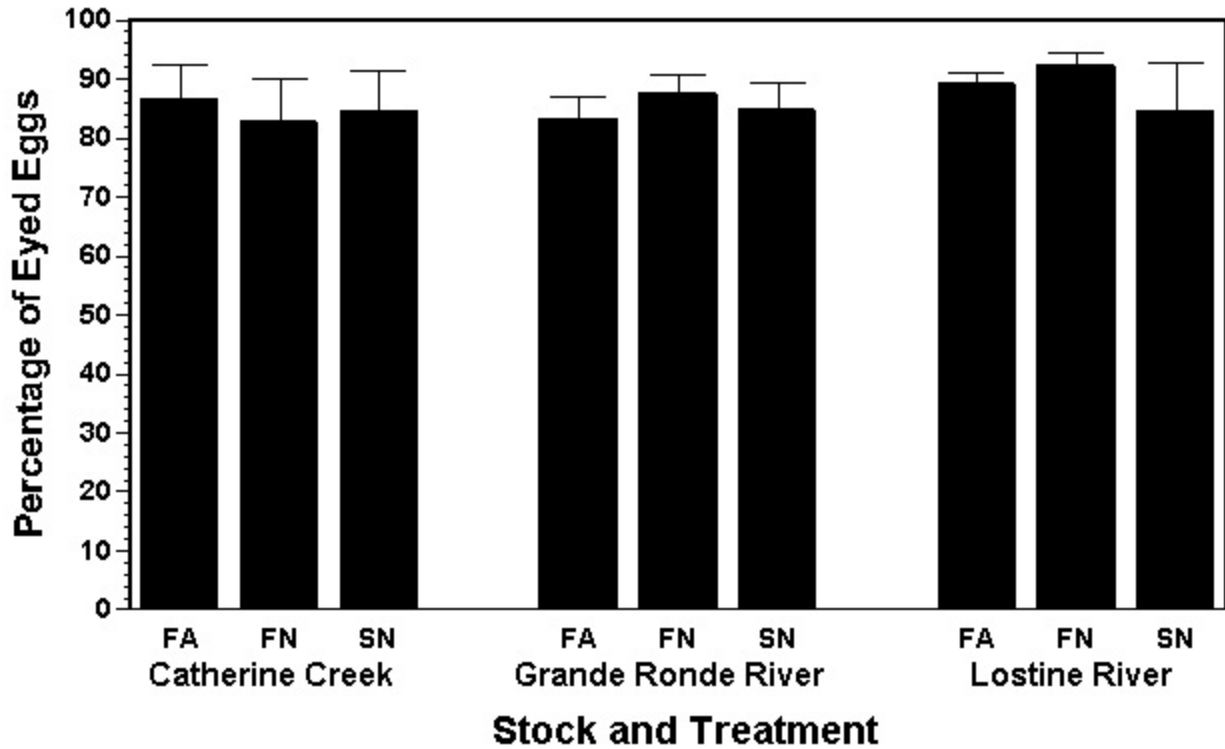


Figure 59 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) from spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) Catherine Creek, Grande Ronde River and Lostine River spawned in 2001.

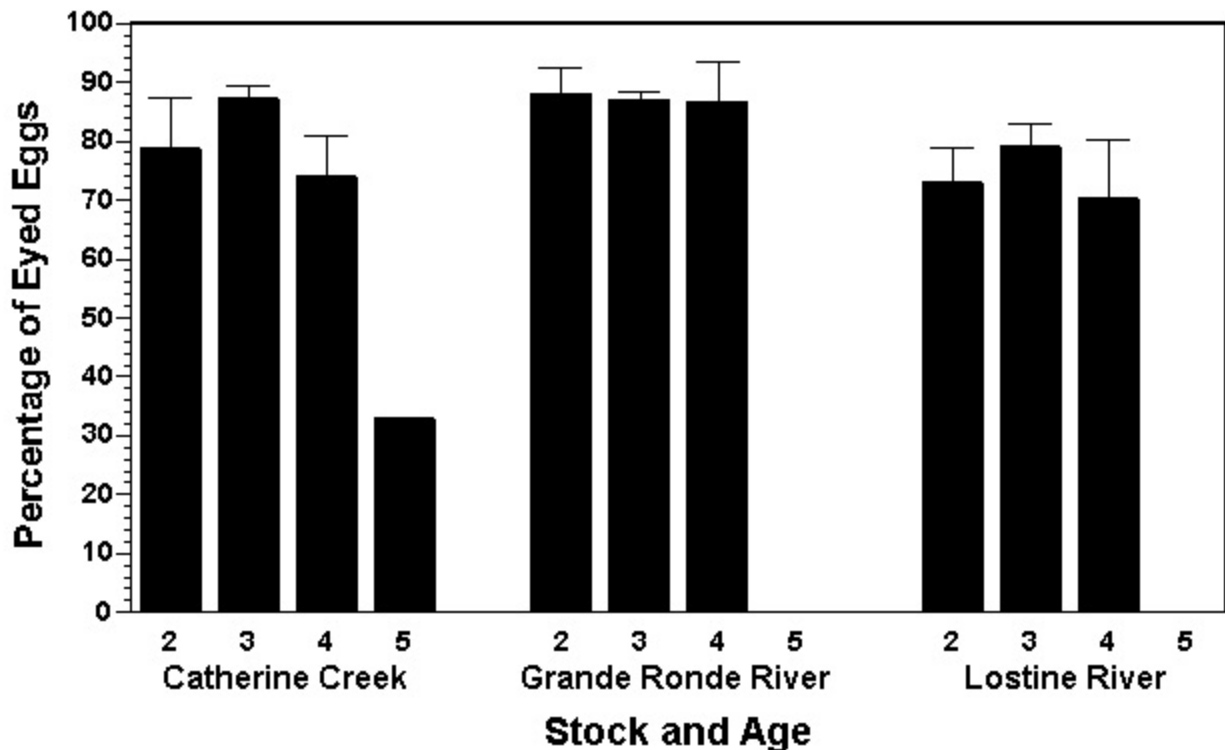
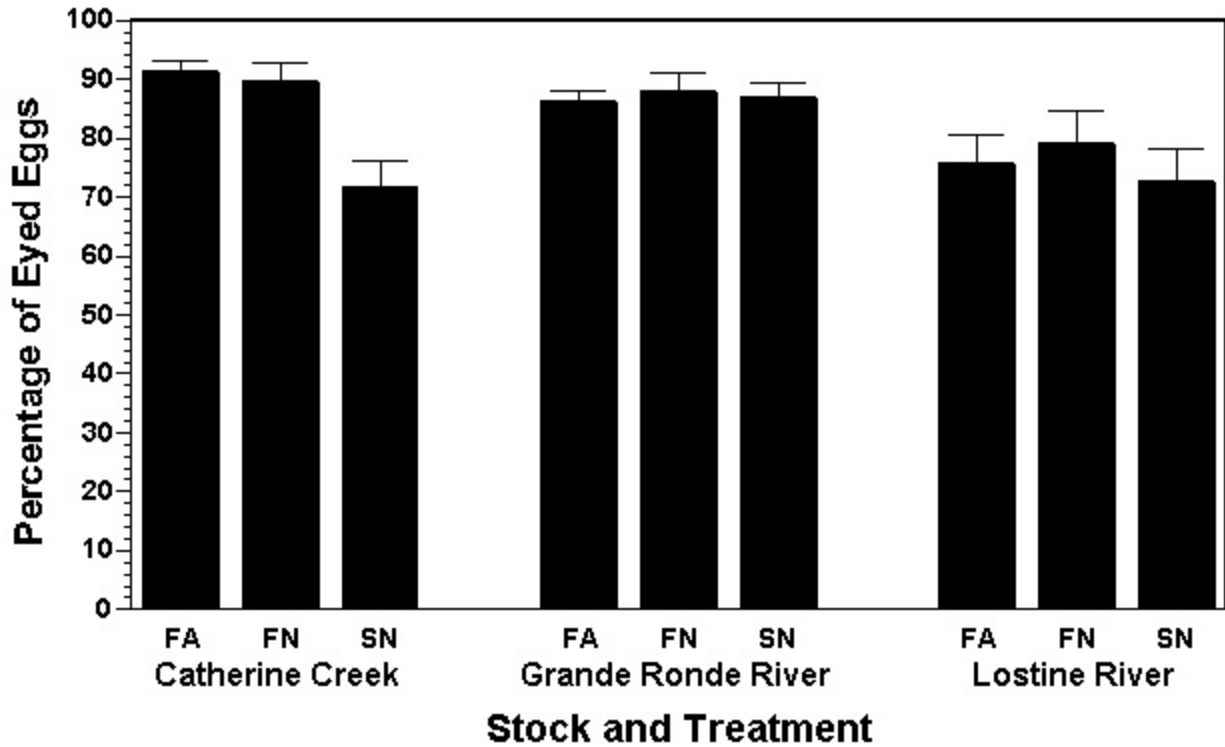


Figure 60 Mean ($\pm 95\%$ CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) in 2000.

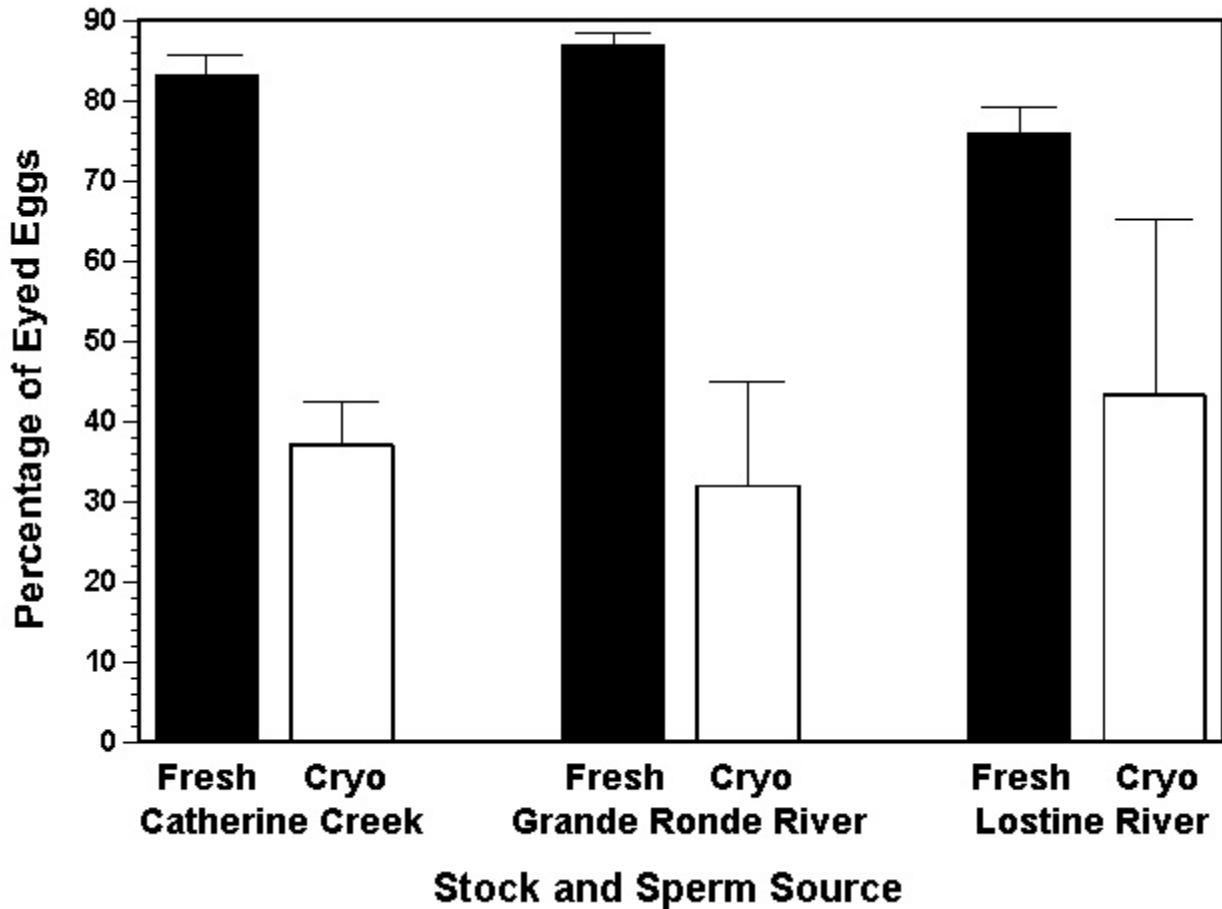


Figure 61 Mean ($\pm 95\%$ CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males, 2000.

analyses can give an overview of how well we are achieving the goals of the program. When analyzing these data, we have included the Freshwater Accelerated treatment group as a separate entity, although it is uncertain whether, due to facility problems at LFH, the Freshwater Accelerated groups of the 1994-1998 cohorts were fully subjected to pre-smolt conditions that differed from the “natural” treatment groups. However, some differences were seen between the 1994-1998 cohort fish subjected to the accelerated and natural growth regimes (e.g., age of maturity). The 1999-2001 cohorts did receive different pre-smolt rearing conditions and as they mature, our evaluation of the pre-smolt growth regimes will be more robust.

Parr collection

Number collected

This program has a goal of collecting 500 parr each year from each stream (Table 7). For the most part, we have been successful in meeting this target. We were able to collect 500 parr from Catherine Creek and near 500 (481 of 1995 cohort) Lostine River parr for each cohort

collected. However, in Grande Ronde River, there were several years in which we didn't reach our target. We only caught 110 of the 1994 cohort and 461 of the 2002 cohort and were unable to collect any fish from the 1995 and 1999 cohorts.

Size at collection

Size at collection of chinook salmon parr varied with population and year. Over all years, Catherine Creek (76.8 mm) and Lostine River (76.4 mm) parr were significantly ($P < 0.0001$) longer than Grande Ronde River (65.2 mm) parr. Lostine River parr (5.93 g) were significantly ($P < 0.0001$) heavier than both Catherine Creek (5.30 g) and Grande Ronde River (3.54 g) parr and Catherine Creek parr were also heavier than Grande Ronde River parr. However, Grande Ronde River parr had a significantly ($P < 0.0001$) higher mean K (1.26) than those from both Catherine Creek (1.23) and the Lostine River (1.22), which did not significantly differ.

Growth

Parr-to-smolt growth

Due to various problems at Lookingglass Fish Hatchery (chiller and water supply), we were unable to achieve acceptable pre-smolt treatments (Natural and Accelerated) to allow us to achieve significantly different growth rates between treatments for the 1995-1998 cohorts - the 1994 cohort was a test group and is not included in these analyses and the 1998 cohort had so many problems that we did not even code the fish as being "natural" or "accelerated" in the database. Target smolt lengths at inception of this program were 186 mm for Accelerated growth fish and 128 mm for Natural growth fish.

The 1995-1997 cohorts had mean lengths at smoltification ranging from 113-134 mm for Natural treatments and 125-133 mm for Accelerated treatments. Although neither length nor weight at smoltification varied among treatments ($P \geq 0.2069$), we did see some effect of the treatments, based on differences seen in age of maturity, fertility of males and females and weight and K of female spawners (see below).

However, for the 1999 and 2000 cohorts we were able to achieve acceptable treatment groups. While we were not able to reach the target size for the Accelerated fish, we did achieve segregation in both length and weight between the Natural and Accelerated treatments ($P \leq 0.0165$). Mean length at smoltification ranged from 119-127 mm for Natural fish and 132-149 mm for Accelerated fish.

Smolt-to-adult growth

Growth of the post-smolt fish has been slower than anticipated. At spawning, captive broodstock females are smaller ($P < 0.0001$) than wild females (naturally-spawned or hatchery-spawned and released as smolts) - approximately 70% as large as fish reared in nature and males are only half as large (partly due to a large percentage of males maturing at ages 2 and 3). When we compare size at maturity within age classes, females are 63-73% as large as wild females and males are 57-78% as large (no data are available for mature wild age 2 males). We appear to be seeing fish reaching a critical length of 500-550 mm at maturity, as there is little difference in mean length of females or males maturing at ages 4, 5 and 6 (Figures 61-63). Those fish that reach a growth threshold (size and/or rate) at a specific time appear to mature, while those that do not reach that threshold wait at least another year before maturing.

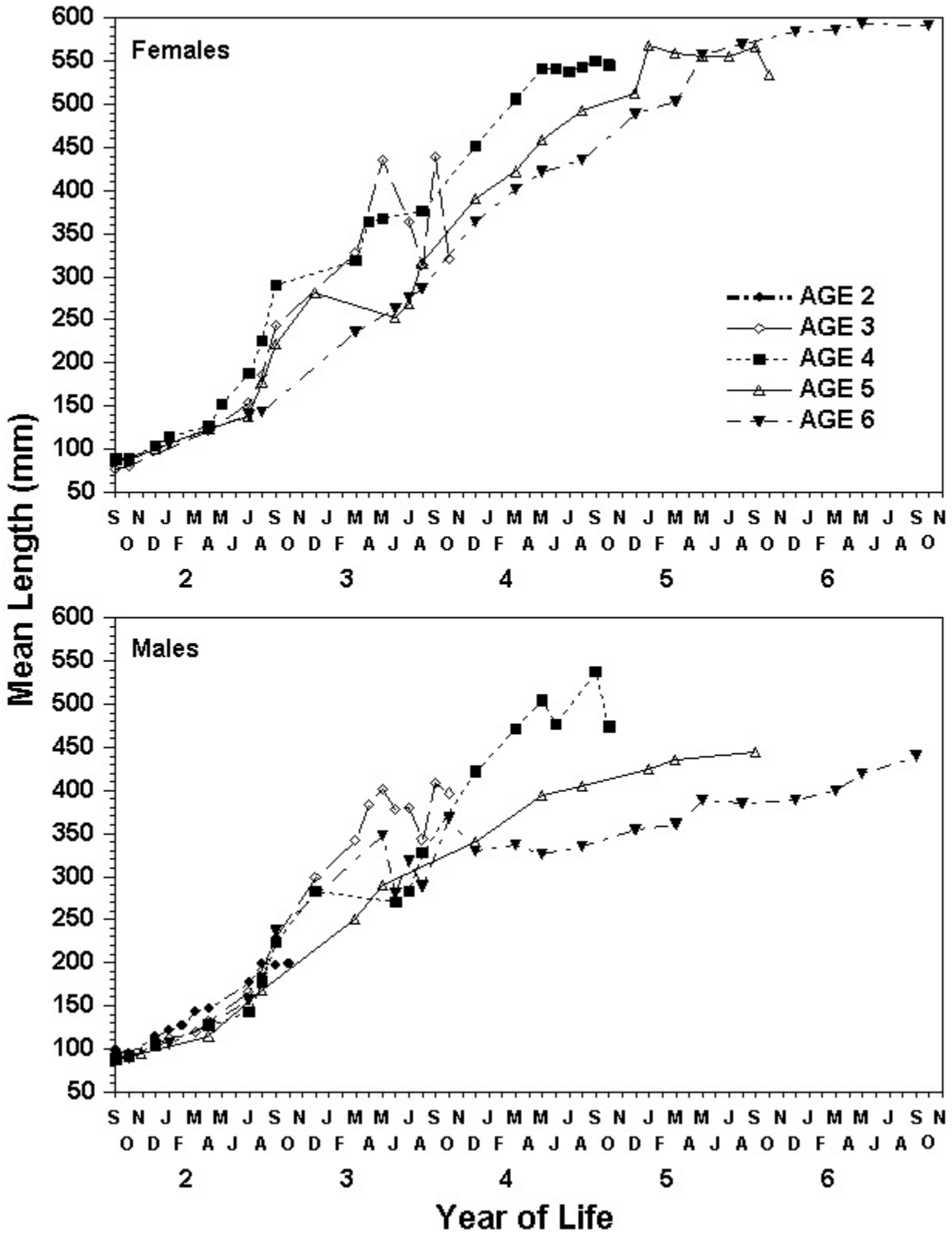


Figure 62 Growth profiles of female (top) and male (bottom) spring chinook salmon reared under the Freshwater Accelerated growth regime and maturing at ages 2 - 6, 1994-1998 cohorts.

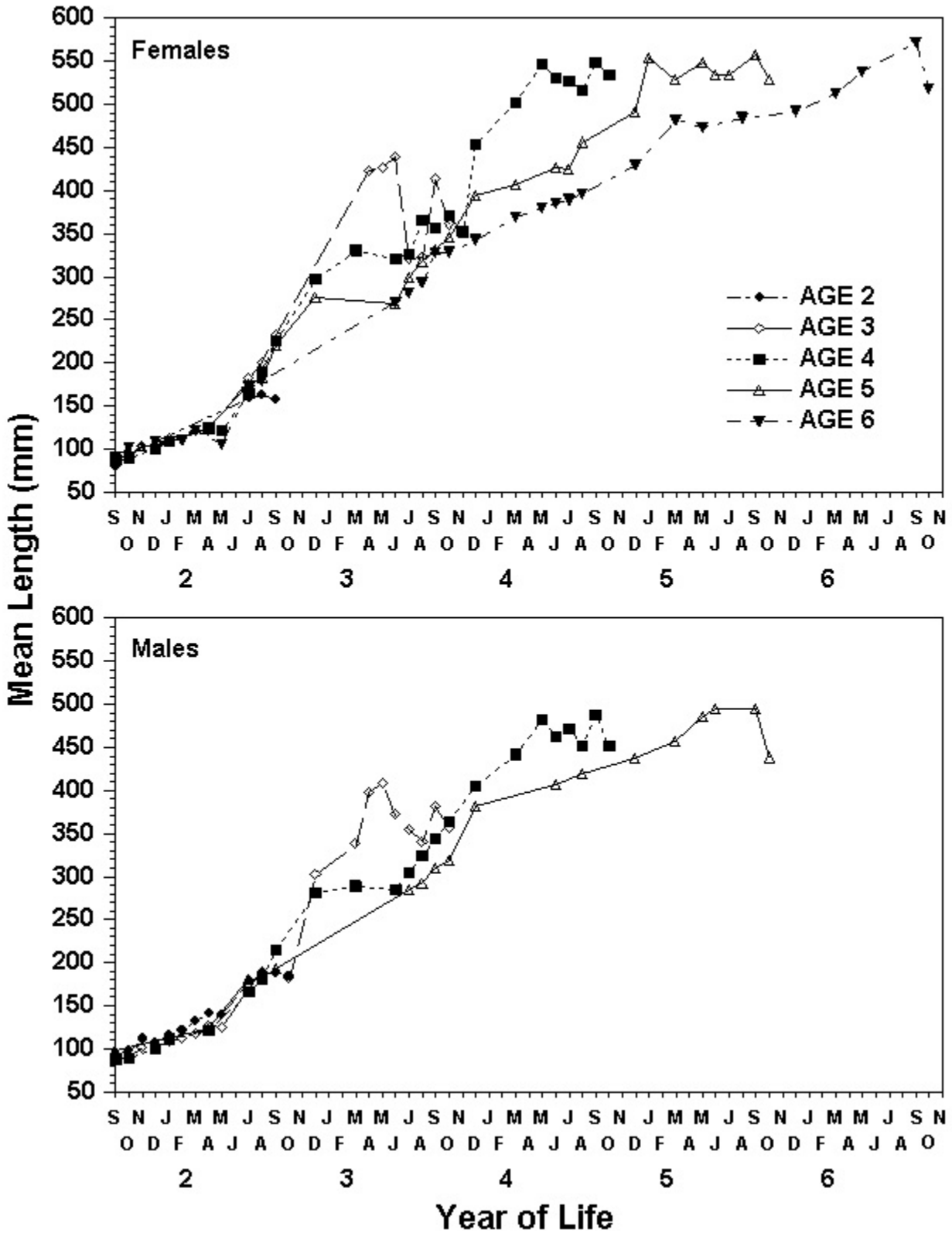


Figure 63 Growth profiles of female (top) and male (bottom) spring chinook salmon reared under the Freshwater Natural growth regime and maturing at ages 2-6, 1994-1998 cohorts.

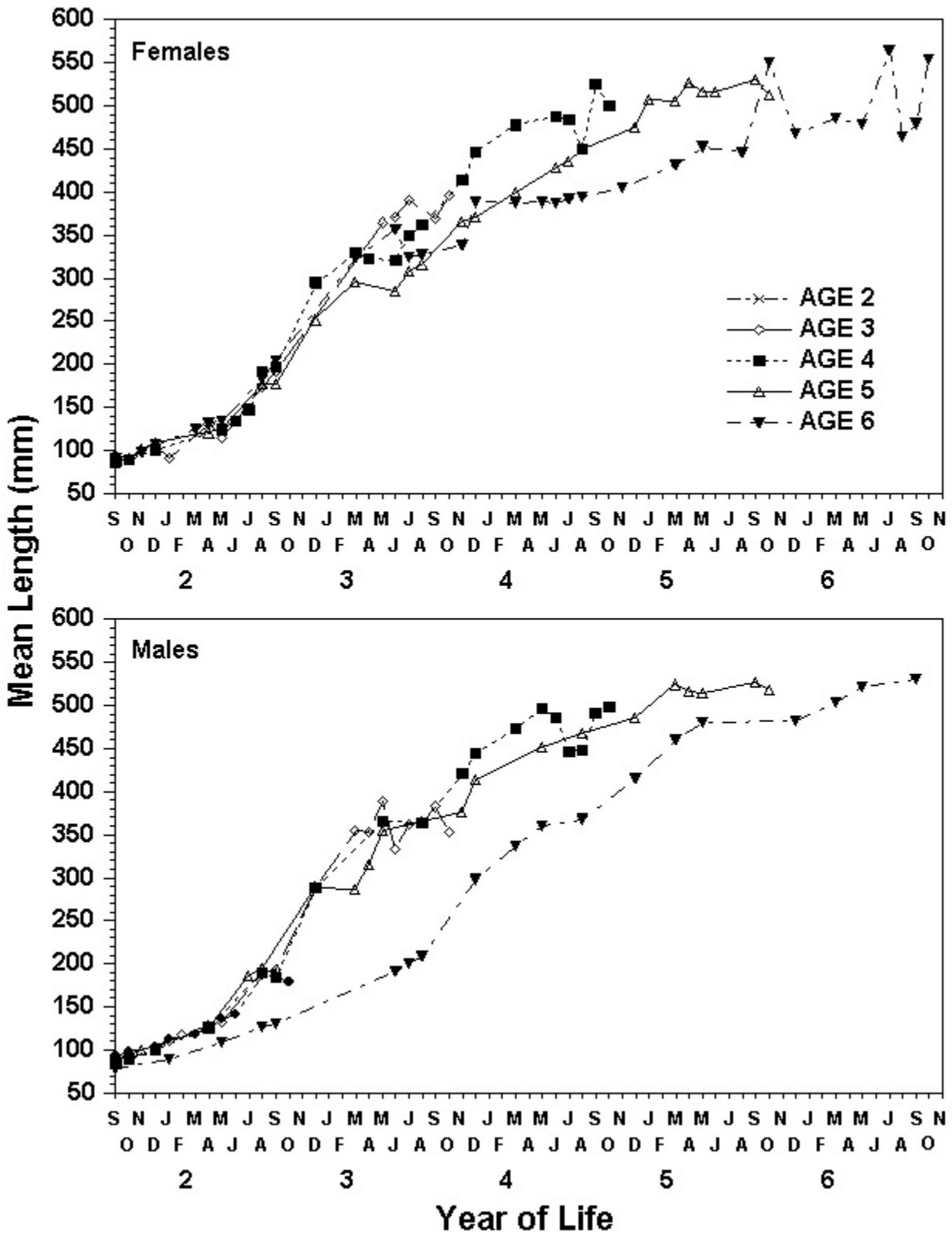


Figure 64 Growth profiles of female (top) and male (bottom) spring chinook salmon reared under the Saltwater Natural growth regime and maturing at ages 2-6, 1994-1998 cohorts.

When comparing growth among treatments, Freshwater Accelerated and Freshwater Natural females did not differ but were larger ($P < 0.0001$) than Saltwater Natural females of the same age (Figures 61 - 63). Saltwater Natural females appear to have grown at a rate nearly identical to that of the Freshwater Natural females until a year (age 4 spawners) or two (age 6 spawners) before maturation, at which time their growth slowed.

Overall, there was no difference ($P = 0.4585$) in male size at maturity among treatments (Figures 61-63). However, Freshwater Accelerated males that matured at ages 2 and 3 were significantly larger than both pre-smolt natural growth groups ($P < 0.0001$) and larger than the Freshwater Natural males at age 4, as well ($P = 0.0037$). It is likely that slower growth allows fish to mature at an older age and, therefore, at a larger size.

Survival

Parr-to-smolt survival

Mean parr-to-smolt survival (97%) was higher ($P = 0.0154$) than the expected rate of 95% and ranged from 86.8% (1994 Lostine River) to 99.4% (1997 Grande Ronde River and Lostine River) (Figure 64). In general, all populations survived well and have been improving since the first two years. Mortality at this stage is generally due to fish not converting to hatchery feed, precocial maturation, adverse reaction to BKD vaccination and operational causes (handling and fish jumping out of rearing tanks).

Smolt-to-adult survival

The expected smolt-to-adult survival rate (55%) was exceeded for 11 of 13 cohorts/treatments, although the mean (62.6%) did not significantly differ ($P = 0.0516$) from expected (Figure 65). Mean smolt-to-adult survival for the Freshwater Natural group (70%) was higher ($P = 0.0181$) than the expected rate, while the survival rates for the Freshwater Accelerated (61%) and Saltwater Natural (57%) did not differ ($P \geq 0.6857$) from expected. Mean survival among stocks ranged from 26.4% (1994 Grande Ronde River) to 83.3% (1997 Grande Ronde River).

Disposition

We assumed 50% survival from parr to spawn and we exceeded this goal ($P = 0.0264$) for each of the first four cohorts (Figure 66). Mean survival to spawn for the 1995-1997 cohorts was 63% and ranged from 52% in the 1995 cohort to 73.7% in the 1997 cohort. Bacterial kidney disease was the largest source of pre-spawn mortality, causing at least 30-52% of the pre-spawn mortalities (some mortalities in the "unknown" category are also likely due to BKD).

Sex Ratio

We anticipated a 1:1 sex ratio at collection for each population. Mean sex ratio for the 1994-1997 cohorts very closely approximated 1:1. Percentage of females for these cohorts ranged from 48.2-52.6% and did not significantly differ from 50% ($P = 0.2126$).

Age of Maturity

Age of maturity differed between sexes and more dramatically than we expected. Males matured at a younger age and females matured at a slightly older age than anticipated. This shift in age of maturity for each sex benefitted the program by helping us avoid sibling crosses during spawning.

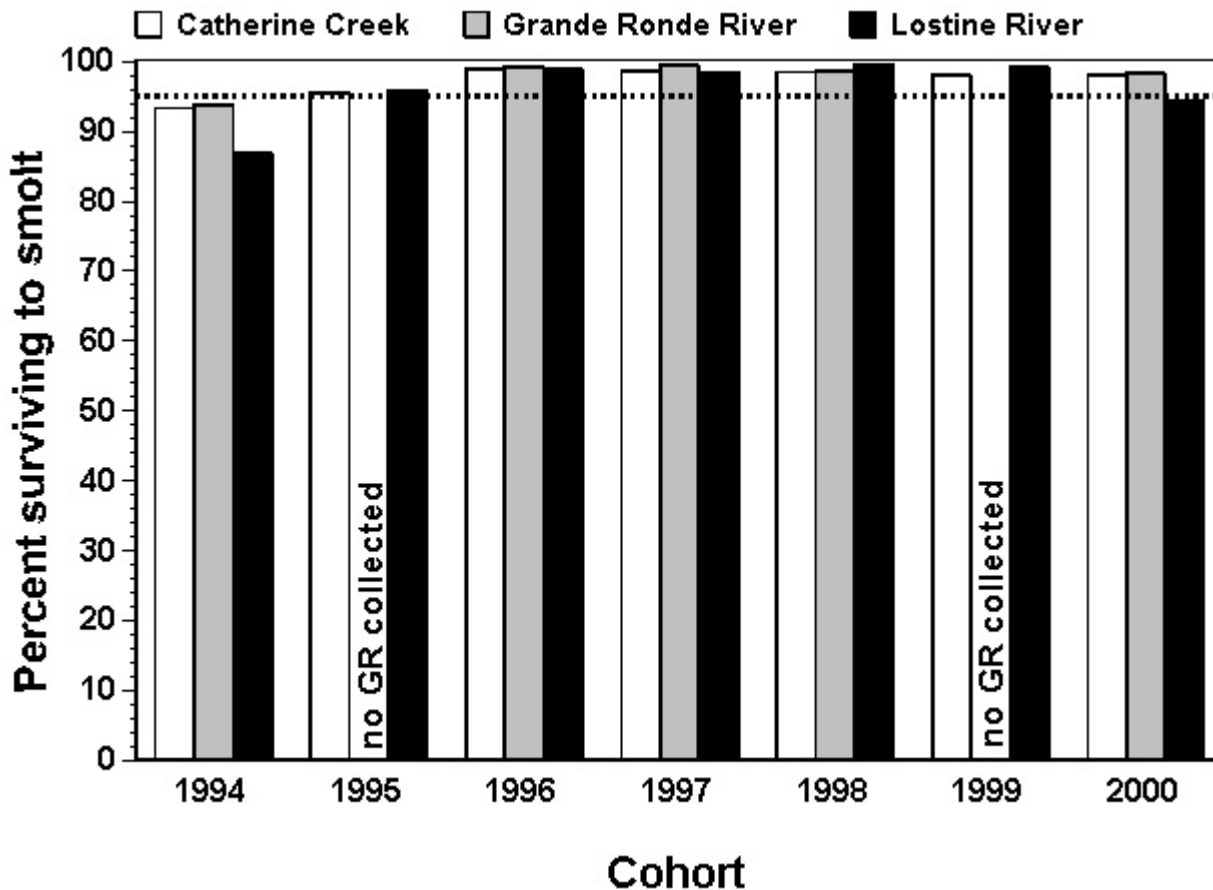


Figure 65 Parr-to-smolt survival for 1994-2000 cohorts of captive broodstock spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River parr collected for the captive broodstock program, 1995-2001. Horizontal reference line indicates anticipated value when the captive broodstock program was initiated.

We predicted that approximately 6% of the females would mature at age 3, 78% at age 4 and 16% at age 5. Our results show females maturing slightly older than expected and there were significantly fewer ($P < 0.0001$) age 3 females and more ($P = 0.0211$) age 6 females (Figure 67). Only 0.4% of females surviving to maturity spawned at age 3. A mean of 82.6% of the females matured at age 4 and 16.4% at age 5. We did not expect any age 6 females but 0.6% of those maturing reached that age. Mean age at maturity was expected to be 4.1 years for females and but was 4.2 years, overall. The Catherine Creek females tended to mature older than the Grande Ronde River and Lostine River females. Age of maturity of females varied among treatment groups ($P < 0.0001$). Females from the Freshwater Accelerated group matured at a younger mean age (4.1 years) than those of the Saltwater Natural (4.2 years) and Freshwater Natural treatment groups (4.3 years). Females in the Saltwater Natural group also matured at a significantly younger age than the Freshwater Natural group.

For males, we expected that 2% would mature at age 2, 35% at age 3, 48% at age 4 and 15% at age 5. Results from the 1994-1998 cohorts showed that males matured at a substantially

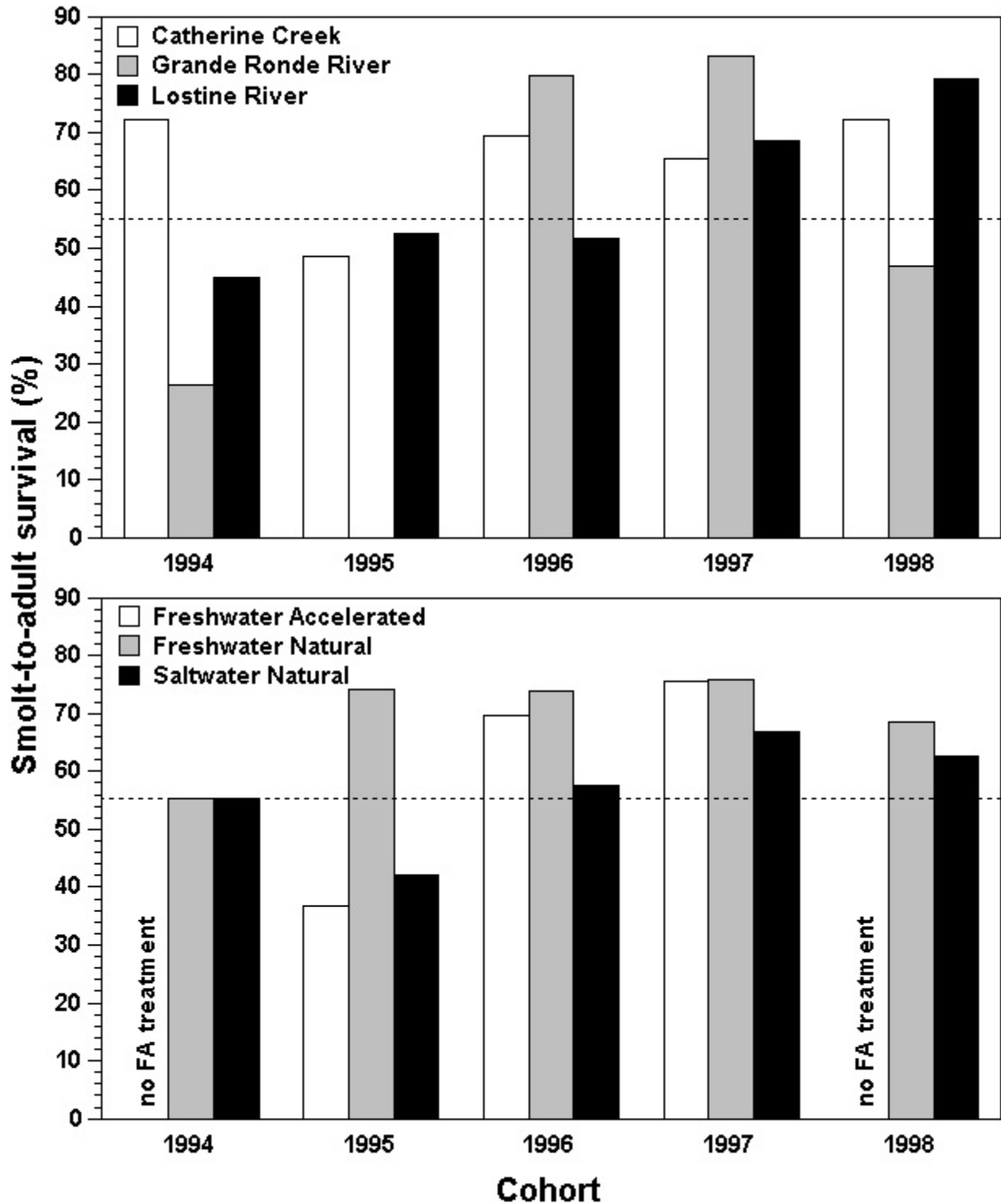


Figure 66 Smolt-to-adult survival for 1994-1998 cohort captive broodstock spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River (top) reared under Freshwater Accelerated, Freshwater Natural and Saltwater Natural treatments (bottom). Horizontal reference lines indicate anticipated value when the captive broodstock program was initiated.

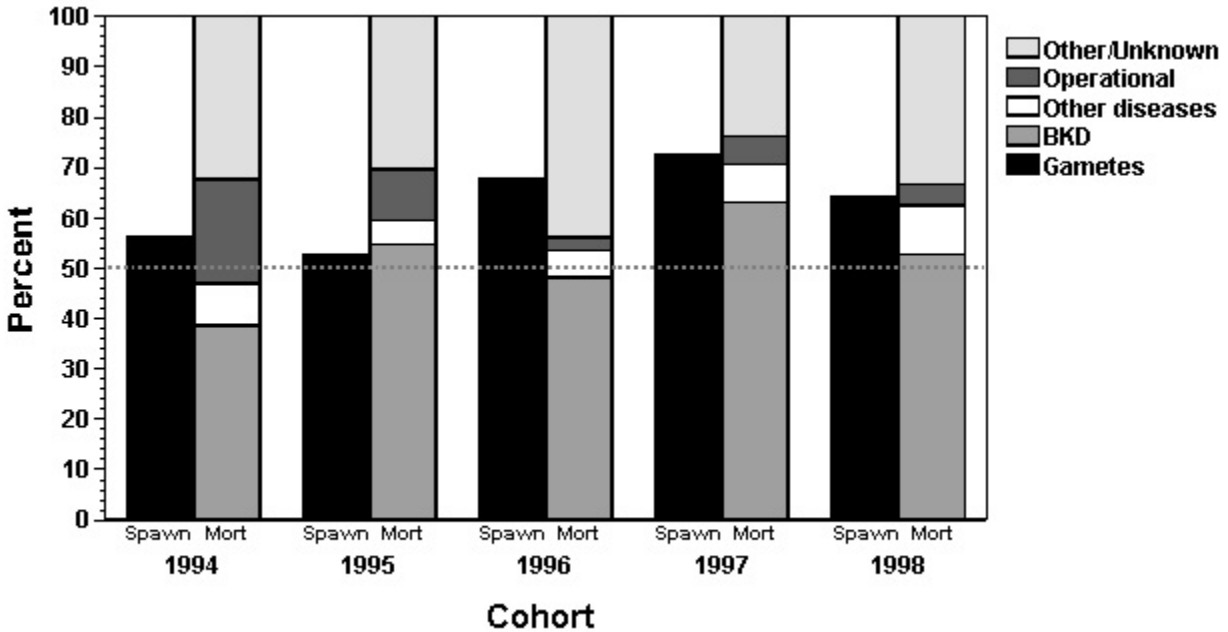


Figure 67Disposition (“spawned” are fish that survived to reproduce) of 1994-1997 cohort captive broodstock spring chinook salmon. Horizontal reference line indicates the anticipated percentage of fish to mature when the captive broodstock program was initiated.

younger age than expected and there were significantly fewer ($P=0.0008$) age 5 males (Figure 68). A mean of 16.7% of the males surviving to spawn did so at age 2, 66.3% matured at age 3, 14.6% at age 4, 2.3% at age 5 and 0.1% at age 6. Mean age at maturity was expected to be 3.8 years for males and but was 3.6 years, overall. The Catherine Creek and Lostine River males tended to mature younger than the Grande Ronde River males. Mean age of maturity of males also varied with treatment group ($P<0.0001$). Males from the Freshwater Accelerated and Freshwater Natural groups matured at a younger mean age (3.0 years) than those of the Saltwater Natural treatment group (3.2 years).

Size of Spawners

We expected that fish would reach the approximate size at maturity of fish reared in nature. However, size at maturity for captive broodstock was significantly ($P<0.0001$) smaller than comparably-aged wild fish (captured at weirs and spawned in the conventional broodstock program) - females were 68% as large and males were 64.5% as large.

Size of captive broodstock female spawners varied among ages and treatment groups ($P<0.0001$). Although there was a statistical difference in length among mature females of different ages, the vast majority of spawners were ages 4 and 5, which did not differ (Figure 69). Mature females of age 6 (543 mm) were also similar in length to those of ages 4 (562 mm) and 5 (572 mm) while age 3 females (445 mm) were significantly smaller. This pattern indicates that as the fish grew and reached a threshold length they became mature. If they had not reached the threshold length, then they delayed maturation for at least another year. Females in the Saltwater

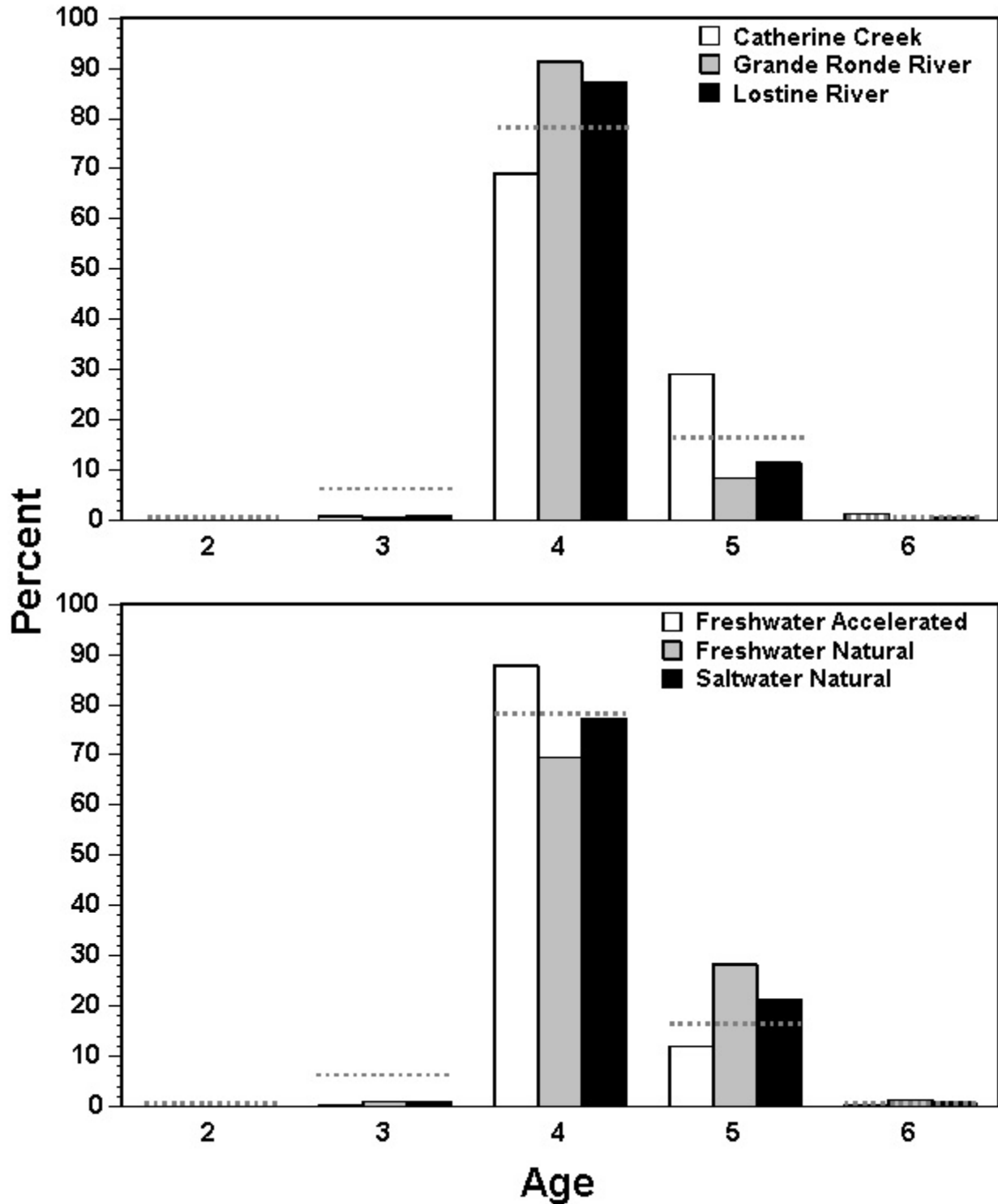


Figure 68 Percent of captive broodstock female spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River (top) and reared under Freshwater Accelerated, Freshwater Natural and Saltwater Natural rearing regimes (bottom) that matured at ages 2-6 (combined 1994-1998 cohorts). Horizontal reference lines show anticipated values for each age when the captive broodstock program was initiated.

Natural group were smaller (518.9 mm) than females in both the Freshwater Natural (549.5 mm) and Freshwater Accelerated (547.6 mm) groups, which did not differ in length.

Size of male spawners varied among ages ($P < 0.0001$) but not among treatment groups ($P = 0.0988$). Ages 4 (520 mm) and 5 (556 mm) males were larger than those of all other ages (Figure 69). Males of ages 3 (419 mm) and 6 (486 mm) were similar size but larger than age 2 (191 mm) males. Mean length did not vary among the Freshwater Accelerated (388.7 mm), Freshwater Natural (393.4 mm) nor Saltwater Natural (384.9 mm) treatment groups.

Fecundity

We expected fecundities to be approximately 1,200, 3,000 and 4,000 eggs for females ages 3, 4 and 5, respectively, approximating that of wild fish. Mean fecundities for ages 3, 4, 5 and 6 females were 1420.7, 1864.6, 1769.3 and 1368.6 eggs / female. Mature females of ages 3 and 6 are rare in this program. Mean fecundity did not reach expected levels, except for the age 3 females (Figure 70). Very few females matured at age 3 and mean fecundity for these fish did not significantly vary from expected ($P = 0.3343$). Fecundity for older fish was much lower than expected and was significantly lower than expected ($P < 0.0001$). Although there was a significant relationship between fecundity and age of females ($P = 0.0133$), multiple comparison procedures did not segregate them, based on age.

Mean fecundity also varied among treatments ($P < 0.0001$). Mean fecundity was higher in the Freshwater Natural (1965.7 eggs/female) and Freshwater Accelerated (1902.7 eggs/female) groups than the Saltwater Natural (1598.9 eggs/female) treatment group. There was no significant difference between the two freshwater groups.

At least a portion of this difference in fecundity is due to differences in size of spawning females among treatment groups and lower than expected growth. Fecundity was positively related to length, weight and K ($P < 0.0001$) and females of ages 4, 5 and 6 were longer ($P = 0.0015$) and heavier ($P = 0.0007$) than age 3 females.

Mean number of eggs / kg female body weight also varied between wild and captive broodstock females ($P = 0.0262$). Wild females (captured at weirs and spawned in the conventional broodstock program) had a mean of 998 eggs / kg whereas captive broodstock females had a mean of 891 eggs / kg body weight. Age 4 females differed ($P = 0.0269$) with captive females having a mean of 908 eggs / kg and wild females having a mean of 1026 eggs / kg. Mean number of eggs / kg did not vary for age 5 females: 810 for captive females and 859 for wild females. Within the captive broodstock program, age 3 females had a mean of 1,300 eggs / kg body weight which was significantly greater ($P < 0.0001$) than that for the ages 4, 5 and 6 females (908, 810 and 671 eggs / kg, respectively), which did not differ.

Egg Weight

Mean weight of eggs varied among treatment groups and ages of females ($P < 0.0001$). Mean egg weight was less in the Saltwater Natural group (0.199 g) than either the Freshwater Natural (0.219 g) or Freshwater Accelerated (0.216 g) groups, which were not different from each other. When combined into Saltwater and Freshwater-reared groups (and including data from the 2002 spawn, in which the fish were spawned only within Freshwater or Saltwater groups), mean egg weight of the Freshwater females (0.212 g) was heavier than that of the Saltwater females (0.197 g). Mean egg weight of 6-year old females (0.254 g) was greater than that of either the 4-year old (0.203 g) or 3-year old (0.138 g) females but not that of the age 5

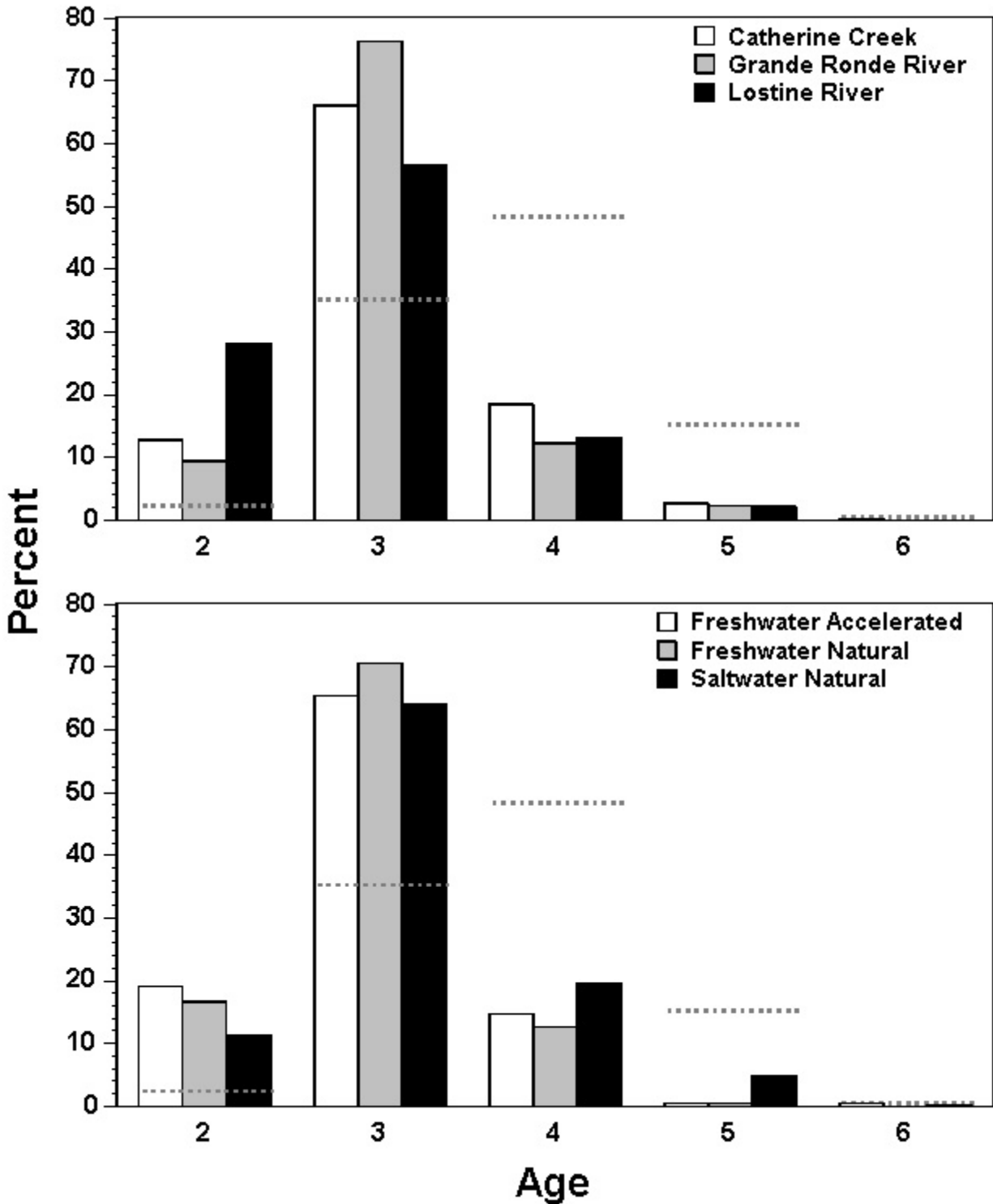


Figure 69 Percent of captive broodstock male spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River (top) and reared under Freshwater Accelerated, Freshwater Natural and Saltwater Natural rearing regimes (bottom) that matured at ages 2-6 (combined 1994-1998 cohorts). Horizontal reference lines show anticipated values for each age when the captive broodstock program was initiated.

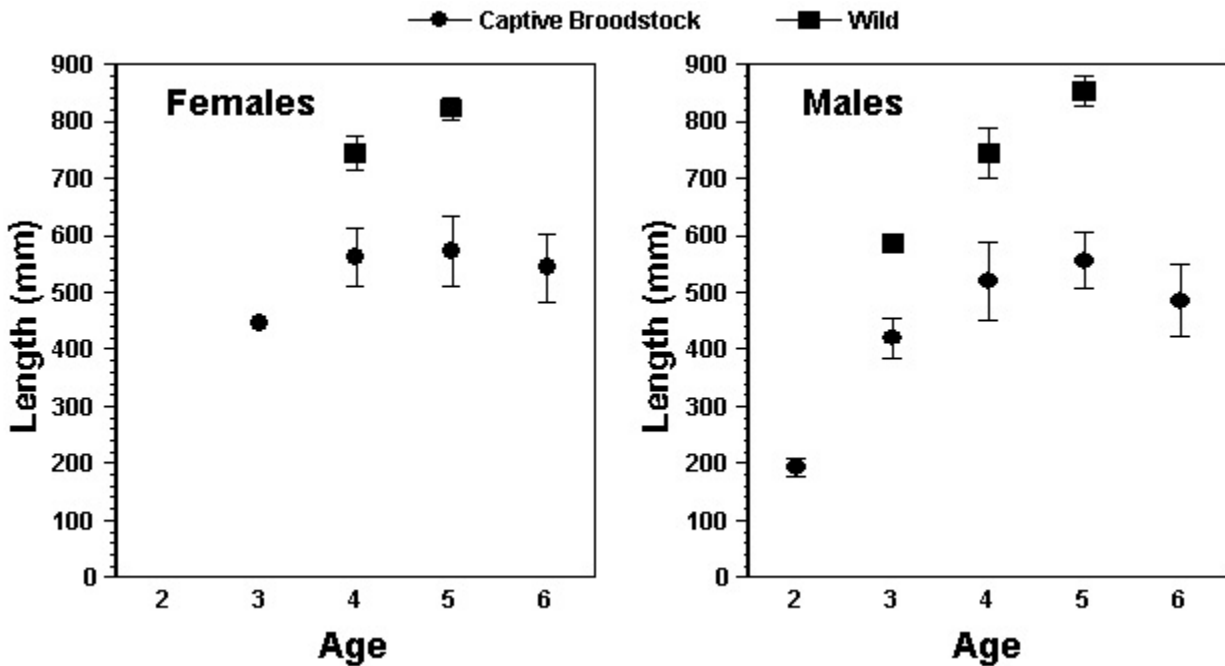


Figure 69. Mean (± 1 SD) length for mature wild and captive broodstock female and male spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River at ages 2 - 6.

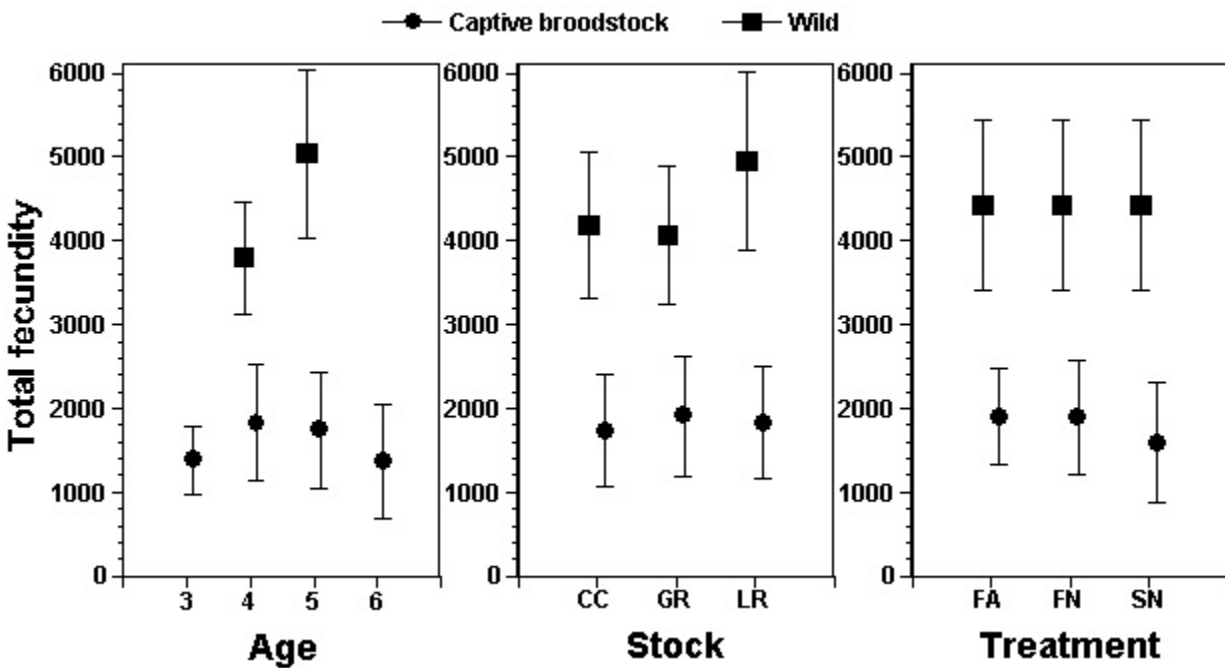


Figure 70. Mean (± 1 SD) total fecundity for wild and captive broodstock spring chinook salmon for each age, population and treatment (treatment values for wild salmon is for all populations, combined).

females (0.228 g). Mean egg weights of the age 4 and 5 females did not differ but were higher than that of the age 3 females. However, there were only eleven 6-year old and six 3-year old females in this analysis, compared with 302 age 5 and 1,396 age 4 females. Egg weight is also positively related to female length, weight and K ($P < 0.0001$). There was no significant relationship between mean egg weight and ELISA OD ($P = 0.5448$) and no difference in mean egg weight between captive broodstock and wild females ($P = 0.6949$).

Fertility

We assumed 75% embryo viability (percent of total eggs that reach the eyed stage). Mean fertility rate has been 78.4% and did not significantly vary from expected ($P = 0.0763$). Spawn year fertility ranged from 55.7% in 1998 to 86.3% in 2001 and has been as low as 38.9% in the 1998 Grande Ronde River fish and as high as 90.8% in the Grande Ronde River fish spawned in 2002.

Female egg fertilization rate varied among treatment groups ($P = 0.0005$) and ages ($P < 0.0001$). Mean fertility was higher in the Freshwater Accelerated group (83.3%) than in either the Freshwater Natural (76.8%) or Saltwater Natural (76.3%) groups, which did not vary from each other. Age 4 females had a significantly higher mean fertilization rate (80.0%) than age 3 (55.7%) and age 6 (40.8%) females but did not differ from age 5 females (74.4%). Age 5 females had a significantly higher fertilization rate than age 6 females but not higher than age 3 females.

There was a significant positive relationship between mean egg weight and fertilization rate ($P = 0.0041$). However, this relationship was poor ($r^2 = 0.0050$).

Fertilization rate (percent of total eggs that reach the eyed stage) of eggs by males varied among the treatment groups ($P = 0.0002$) but not with age ($P < 0.0870$). Mean fertility was higher in the Freshwater Accelerated group (83.6%) than either the Freshwater Natural (77.0%) or Saltwater Natural (79.0%) groups, which did not vary.

Mean fertility also varied with the use of fresh vs. cryopreserved semen ($P < 0.0001$). Use of fresh semen resulted in a mean fertilization rate of 79.4%, while using cryopreserved semen resulted in only 34.0% fertilization.

The relationship of spermatocrit vs. fertility was also examined. Mean spermatocrit in the Freshwater Accelerated group (32.9) was higher than either of the other two groups and spermatocrit in the Freshwater Natural group (26.9) was higher than that of the Saltwater Natural group (24.4). However, there was no relationship ($P = 0.6726$) between spermatocrit and fertilization rate (percent of eggs reaching the eyed stage).

Captive vs. wild spawners

Captive broodstock chinook salmon are substantially and significantly ($P < 0.0001$) smaller than wild chinook salmon at age of maturity (Figure 69). Salmon that mature at an older age are larger in wild salmon but length of captive females did not vary with age and the variation was reduced for captive males. Due to larger body size, wild females have greater fecundity than captive broodstock females (Figure 70). Captive broodstock salmon spawn an average of four weeks later than wild salmon that were collected from Catherine Creek, Grande Ronde River and Lostine River and held for conventional spawning at Lookingglass Fish Hatchery (Figure 71).

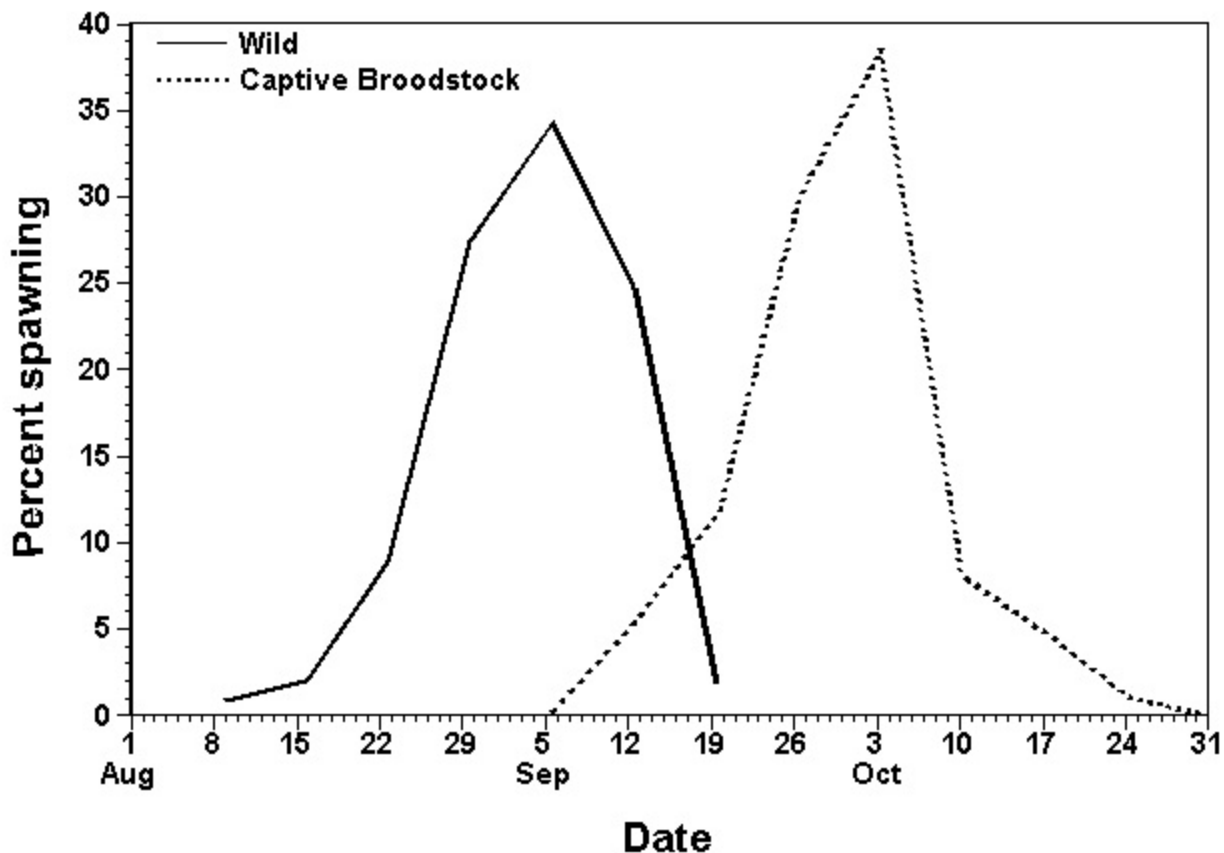


Figure 72 Percent of wild (collected for conventional broodstock spawning at Lookingglass Fish hatchery) and captive broodstock spring chinook salmon spawning at weekly intervals.

F₁ Survival

Eyed egg-to-smolt survival

We assumed 80% viable embryo-to-smolt survival when developing this program. Mean eyed egg-to-smolt survival has been 75% for the 1998, 1999 and 2000 cohorts and stocks, which is significantly lower ($P=0.0267$) than our expected value (Figure 72). We exceeded 80% survival for the 1998 and 2001 cohorts of Catherine Creek and 1998 cohort Lostine River salmon.

Smolt-to-adult survival

To date, only two years of captive broodstock F₁'s have returned as adults. The 2001 and 2002 return years have been comprised of the 1998 (ages 3 and 4) and 1999 (age 3) cohorts. Typically, chinook salmon reared at and released as smolts from LFH return at a mean rate of 0.1% smolt-to-adult survival (ODFW 1995). We have exceeded that rate for the 1998 cohort, even without the age 5 returns (Table 16). Mean return rate is 0.45% and ranges from 0.2% in the Grande Ronde River to 0.79% in the Lostine River.

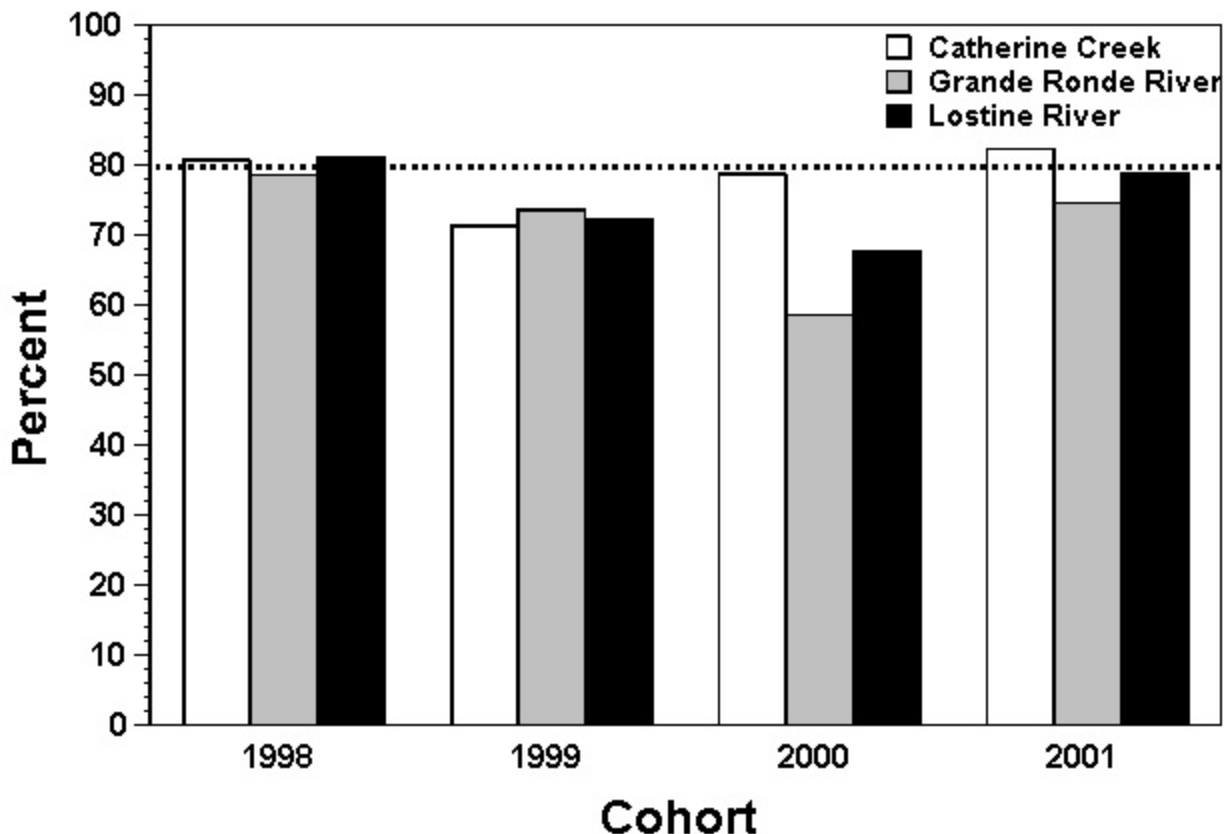


Figure 73 Eyed egg-to-smolt survival for 1998 - 2000 cohort captive broodstock F₁ spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River populations. Horizontal reference line indicates the anticipated value when the captive broodstock program was initiated.

Table 16. Estimated number (based on recovery of marked carcasses on spawning ground surveys) of captive broodstock progeny returning as adults and return rate for each population and cohort, as of 31 December 2002.

Stock	1998 cohort					1999 cohort	
	Age 3 males	Age 4 males	Age 4 females	Total return	Return rate	Age 3 males	Return rate
Catherine Creek	85	96	106	287	0.76%	16	0.01%
Grande Ronde River	0	0	3	3	0.20%	0	0%
Lostine River	34	159	189	382	1.09%	21	0.02%
Numbers released:	Catherine Creek:		1998 cohort: 37,980		1999 cohort: 136,833		
	Grande Ronde River:		1998 cohort: 1,508		1999 cohort: 2,560		
	Lostine River:		1998 cohort: 34,987		1999 cohort: 133,883		

F₁ Age Structure

Based on age structure data from previous LFH returns, we assumed that 10%, 60% and 30% of the smolts released into the wild would return as adults at ages 3, 4 and 5, respectively. While we cannot make any conclusions based on only two years of returns, so far we seem to have fewer age 3 males than anticipated since only 7% of the Catherine Creek and 6.8% of the Lostine River 1998 cohort returns were jacks and this percentage will decrease as the age 5 fish return in 2003.

DISCUSSION AND MANAGEMENT RECOMMENDATIONS

The Grande Ronde River Captive Broodstock Project is designed to quickly increase the number of endemic adult chinook salmon returning to this river system while maintaining the genetic diversity and identity of the endemic Catherine Creek, Lostine River and Grande Ronde River and unsupplemented populations (Minam and Wenaha rivers). Over the eight years that this project has been underway, we have modified some practices to improve our ability to reach our production goals within a gene conservation framework. The following is a discussion of some of the successes, failures and uncertainties about the program and subsequent modifications that have or will be made to the program to address these issues.

Program Operation

Protocols for the program have evolved as the captive broodstock program progressed. We have modified fish culture and monitoring practices to reduce handling and subsequent stress on the fish. Our protocols for prevention and treatment of disease, particularly BKD, continue to be modified to improve the health of the captive broodstock fish. We have also modified the design of the program in order to improve our ability to evaluate results.

Fish Culture Practices

Optimal fish culture techniques and only minimal and essential handling are vital to long term survival of chinook salmon in confinement. Modifications to conventional fish husbandry methods are already designed into the captive broodstock project and other adjustments have been and may be implemented, as needed. Early in the program, we sampled the fish frequently to monitor growth and condition in order to gain a better understanding of their performance. However, each set of measurements required handling and starvation of the fish, which increased stress and reduced growth. We have now reduced the amount of handling by reducing the amount of sampling to quarterly and combining other activities, such as tagging, inoculations and prophylactic treatments, with scheduled handling, whenever possible. Based on our trend of increasing egg survival, we feel that this reduction in handling has been beneficial. In addition, we anticipate that the use of ultrasound or near infrared spectroscopy to examine fish for maturation will allow us to conduct a single maturity sort and determine sex of each maturing fish early in the year (March/April), instead of two or three maturity sorts and later sorting to determine sex. We have tested the use of these methods to determine sex and maturity of chinook salmon during the 2001 and 2002 spawning seasons and will continue to do so in 2003. Initial results are promising for both methods.

We have also used our experience to improve fish culture practices and survival of the captive broodstock. Losses due to operational causes, such as handling and fish jumping out of tanks, has been greatly reduced. Additionally, the installation of light timers at Lookingglass Fish Hatchery has allowed us to simulate the natural photoperiod, which improves the ability of juveniles to smoltify more naturally (Hoffnagle and Fivizzani 1998).

Pre-smolt Growth

Due to problems with chillers and water supply at Lookingglass Fish Hatchery, we have

been unable to achieve a separation in size of fish raised under the “natural” vs. “accelerated” pre-smolt growth regimes until the 1999 cohort. Prior to the 1999 cohort, we raised fish whose growth was between the two planned experimental pre-smolt growth regimes and there was little difference in mean size between them (although we did see differences in some parameters - see Results, above). However, those problems appear to be solved and the 1999 and 2000 cohorts have shown growth rates resulting in the mean size at smoltification of natural fish being similar to that of wild smolts in this system and the accelerated growth fish are significantly larger (but still not to the size anticipated at the beginning of the program - these growth rates may be unattainable). The 2001 cohort is on a similar growth trajectory. This will allow us to truly evaluate the effect of hatchery growth rate on the parameters outlined in our study plan (e.g., survival, growth, age of maturation, fecundity and egg fertilization rates).

We have also modified the experimental design to rear fish at equal densities at BOH and MML. The addition of a Saltwater Accelerated group in the 2000 cohort means that as many as 250 fish are sent to each post-smolt rearing facility. Therefore, we now have FA, FN, SA and SN groups which will allow us to better compare both pre- and post-smolt rearing treatments. Also, with the addition of this group we have simplified our spawning activities to spawn fish within post-smolt (freshwater or saltwater) rearing groups instead of pre- and post-smolt treatments.

Bacterial kidney disease is the largest cause of mortality in the captive broodstock program. We have used erythromycin, injected and oral, as a prophylaxis and to treat outbreaks of the disease, with some success. The 1998, 1999 and 2000 cohorts were given injections (as parr) of a BKD vaccine (Renogen, Aqua Health, Ltd.) which uses *Arthrobacter* sp., a closely related bacterium to *R. salmoninarum* (the causative agent of BKD) to induce an immune response. Preliminary data were promising and suggested that mortality due to BKD of vaccinated fish was lower than that experienced by previous cohorts of unvaccinated fish for the first 20 months after vaccination. However, severe mortalities in late 2001 in the 1998 cohort of Grande Ronde River salmon (and lesser, but still high, levels of mortality in other populations) indicated the vaccination may have only delayed the onset of BKD. In addition, studies of Renogen in chinook salmon showed it to be ineffective (Rhodes et al *in press*). Therefore, we decided to not vaccinate the 2001 cohort and future cohorts until a better vaccine is developed - Rhodes et al showed promising results with another vaccine.

Saltwater Transfer

Early in the program, saltwater tolerance tests were conducted at Lookingglass Fish Hatchery as a means of determining when to transfer smolts to Manchester Marine Laboratory for rearing in saltwater. However, these tests proved inconclusive and were not useful in determining the proper time of smoltification. Therefore, they were discontinued in 1997. Currently, we ship ten sentinel fish from each population to MML at the time of migration of wild fish. If these fish survive and begin to feed within seven days, then the remainder of the Saltwater fish are transferred. If they do not survive and actively feed within seven days, a new set of sentinels is transferred and tested. This method has worked well, with few mortalities and will be continued.

Post-smolt Growth

Post-smoltification growth has been substantially lower than expected, mature fish are much smaller and females have fewer eggs than comparably-aged wild fish, especially for those

captive broodstock fish raised in saltwater. Higher than expected survival to maturity of the captive broodstock fish has offset the reduced fecundity. However, in our effort to develop recommendations for future captive broodstock programs, we would like to determine the reasons for this reduced growth and improve it, if possible. The cause of this may be a deficiency in feeding or nutrition. Very little information is available on rearing chinook salmon to maturation in captivity so specific research will be needed.

Tagging

All captive broodstock fish receive PIT tags and VI tags as a means of identifying individual fish and following their growth and survival. However, these tagging methods have not been without problems. At times, the older (400KHz) PIT tags were difficult to read due to their limited range. The newer (134 KHz) PIT tags have a longer range and have proven more reliable. Loss of PIT tags can also be a problem, particularly if they are not implanted properly or are expelled just prior to spawning. VI tags have worked fairly well as a secondary tag but are sometimes difficult or impossible to read due to migration and/or growth of opaque tissue over them. We will continue to use these tags, as the combination of the two makes it possible to track nearly all fish individually, and try to ensure that they are implanted correctly.

Spawning

With increased experience and improved ability to determine sex of fish (using ultrasound and/or near infrared spectroscopy) we can now develop spawning matrices earlier in the maturation season. This improves the efficiency of spawning procedures and allows us to maintain the highest amount of genetic diversity possible within our logistical constraints. This also allows us to use more fresh sperm, which increases egg fertilization rates and resulting smolt production. While sperm cryopreservation allows us to insure that there will be sufficient sperm to fertilize eggs, use of cryopreserved sperm has resulted in lower mean fertilization rates (although there is wide variation) than when using fresh sperm (34% vs 79%). Therefore, cryopreserved sperm is used only when fresh sperm from dissimilar cohorts (to eliminate the chance of sibling crosses) is not available.

Egg fertility rate was determined by two methods from 1999-2001: 1) percentage of a ten egg sample showing cell division approximately 20 hours after fertilization and 2) percentage of all eggs reaching the eyed stage. Method 1 gave a result that was higher, by a mean of 6.1% (SD=20.51%), than Method 2 and linear regression showed that the relationship between these two methods was significant ($P<0.0001$) but not as precise as we would have liked ($r^2=0.3001$; Figure 73). Given that we enumerate live and dead eggs as part of the evaluation of this program and if we assume that eggs counted as dead were not fertilized (a relatively safe assumption), then using Method 2 is an accurate method to measure fertilization rate for our purposes and is preferred because it is much easier to determine. Therefore, in 2002 we discontinued measuring fertilization based on the number of eggs showing cell division and future analyses will calculate fertilization rate using the percentage of eggs reaching the eyed stage.

In 1997-1999, we measured spermatocrit and sperm viability of males from which sperm was collected with the thought that variation in these parameters might relate to egg fertilization rate. There was a significant, but weak relationship ($P<0.0001$; $r^2=0.1419$) between spermatocrit and sperm viability. However, spermatocrit was found to be not significantly related to either measure of egg fertilization rate: percentage of ten eggs showing cell division ($P=0.3093$) nor

percentage of eggs reaching the eyed stage ($P=0.6037$). Likewise, sperm viability was found to

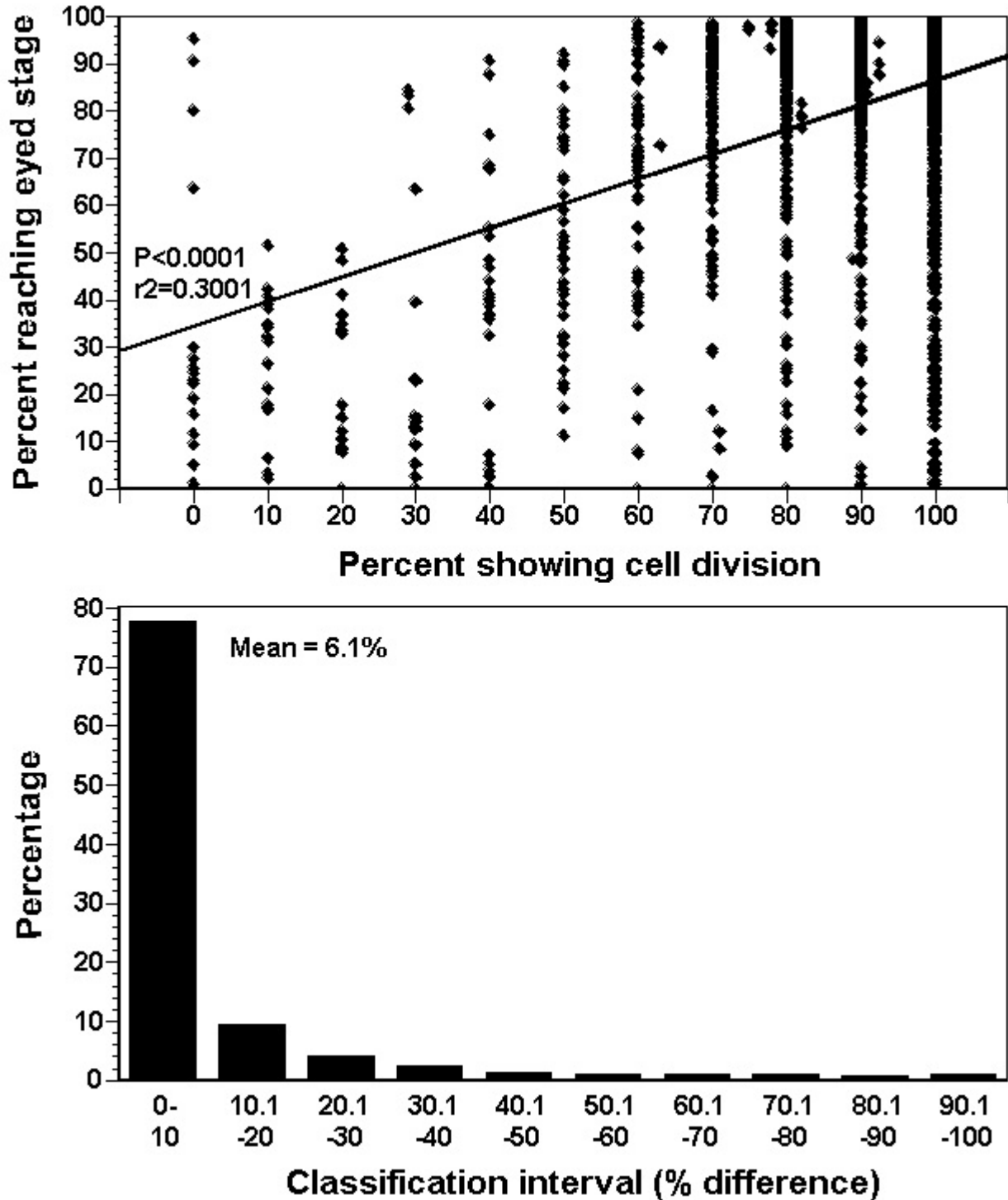


Figure 74 Relationship between percentage of eggs showing cell division and percentage of eggs reaching the eyed stage (top) and distribution of differences in fertilization estimates between the two methods (bottom).

be unrelated to either the percentage of ten eggs showing cell division ($P=0.3816$) or percentage

of eggs reaching the eyed stage ($P=0.8681$). Therefore, these activities were discontinued.

Database

Finally, the captive broodstock database (a large number of spreadsheets and databases in various formats) is in the process of being compiled and organized into a relational database (in one format - Microsoft Access¹). The data collected by this project will be used for many reports and publications and must be accessible to all collaborators in a useful condition and free of errors. This database can then be accessed by all who need it for data summarization, statistical analyses and reporting. The database is nearly completed and parts of it are being used at this time. The remainder of the database will be constructed as the remaining groups begin to use and enter their data into the captive broodstock database rather than other programs.

Future considerations

We still have some things to learn. The co-managers of the populations used in this program (ODFW, NPT and CTUIR) have developed an agreement for deciding what to do when we produce more captive broodstock offspring than we can rear or have targeted for release as smolts. The Grande Ronde Hatchery Management Plan will be implemented in 2003.

Bacterial Kidney Disease

As previously stated, BKD is the largest source of mortality in this program. We often spawn females with relatively high infections of *R. salmoninarum* (based on ELISA). For the F₁ 2000 - 2002 cohorts, we have culled eggs at a rate that has resulted in a severe program size reduction for one or more stocks. The rationale behind this culling is to decrease the prevalence of BKD in the F₁ generation (by curtailing vertical transmission of this disease) and reduce the mortality that it causes in hatchery (vertical and horizontal transmission) and wild fish (horizontal transmission) upon their release. This may also reduce the amount of BKD in nature when these fish return as adults. Indeed, raceways with progeny from high BKD females are more likely to suffer from BKD outbreaks but it is by no means predictable. The risks of vertical and horizontal transmission are poorly understood in hatcheries and appear to be even less understood in nature. Vertical transmission is likely a function of the severity and location of an infection in the female - infections confined to the kidney are unlikely to be passed on while systemic infections are likely to include eggs. Horizontal transmission is likely a function of fish density, pathogen prevalence and fish resistance. Culling entire lots of eggs removes the genetic contribution of that female and reduces or eliminates the genetic contribution of the males which fertilized those eggs, which is counter to the genetic conservation goal of this program.

Therefore, we need to further examine the risk of raising progeny from high BKD females by answering several questions. First, what is the correlation between prevalence of *R. salmoninarum* in the female vs. her eggs? Second, what is the relationship between maternal BKD infection and likelihood of offspring developing and spreading this disease? Third, can hatchery practices (e.g., rearing density, prophylactic treatments) affect the likelihood of a BKD outbreak or its severity? Fourth, what is the risk of progeny from high BKD females to wild fish via horizontal transmission, both before (from hatchery effluent) and after release? Answering

¹Used for information purposes only and does not constitute an endorsement of this product.

these questions will allow us to better evaluate the risks of raising progeny from high BKD parents and allow us to balance the risk of BKD vs. loss to the gene pool of these endemic populations. In addition, we must consider the risk of releasing fish that carry *R. salmoninarum* to other fish when they are captured and transported in barges (usually at high density) around dams on the Snake and Columbia rivers. We have reared some offspring from high BKD (ELISA OD >0.8) females mixed with those from lower ELISA groups and the results have been mixed. For the 2002 cohort of F₁ fish, we are rearing an entire raceway of offspring from high BKD females (ELISA OD > 0.8) from the Grand Ronde River. These fish are being reared at low density in the hope that will improve survival. This will give us more information concerning our ability to rear these fish with the conflicting risks of culling to reduce BKD or rearing potentially sick fish to maintain genetic diversity.

F₁ Disposition

If sufficient hatchery space is available, all offspring (within management plan guidelines - see CTUIR et al 2002) will be raised to smoltification and acclimated at and released into their parental home stream or one of its tributaries presently with few or no chinook salmon. If hatchery space is unavailable to raise them to smoltification, then they can be direct stream released as fry or parr into specific outlet streams (tributaries of the program streams or other nearby streams previously designated and agreed upon by co-managers). In 2002, we released fish (2001 cohort) as parr into the following outlet streams: Lostine River fish into Bear Creek, Grande Ronde River fish into Sheep Creek and Catherine Creek fish into Lookingglass Creek.

Another option is to release mature adults into their natal stream or an outlet stream to spawn naturally, preferably with wild fish. However, captive broodstock fish have been spawning approximately three weeks later than wild fish (Figure 71). This suggests that it would be unlikely that they would spawn with wild fish. Additionally, any eggs deposited this late would not receive the advantage of beginning their development in relatively warm water of late summer and hatching time for these fish will be much later than wild fish, thereby dramatically reducing their likelihood of surviving. We need to determine the reasons for late spawning in captive broodstock fish and correct it before any fish can be released to spawn in nature.

If excess eggs are collected, they can be planted into outlet streams and allowed to develop and hatch in the wild. The problem of late spawning can be corrected by incubating these eggs at a higher temperature to allow them to catch up in their development with wild eggs. They would then be placed in the stream at approximately the same stage of development as wild eggs and should hatch at the same time.

Parr Collection

Lastly, if we are able to consistently produce more smolts than targeted for this program, a reduction in the number of parr collected will be warranted. This contingency was expected when the program was developed. It was decided that we could reduce the number of parr collected to as low as 300 and still maintain statistically valid comparisons among our experimental treatments. Also, if sufficient numbers of adults return to these streams, the captive broodstock program may be reduced or terminated for a given stream, in deference to a conventional broodstock program.

CONCLUSION

Although there are areas in which improvement is needed, the Grande Ronde Basin Captive Broodstock Program has been more successful, at this early stage, in producing smolts for release than anticipated. We have also been aggressive and successful in exploring new methodologies for use in the program, such as ultrasound and near infrared spectroscopy for early detection of maturation and the use of azithromycin for prevention and treatment of BKD. We have shown that captive broodstocks are a viable option for producing large numbers of smolts in a short period of time to increase size of populations that are in danger of extinction. However, we still do not know whether this will translate into long-term success in increasing naturally spawning populations and what affect these programs may have, if any, on the genetic diversity of target populations. These factors will be examined as we continue to release captive broodstock progeny and have them return to create the F₂ generation. We will not claim success for the program until we have a sustained increase in the number of wild chinook salmon spawning in the program streams.

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