Fish Research and Development Project

Oregon

Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Program

Project Status Report

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and

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INTRODUCTION

Historic and Present Population Status

The Grande Ronde Basin once supported large runs of chinook salmon *Oncorhynchus tshawytscha*, with estimated escapements in excess of 10,000 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 stocks. 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 habitat, 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 1799 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 all 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 estimated number of redds in 1957 was 374 (not including North Fork Catherine Creek), 478 and 893, respectively. Redd counts for index count areas (a portion of the total stream) in Grande Ronde Basin streams are presented in Table 1.

The Minam and Wenaha rivers are tributaries of the Grande Ronde River located 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 has 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 and is listed as such as a requirement of our Endangered Species Act (ESA) permits. 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 F_1 and F_2 generations spawn and complete their life cycles in the wild.

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

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 goals are to prevent extinction of the

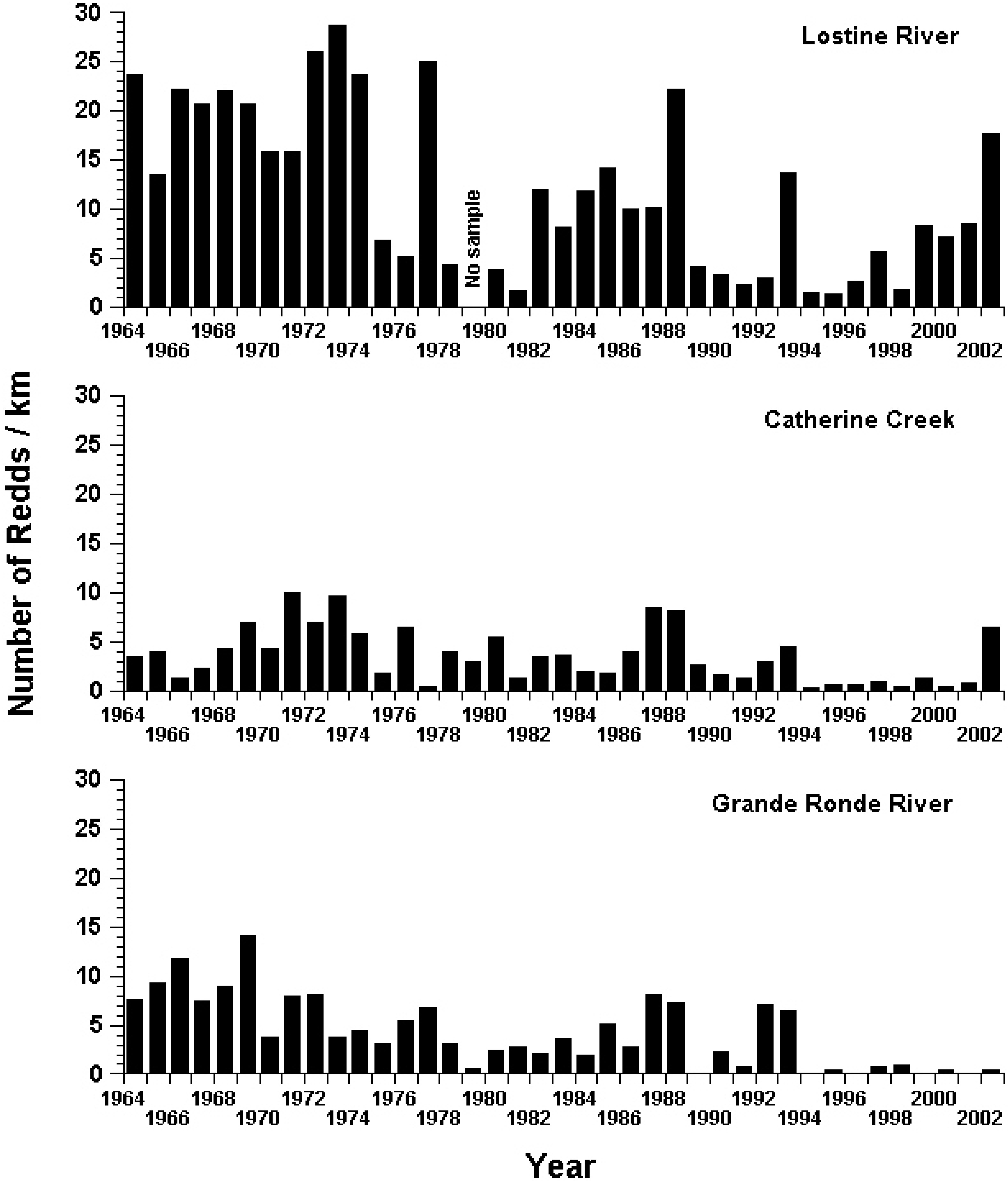


Figure 1. Number of spring chinook salmon redds / km within index areas on the Lostine River (4.8 km surveyed), Catherine Creek (12.1 km) and Grande Ronde River (13.7 km), 1964-2002. Note: in Grande Ronde River, 22.5 km were surveyed in 1964, 12.1 km in 1966, 29 km in 1967, 33.8 km in 1968 and 16.1 km 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, 1964-1995.

Stream	River km Surveyed	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	4.8	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	167	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	47	15	27	51	85	51	121	85	116
N. F. Catherine Cr.	4.8	--	--	--	31	15	19	19	28	38	73
S. F. Catherine Cr.	3.2	--	--	--	17	7	43	3	86	21	33
Grande Ronde River[a]	13.7	172	128	143	216	304	194	51	129	110	52
Sheep Creek	9.7	--	4	--	24	13	106	74	58	69	21
Minam River	16.2	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	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	4.8	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	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.	3.2	19	12	21	--	26	5	0	3	7	4
Grande Ronde River[a]	13.7	61	42	75	92	42	7	32	38	29	49
Sheep Creek	9.7	19	22	18	--	--	0	8	8	18	5
Minam River	16.2	51	50	52	14	137	9	10	14	22	21
Little Minam River	2.4	22	13	--	--	--	--	--	--	--	--
Indian Creek	4.8	1	0	9	--	11	--	--	--	--	--

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	2	2	0	9
Hurricane Creek	4.8	0	20	5	17	9	2	0	4	1	19
Wallowa River	7.2	12	3	7	15	7	0	1	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.	3.2	4	7	21	35	39	17	7	1	0	2
Grande Ronde River[a]	13.7	26	70	37	112	99	0[b]	31[c]	10	97	88
Sheep Creek	9.7	18	30	4	7	0	0	0	0	5	0
Minam River	16.2	23	116	63	90	87	33	53	37	16	63
Little Minam River	2.4	--	--	--	--	--	--	--	--	--	--
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	4.8	0	0	3	--	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	2	28	26	24	5	25	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	0	24	1	0	0	0	0
Catherine Creek	12.1	4	7	8	24	5	16	5	9	79
N. F. Catherine Cr.	4.8	0	0	1	7	4	1	2	10	6
S. F. Catherine Cr.	3.2	0	0	0	2	0	0	0	1	1
Grande Ronde River[a]	13.7	1	5	2	10	12	0	6	2	6
Sheep Creek	9.7	0	0	--	--	--	--	--	--	--
Minam River	16.2	5	10	57	36	31	26	63	44	131
Little Minam River	2.4	--	--	5	5	4	5	0	8	12
Indian Creek	4.8	0	0	--	--	--	--	--	--	--

[a] 22.5 km surveyed in 1964, 12.1 km in 1966, 29 km in 1967, 33.8 km in 1968, 16.1 km in 1971.

[b] 1989 Grande Ronde survey conducted after flash flooding on 8 August 1989.

[c] Supplemental survey data used because original index count was conducted too early.

Catherine Creek, Lostine River and upper Grande Ronde River chinook salmon populations, provide a future basis to reverse the decline in stock abundance of endemic Grande Ronde Basin chinook salmon and ensure a high probability of population persistence well into the future once the causes of basin wide population declines have been addressed. Associated long term objectives include:

1) Reduce the demographic risks associated with the decline of native wild populations in Lostine and upper Grande Ronde rivers and Catherine Creek.
2) Maintain genetic diversity of both wild and artificially-propagated indigenous chinook salmon populations in the Grande Ronde Basin.
3) Ensure a minimum annual threshold escapement of 150 adults in Catherine Creek, Lostine River and Grande Ronde River.
4) Develop indigenous broodstocks for a Grande Ronde Basin chinook salmon hatchery program.
5) Maintain health of captive broodstock fish and reduce incidence of diseases such as bacterial kidney disease in captive broodstock populations.
6) Determine the most effective treatments (e.g., strategies for rearing, spawning and disease prevention/management) for captive broodstock programs.
7) Assess captive broodstock program performance in achieving management objectives of adult broodstock, smolt production and adult return goals.
8) Assess the effectiveness of captive broodstock programs for use in recovery of salmonid populations.
9) Coordinate ESA permit activities and participate in basinwide (Snake River) captive broodstock planning and oversight.

Background

The Grande Ronde Basin once supported large runs of chinook salmon and escapement may have exceeded 10,000 fish as recently as the late 1950's (U.S. Army Corps of Engineers 1975). However, natural escapement has declined in the Grande Ronde Basin, as have those of other Snake River stocks. Catherine Creek and Grande Ronde and Lostine rivers historically contained three of the most important populations in the Grande Ronde Basin. Escapement levels in these three streams dropped to alarmingly low levels in 1994 and 1995 (Figure 1; Table 1). Present escapement levels and recent trends indicate that Grande Ronde Basin spring chinook salmon stocks show some improvement but these stocks (particularly the upper Grande Ronde) remain in imminent danger of extinction. We are presently in an emergency situation where dramatic and unprecedented efforts are needed to prevent extinction as well as preserve any future options for use of natural fish for artificial propagation programs. The initial management plan under the Lower Snake River Compensation Plan (LSRCP) program called for hatchery supplementation of four chinook populations in the basin: Catherine Creek and Wallowa, Grande Ronde and Lostine rivers. In 1995, the Oregon Department of Fish and Wildlife (ODFW), U.S. Fish and Wildlife Service (USFWS) and Nez Perce Tribe (NPT) began immediate development of 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) joined in this program at a later date. The decision to begin a captive broodstock program was a result of a number of factors including: increased emphasis on natural production and endemic stock recovery; 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 will provide 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, Redfish Lake sockeye salmon *Oncorhynchus nerka*, Salmon River spring chinook salmon and White River, Dungeness River and Tucannon River chinook salmon. 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 culture, research, monitoring and evaluation protocols for this program.

Recovery of these populations is dependent upon improved juvenile and adult survival through mainstem Snake River and Columbia River reservoirs and dams. Project success is dependent upon achievement of adequate survival, growth, maturation, gamete viability, smolt-to-adult survival and reproductive success objectives. In order to promote recovery, we must first ensure survival of these populations into the future. Our current efforts using captive broodstock are focused on ensuring short term persistence of the populations.

The Biological Requirements Work Group (1994) developed "threshold" escapement levels for use in their analyses, based on considerations of demographic and genetic risk. Application of these risks provide information on salmon population structure and uncertainty in available observations of the abundance and production. 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 stocks. Lostine River, Catherine Creek and upper Grande Ronde River are all classified as small stocks. Our take of 500 parr each year from each stream is based on reaching a threshold population goal of 150 spawning adults returning to each stream annually.

We have reviewed escapement patterns for Lostine River, Catherine Creek and upper Grande Ronde River to assess the number of years that spawning escapements have exceeded 150 adults (threshold). During 1964-1974, spawning escapements on all three streams exceeded 150 adults nearly 100% of the time. Since 1975, escapement in these streams exceeded 150 adults only 54-61% of the time and escapement has exceeded 150 fish 0-56% of the years since 1994. The reduction of spawning escapements below this threshold indicates a high and unacceptable risk to the survival of these populations, resulting in the development of the captive and conventional broodstock programs 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 2001) and in 2001 in Catherine Creek and Grande Ronde River. The captive broodstock program proved to be successful more quickly than anticipated and conventional programs are underway. This has forced the comanagement agencies to develop management strategies for these streams. In late 2002, management strategies for each stream were negotiated and will be implemented beginning in 2003. The upper Grande Ronde River has the lowest number of returning adult chinook salmon and will be managed for maximum production from both captive and conventional broodstock programs in order to maintain this stock. Adult collections in Grande Ronde River will be aggressive, with the purpose of collecting enough adults (within our ESA permit) to maximize smolt production at the permitted level of 250,000 (Table 2). Adult collections from both Catherine Creek and Lostine River for the conventional broodstock programs will be more conservative and follow the original sliding scale developed at the initiation of the captive broodstock program. We will also maintain the Minam and Wenaha rivers as wild salmon streams, which we do not supplement with hatchery fish, 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 is focused on an assurance of survival of the populations, using the return of 150 adults as an indicator. At initiation of the program, proposed release of F_1 generation smolts was based on a sliding scale and was more aggressive at lower levels of escapements and reduced as spawning escapements approached or exceeded 150 adults (Table 2). The proposed releases continued at a reduced level at 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 captive brood is based on a series of assumptions (survival, fecundity, etc.), the continuation (at escapements above threshold) of lower levels of hatchery releases (from a conventional program) offers some hedge in case our experience results in less fish than we assumed would return.

Implementation of a conventional hatchery supplementation program, using naturally-produced adults, has now begun for all three populations. Maximum wild and hatchery (captive and conventional) smolt production levels for each stream are part of the management strategies that have been negotiated by the comanagement agencies. This agreement calls for use of the original sliding scale for juvenile releases in Catherine Creek (Table 3). In Grande Ronde and Lostine rivers, no 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

Table 2. Sliding scale for collection of adults for conventional broodstock spawning in Catherine Creek, Grande Ronde River and Lostine River.

Estimated adult escapement	Catherine Creek		Grande Ronde River		Lostine River	
	Wild	Hatchery*	Wild	Hatchery*	Wild	Hatchery*
≤250	≤40%	≤40%			≤40%	≤40%
251-500	≤20%	≤20%	≤50%	≤100% (up to that needed for collection goal)	≤20%	≤20%
>500	≤20%	0%			≤20%	0%

*Not to include any Captive Broodstock F_1 adults

Table 3. Proposed sliding scale for release of captive broodstock F_1 generation smolts. This scale assumes 4000 eggs/female, 3.1 adults/redd and 11.9% egg-to-smolt survival for wild fish.

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	150,000	97	154,760
16	7600	4	150,000	96	157,616
32	15,200	11	120,000	89	135,232
48[a]	22,850	19	100,000	81	122,848
65	30,950	34	60,000	66	90,940
80	38,080	43	50,000	57	88,080
100[b]	47,600	66	25,000	34	72,600

[a] represents 150 adults

[b] represents 310 adults

come from the Captive Broodstock Program while in Grande Ronde River there is a preference for conventional fish but no limit on origin of the hatchery fish.

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 and production 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 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). The 90% survival from parr to smolt at 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) suggest it is unlikely we will need to collect more than 500 juveniles. 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 a 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) Based on age structure data from LFH returns, 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 program fish will be made as smolts as similar in size to natural fish as possible (approximately 125 mm fork length). Because of the critical need to promote the survival of these stocks into the future, smolts were chosen as the preferred life stage to release fish since they have proven to provide a substantial egg-to-adult survival advantage over pre-smolt releases in the Grande Ronde system (Messmer et al 1992; 1993) and is the most effective short term strategy. Fish will be acclimated for 20-30 days prior to release, at acclimation sites supplied with river water. Acclimation sites are 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 program smolt goals) will be released into suitable, unseeded habitat. Potential suitable sites include: in the Wallowa River drainage, the Wallowa River between RK 64-76, Hurricane Creek between RK 0-8 and Prairie Creek between RK 0-8; in the upper Grande Ronde River drainage, Sheep Creek between RK 0-8; in the Catherine Creek drainage, Little Catherine Creek between RK 0-3. Additionally, reestablishment of a naturally spawning population in Lookingglass Creek may be accomplished using Catherine Creek fish.

All efforts will be made to reassess the survival/mortality/fecundity estimates for the captive brood program to modify the number of juveniles collected from the wild each year to produce the desired number of fish, while considering the gene conservation objectives of the program. The use of fish not identified 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.

Disease continues to be a concern in the Captive Broodstock Program. By far, the most survival-threatening infection has proven to be bacterial kidney disease (BKD). Other infectious diseases that could or have occurred include: 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, particularly for maturing fish, and infestations by ectoparasites are also possible.

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 (FWP) (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." Our goal is to prevent extinction of the three populations, provide a future basis to reverse the decline in stock 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 U.S. vs. Oregon Grande Ronde chinook salmon dispute resolution in 1996 (Currens et al. 1996). This project is directed by the conceptual premise that identifies maintenance within and between population variations in genetic and life history characteristics as essential for long term fitness and persistence. It 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 Grande Ronde Basin Chinook Salmon LSRCP. 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.

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. We take all opportunities to maximize efficiency.
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. Extensive

be providing information to assess the success of the captive broodstock project.

3) The Northwest Power Planning Council's Fish and Wildlife Program: The Council's1994 report calls for initiation of captive broodstocks and associated research. Measure 7.4.D.2 requests Bonneville Power Administration to "fund captive brood stock 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 stocks.

Evaluation Objectives

The primary objective of the evaluation is to assess the effectiveness of using captive broodstock in recovery of ESA listed salmonids. Each captive broodstock cycle begins when 500 natural parr are collected from each stream in August/September, approximately 12 months after fertilization. Experimental treatments begin at Lookingglass Fish Hatchery when a portion of the juveniles are reared under simulated natural growth rates and one-third reared at an accelerated rate. The second treatment occurs at smolt transfer 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. Therefore, 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. Originally, there were three experimental groups. 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. 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 a fourth evaluation group: Saltwater Accelerated (SA), which is reared at the accelerated pre-smolt rate and then transferred to saltwater.

Because there are numerous uncertainties associated with captive broodstock programs, there is a need for careful assessment of the program at key periods in the production cycle.

Captive fish are reared through maturation, spawned and their progeny are reared to the smolt stage and released, progeny return and spawn naturally and their offspring complete a natural cycle. This cycle typically requires 3-5 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 progeny production. Logical evaluation points are during and at the end of these periods of the cycle. Hence, to completely evaluate a cycle requires up to 11 years. The experimental design requires a minimum of five cycles, thus requiring 16 years for completion.

We have divided the cycle into four phases: the Captive Juvenile Phase begins at collection and ends when fish are transported as smolts to Bonneville Fish Hatchery or Manchester Marine Laboratory; the Captive Adult Phase begins at transfer to Bonneville Fish Hatchery or Manchester Marine Laboratory and continues until the fish have been spawned or die; the F_1 Generation Phase runs from fertilization of eggs from captive fish through the death of the fish from these embryos; and the F_2 Generation Phase begins once eggs from F_1 generation females are fertilized and ends when fish from these embryos die.

We measure an array of variables in each period of the cycle. The information we collect and analyze will allow us to compare our experimental treatments (Freshwater Natural, Freshwater Accelerated, Saltwater Natural and Saltwater Accelerated), to develop relationships between treatments and performance, to monitor the basic progress in fish culture, to detect areas of concern that may need our immediate attention and to judge the adequacy of the benchmarks we have used to design the overall captive broodstock program.

DESCRIPTION OF STUDY AREA AND FACILITIES

Grande Ronde Basin

The Grande Ronde River drains approximately 10,700 km^2 of northeast Oregon (Figure 2). It's headwaters are above 2100 m in the Blue and Wallowa mountains, with some peaks reaching 3,000 m, and joins the Snake River at RK 272, near Asotin, Washington. The Grande Ronde River is fed largely by snowmelt and peak runoff occurs from Aril through June. Catherine Creek and Lostine River are two of its larger tributaries. Catherine Creek enters the Grande Ronde River near the town of Cove (RK 224). Lostine River joins Wallowa River at RK 26, near the town of Wallowa, and Wallowa River joins Grande Ronde River at RK 132. Salmon from the Grande Ronde Basin must migrate up to 1120 km between the spawning/rearing grounds and the ocean. This migration also 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.

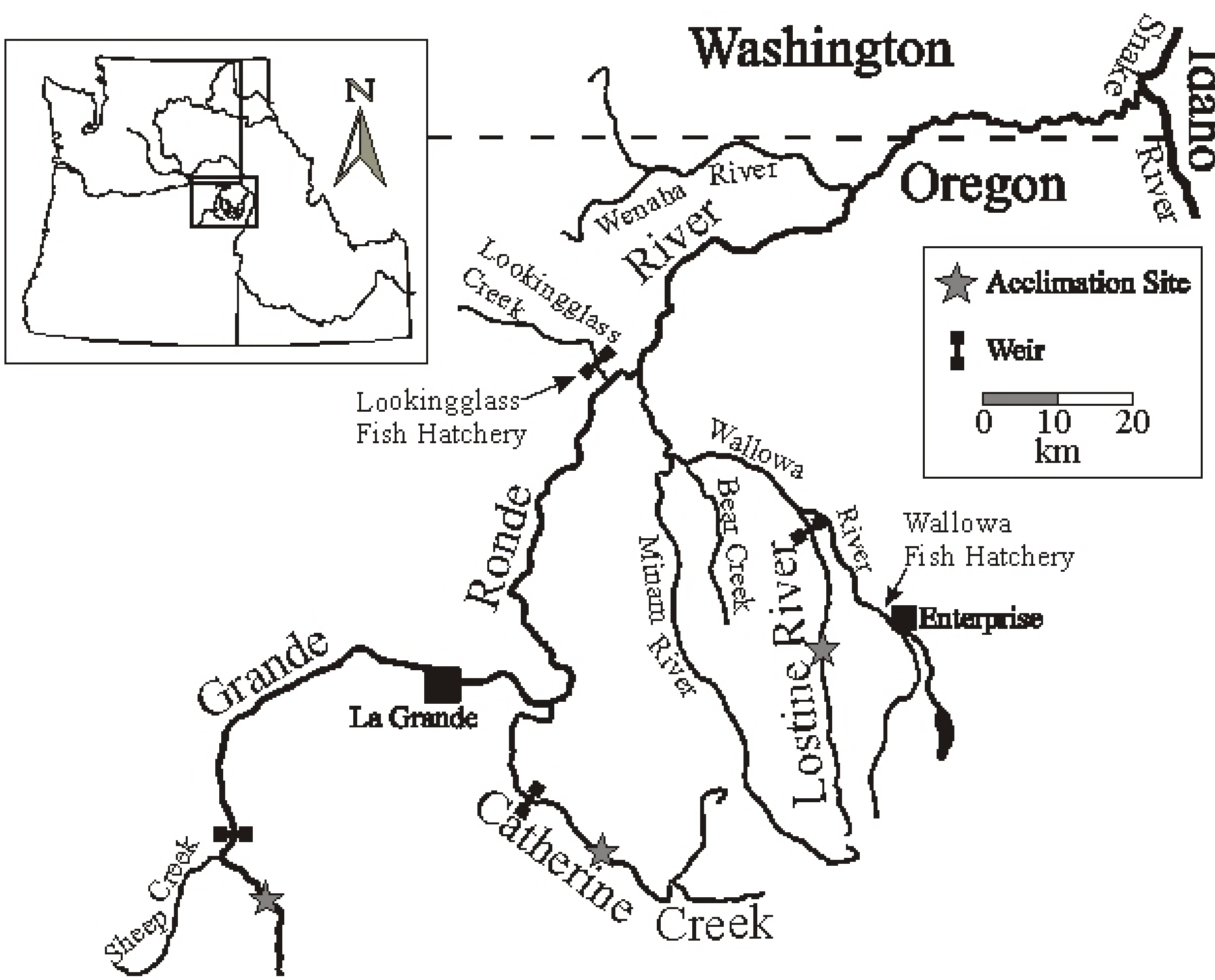


Figure 2. The Grande Ronde Basin, Oregon and Washington, with major tributaries, hatcheries, weirs and acclimation sites used in the Captive Broodstock Program.

Wallowa Fish Hatchery

The captive broodstock program will use Wallowa Fish Hatchery instead of Lookingglass Fish Hatchery 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 brood program will use at least 6 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 and 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 spring is treated each year to kill any fish. We are also working to cap the spring to further decrease the likelihood of pathogens. We have reared sentinel chinook salmon in the spring water without disease problems.

Bonneville Fish Hatchery

Bonneville Fish Hatchery is located below Bonneville Dam on Columbia River (RK 234). A Captive Broodstock Facility is located at the hatchery and includes a 972 m^2 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. 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 facilities for the Oregon and Idaho Snake River chinook and sockeye salmon captive broodstock programs. A 400 m^2 building houses six 4.1 m circular tanks and a 1280 m^2 building houses twenty 6.1 m diameter rearing tanks. Portable tanks (0.8-2.3 m^2) are also available for use. Salmon here are raised in 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 Annual Operating Plans for details of annual rearing strategies.

Fish Collection

We attempt to collect up to 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 in the stream the previous spawning season (based on the number of redds counted during spawning ground surveys). To estimate the number of available parr, we assume 4,000 eggs / redd (OFC 1958-1964; 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 determine from where to collect fish.

Collections are made from throughout the drainage to maintain the genetic diversity of the captive broodstock. Fish are collected using a method in which snorkelers herd fish into a seine. Fish are then transferred into 19 L live cars until they are transferred into 151 L coolers containing well water from Lookingglass Hatchery. To reduce stress, fish collections are made only when stream water temperature is <15˚C.

Captive Juvenile Phase

At the end of each day's collection efforts, the fish are transported to LFH 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).

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 23 g). 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. Treatment of natural and accelerated groups differs based on water temperature and amount of food fed (which is also based on the ability of fish to metabolize food at a given temperature; Table 4). 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. A naturally increasing photoperiod is important 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.

At LFH, the Captive Broodstock are reared in 2200 L troughs (5.4 m x 79 cm x 48 cm) which receive water at a rate of 114 L/min. They 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 also 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 one of the following prophylactic treatments for BKD: 1) an intraperitoneal injection of erythromycin on the day of capture; 2) an intraperitoneal injection of azithromycin on the day of capture; or 3) 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, the inoculation is given to all fish to maintain consistency of treatment between experimental groups.

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

	Photoperiod			Temperature (˚C)	
Beginning date of treatment	Time on	Time off	Total hours of light	Natural treatment	Accelerated treatment
17 Aug	423	1928	8.9	12.2	12.2
31 Aug	442	1903	9.7	12.2	12.2
14 Sep	500	1835	10.4	12.1	12.2
28 Sep	517	1808	11.2	11.6	12.2
12 Oct	535	1742	11.9	10.0	12.2
26 Oct	553	1720	12.6	6.0	12.2
09 Nov	611	1701	13.2	6.0	12.2
23 Nov	629	1649	13.7	6.0	12.2
07 Dec	644	1643	14.0	6.0	12.2
21 Dec	654	1646	14.1	6.0	12.2
04 Jan	658	1657	14.0	6.0	12.2
18 Jan	654	1712	13.7	6.0	12.2
01 Feb	642	1731	13.2	6.0	12.2
15 Feb	624	1750	12.6	6.0	12.2
01 Mar	601	1809	11.9	6.0	12.2
15 Mar	536	1827	11.2	6.0	12.2
29 Mar	509	1846	10.4	6.8	12.2
12 Apr	443	1905	9.6	7.6	12.2
26 Apr	417	1924	8.9	9.4	12.2
10 May	355	1943	8.2	11.0	12.2

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, for a stock, survived for one week, then the entire stock was transferred. If there was mortality, then another six fish were tested the following week. This method did not work well and one group never had 100% survival and was finally transferred in mid-June, after they had smolted, and suffered high mortality. Currently, transfer of the majority of the saltwater fish is preceded by the transfer of ten sentinel fish, in early May, to ensure that they have smolted and will thrive in saltwater. Sentinels are 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. 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

Fish at both BOH and MML are reared in separate tanks for each stock and cohort, except for remaining five- and six-year old fish which are combined within each stock. 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 100mg / 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 from each stock, 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 worry.

<u>Bonneville Fish Hatchery</u>

At BOH, Captive Broodstock fish are reared in unfiltered, but 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 / m^3. 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.

<u>Manchester Marine Laboratory</u>

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 / m^3. 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 F_1 population 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 cyropreserved semen, as well as balancing the logistic limitations associated with spawning. All fish are spawned within stocks and treatments. Furthermore, we are concerned about potential sibling crosses and inbreeding. Based on four 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 four years of spawning captive broodstock fish at BOH.

<u>Sorting of maturing fish</u>

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) was unacceptable.

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 - continued rearing in saltwater is likely to stress kidney osmotic function and may affect egg quality. We are testing ultrasound and near infrared spectroscopy to allow us to determine maturation status and sex of each fish. We hope that we will 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 2002 was very successful but some fish were incorrectly classified. The need for additional maturity sortings in June or July are 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 'mature tanks' at BOH.

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. We attempt to estimate the sex ratio (female:male) for each stock and treatment at the ripeness sort during early September (we hope that ultrasound or NIR will provide conclusive sexing information). These estimated sex ratios are used to determine the type and number of matrices to be used for each stock/treatment during spawning. Additional ripeness sorts continue on a weekly basis throughout the spawning season. These sorts provide information on fish available for spawning each week for each cohort, stock, treatment and sex.

<u>General spawning matrix development guidelines</u>

Our objective is an equitable contribution to the next generation by all mature fish, within disease and survival constraints. We have focused on making each parent's contribution to the next generation as equal as possible 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, 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 the likelihood of sibling crossing. 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. We begin by assigning females, then males to matrices.

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 eleven categories and each category is associated with a particular spawning matrix (Table 5).

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

Preferred matrix development protocol

If accurate estimates of sex ratios can be made prior to the first spawn (we hope to improve this by using ultrasound or NIR), the 'preferred matrix development protocol' is employed throughout the spawning season. Ripeness sorts are conducted on a weekly basis throughout the spawning season. These sorts provide information on fish available for spawning each week by cohort, stock, treatment and sex: they are not used to determine sex ratios for matrix development purposes.

The sex ratios for each stock 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 stock/ 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. These remaining four fish fall into category 'F' and would be spawned in a 2 x 2 matrix.

Back-up matrix development protocol

If ultrasound/NIR or first ripeness sorting in early September produced a high number of fish of undetermined sex the 'back-up matrix development protocol' will be employed for the remainder of the spawning season or until nearly all fish are able to be sexed. This protocol is also employed when too few fish of either sex are available to meet the target sex ratio under the 'preferred matrix development protocol'.

Ripeness sorting is conducted throughout the spawning season to provide information on cohort, stock, treatment and sex of fish available for spawning. Each week, when this process is completed, we determine the female:male ratio by stock and treatment of fish that are ready to spawn. For each stock and treatment, we assign a spawning category (A-K; Table 4) 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 stock 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.

Criteria for using 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 stock and treatment for a matrix, then three cryopreserved semen samples of the same stock 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 from three separate males is used for each female in the matrix in order 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 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. For example, if one fresh male and three cryopreserved samples are used, then 50% of the eggs will be fertilized by the fresh male and 16.7% of the eggs will be fertilized by each of the three cryopreserved semen samples.

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 6% of the eggs while fresh males fertilize approximately 45%. 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.

Guidelines for selecting cryopreserved semen for spawning

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

Too few fresh males and cryopreserved semen samples

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 any 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 stocks, 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 yet not occurred in this program and all efforts will be made to avoid them.

Spawning procedures at BOH

Mature fish are given an injection of erythromycin and placed in separate tanks at BOH based on stock and age. Mature 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 give them more natural seasonal and diel temperature changes and chemical cues for maturation. 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 1:6000 for one hour. In 2001 and 2002, hydrogen peroxide (1:3500 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 insure identification in case of loss of PIT and VI tags. 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 stocks and treatments and not usually within cohorts, to prevent sibling crosses (see matrix development sections above).

Males are spawned first. Each male is anesthetized and scanned for its PIT tag, VI tag or jaw tag (in that order) to obtain corresponding information from the database. Fork length and weight are measured. The male is then placed on the spawning rack, quickly wiped down with an iodiphor solution (200 ppm) and wiped dry. Semen is 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. If no semen is collected, (i.e., male was still green), it is returned to the pond and used later in the spawning season. 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 then lined up on the spawning rack. 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 iodiphor solution and then dried. The first female is spawned 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 70 ppm iodiphor 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 F_1 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 similar sizes. 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 when 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 until smoltification and release, after acclimation, into their parents' natal stream.

Health Monitoring and Disease Treatments

There are several infectious diseases that could occur in the spring/summer chinook collected and maintained in the Oregon captive brood stock project. Among the infectious diseases that could or have occurred are: bacterial kidney disease (BKD), 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 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 ≥ 1.2% (3/250) attributable to BKD occur in any rearing unit. This does not apply if the fish have received a treatment within the prior 30 days.

Dietary treatments (i.e., medicated feed and pills as aquamycin or erythromycin) were 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 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.

The 1998-2000 cohorts have been 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 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 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 was 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.

<u>Other diseases</u>

Monitoring for cold water disease, columnaris, enteric redmouth disease, aeromonad-pseudomonad septicemia and furunculosis was done by streaking smears from kidneys for 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 will be 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 will monitor the fish daily for signs of ectoparasititic infestation, such as flashing. Fish pathology personnel will collect gill clip and skin scrape samples from freshly dead or moribund fish. Also, a subsample of incoming parr will be visually inspected for the presence of *Salmincola* sp., a parasitic copepod. If copepods are present, a subsample will be 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. The fish pathology section will decide the form of treatment: 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 and infestations. 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 will need to 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 disease conditions might reduce survival. Second, acquisition of infections or disease conditions 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. Therefore, 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 were 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 stock 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 and, as such, has an extensive monitoring and evaluation component, which is used to determine the effectiveness of the 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. Measurable objectives include:

1) Collect 500 parr for captive broodstock annually from Catherine Creek, Lostine River and upper Grande Ronde River populations.
2) Monitor growth, development and survival to smoltification.
3) Mark all individuals in each cohort and stock with primary (PIT tag) and secondary (VI tag) marks.
4) Determine when fish are ready to be transferred to seawater.
5) Rear all fish to maturation.
6) Implement prophylactic erythromycin treatments for bacterial kidney disease under INAD protocols.
7) Assess maturation and characterize length, weight and survival of all stocks and treatments.
8) Determine etiology of captive broodstock morbidity and mortality.
9) Monitor fish culture practices and fish handling for situations that may contribute to impaired fish health or exacerbate disease.
10) Develop and implement complex matrix spawning protocols and oversee and facilitate the spawning of all ripe fish.
11) Coordinate Endangered Species Act permit activities and participate in captive broodstock planning and oversight.
12) Develop and maintain a comprehensive database for the program.
13) Analyze and summarize data and prepare reports describing our findings.

We rear the fish under one of four (originally three) treatments: two pre-smolt and two post-smolt treatments. One pre-smolt treatments 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 14˚ C to 5˚ C, to simulate that seen 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 14˚ C in order to grow the fish as large as possible before smoltification. The post-smolt treatments involve rearing the fish to maturation in either freshwater (at BOH) or saltwater (at MML). This creates four evaluation groups: Freshwater Natural (FN), Freshwater Accelerated (FA), Saltwater Natural (SN) and Saltwater Accelerated (SA). Variables other than environmental salinity and juvenile growth rate will remain as similar between treatments as possible. For example, at all times, all fish will be reared under a simulated natural photoperiod.

Stocks 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 stocks, cohorts, age classes and treatments, as appropriate, using statistical tests at α=0.05.

Captive broodstock fish are reared through maturation, are spawned and then their progeny are reared to the smolt stage and released, progeny return and spawn naturally and their offspring complete a natural cycle. This cycle typically requires 3-5 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 progeny production. Logical evaluation points are during and at the end of these periods of the cycle. Hence, to completely evaluate a cycle requires up to 11 years (Figure 3; Table 6). The experimental design requires a minimum of five cycles, thus requiring 16 years to completion.

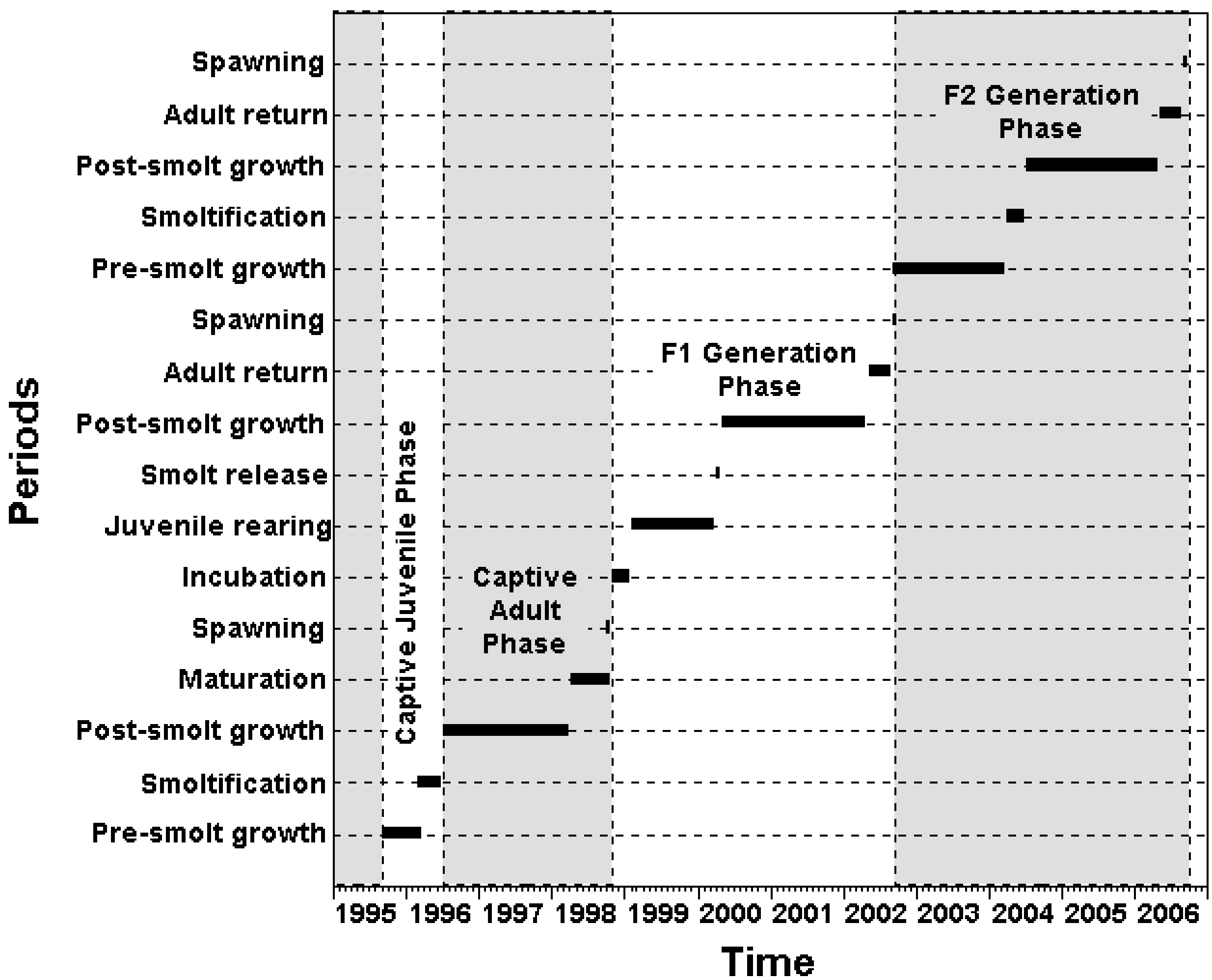


Figure 3. Captive broodstock monitoring and evaluation terminology and example time scale for fish maturing at age four.

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</u>	August 1995 - June 1996
pre-smolt growth period	August 1995 - April 1996
smoltification period	April - June 1996
<u>Captive Adult Phase</u>	June 1996 - September 1998
post-smolt growth period	June 1996 - April 1998
maturation period	April 1998 - October 1998
spawning period	September - October 1998
<u>F_1-Generation Phase</u>	September 1998 - September 2002
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>F_2-Generation Phase</u>	September 2002 - September 2007
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

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

Captive Juvenile Phase: This phase begins at collection and ends once fish have been transferred to BOH or MML. It is composed of two phases: <u>pre-smolt growth</u> and <u>smoltification</u>. 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.

Captive Adult Phase: The captive adult phase begins at transfer to either BOH or MML and ends when the fish die - either before or at spawning. It 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: size at maturity, sperm viability, fertility, egg size, fecundity, spawn timing, size at spawning, age at spawning and disease profile.

F_1 Generation Phase: This 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 phases. 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, prespawn survival in nature, spawning distribution in nature, spawning success and straying.

F_2 Generation Phase: The F_2 Generation Phase begins once embryos resulting from F_1 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 phases. 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, smolt migration patterns, parr and smolt production, smolt-to-adult survival, catch distribution, run timing, age structure at return, size-at-age, sex ratios, prespawn survival in nature, spawning distribution in nature, spawning success, straying and productivity (progeny to parent ratios).

F_1 and F_2 Generations

All spawning is conducted at BOH - 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. F_1 generation embryos from a given male-female pairing are kept separate from embryos from other pairs at Oxbow and Irrigon hatcheries. Embryos are kept distinguishable by female until the eyed stage. Fry are transported to LFH for completion of rearing to smoltification.

F_1 generation fry are reared in outdoor ponds and in Lookingglass Creek water at LFH. They are fin-marked (adipose-clipped) and coded-wire-tagged during June and July. Progeny from each treatment group are tagged to permit later identification. As many as 50,000 fish are reared in each pond. 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 stock 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 20-30 day period of acclimation, fish are released into the stream.

We anticipate that some of these fish will mature two, three or four 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 F_1 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 F_1 generation adults will be incorporated into any conventional supplementation program.

F_1 generation fish that survive to spawn may reproduce with other natural fish or other F_1 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 F_2 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 will be 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. This is an interim report, so 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 project was initiated in the Grande Ronde Basin in 1995. We have collected six cohorts (1994-2000) of spring chinook salmon juveniles from Catherine Creek and Lostine River in 1995- 2001 and from the upper Grande Ronde River in 1995, 1997-1999 and 2001 (collections were attempted in 1996 and 2000 but were unsuccessful). Each year, we have collected 500 (or nearly) fish from Catherine Creek and the Lostine River (Table 7). Only 110 fish were collected from the Grande Ronde River 1994 cohort and no fish were collected from the 1995 and 1999 Grande Ronde River cohorts. Fish are reared at LFH until the yearling smolt stage and then transferred to BOH and MML for the Captive Adult Phase. Fish from the 2001 collections (2000 cohort) are presently being reared at LFH.

1994 Cohort

<u>Collections</u>

The 1994 cohort was collected from Catherine Creek from 29 August-1 September 1995 between river kilometers (RK) 29.8-52.0. Grande Ronde River fish were collected from 18-22

Table 7. Number of spring chinook salmon parr from the 1994-2000 cohorts collected from Catherine Creek, the upper Grande Ronde River and Lostine River annually from 1995-2001, number of mortalities and number spawned, as of 31 December 2001. Note: 1994 cohort was collected in 1995, etc. “Gametes collected” means that the fish survived to spawn although gametes may not have been collected - e.g., we attempted to spawn some fish too early, etc.

	Stream		
Cohort/variable	Catherine Creek	Grande Ronde River	Lostine River
1994			
Collected	498	110	499
Mortality	138	81	268[1]
Gametes collected	360	29	231
1995			
Collected	500	0[2]	481
Mortality	259	0	210
Gametes collected	241	0	270
1996			
Collected	500	500	501
Mortality	154	101	244
Gametes collected	346	399	257
1997			
Collected	500	500	500
Mortality	217	92	164
Gametes collected	322	415	342
1998			
Collected	500	500	498
Mortality	275	306	268
Gametes collected	325	221	367
1999			
Collected	503	0	500
Mortality	205	0	185
Gametes collected	117	10	171
2000			
Collected	503	502	503
Mortality	38	24	105
Gametes collected	25	7	49
2001			
Collected	500	461	500
Mortality	3	4	8
Gametes collected	0	0	0

[1] Includes 49 fish that jumped out of the tank soon after collection.

[2] One Grande Ronde River chinook salmon parr was collected but returned to the river.

September between RK 296-325. Lostine River parr were collected from 14-17 August between RK 1.3-31.0. There were no collection-related mortalities in any of the stocks.

<u>Size at Collection</u>

Size at collection of chinook salmon parr varied with stock (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).

<u>Growth</u>

The Saltwater Natural growth groups were larger until about the age of four years in both the Catherine Creek and Lostine River stocks - no Grande Ronde River fish were raised in saltwater (Figure 5). All stocks also grew similarly.

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 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 July1996, 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.

<u>Mortality</u>

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

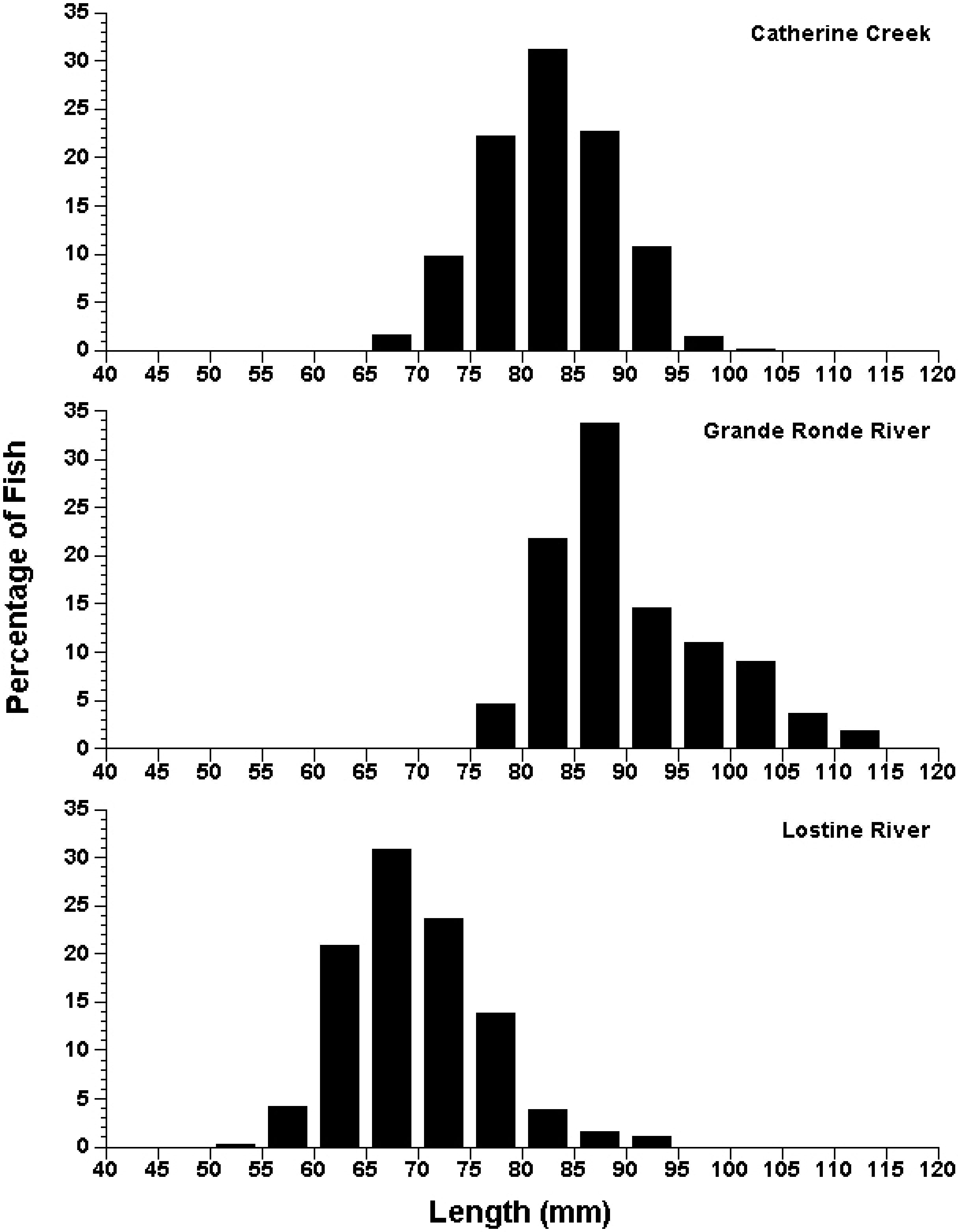


Figure 4. Length frequency distribution for 1994 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1995.

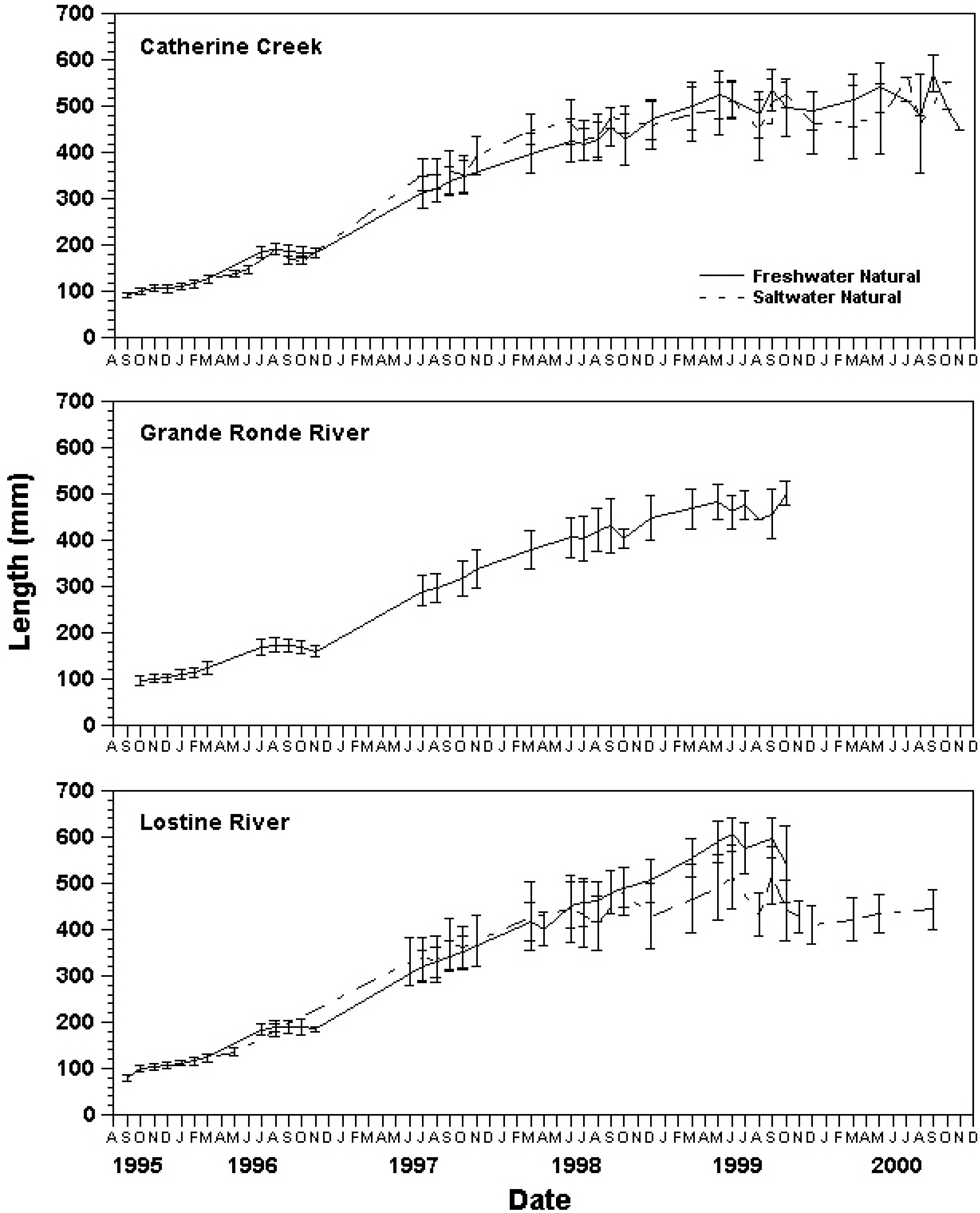


Figure 5. Mean length (±1 SD) of 1994 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Natural and Saltwater Natural growth regimes, 1995-2000.

Table 8. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
CC	360	72.1	20	4.0	29	5.8	21	4.2	3	0.6	66	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
Treatment												
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	86	25.1
Age												
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	54	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	157	79.3	0	0.0	20	10.1	6	3.0	2	1.0	13	6.6
6	9	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

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 3 (50.1%), 4 (70.3%), 5 (59.0%) and 6 (77.8%). Bacterial kidney disease was a large mortality factor for the 4 year old fish (32.7%), particularly those from the Grande Ronde and Lostine rivers. Unknown causes accounted for a large percentage of the mortalities at ages 1-3 (15.4%, 52.7% and 19.5%, respectively). Operational causes were the cause of 84.6% of the age 1 mortalities and 76.5% of the age 6 mortalities.

<u>Survival to Spawning and Age of Maturity</u>

All 1994 cohort fish have died and 621 of 1107 parr collected (56.1%) survived to contribute gametes. Within treatments, 38.1% 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 39.3% 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.

Sex ratios of spawners shifted with age (Figure 7). Of the mature fish, 10.4% matured at age 2 and 35.4% at age 3; 100% of these age classes were males from which semen was cryopreserved. At age 4, 27.5% matured (67.0% were females, 24.3% were males that were spawned and 8.7% were males from which semen was cryopreserved), 25.6% 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 was collected from Catherine Creek from 26-29 August-1 September 1996 between RK 29.8-52.0. Lostine River parr 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 stocks.

Size at Collection

Size at collection of chinook salmon parr varied with stock (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. As a result, there was no 1995 Grande Ronde River cohort in the Captive Broodstock Program.

Growth

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

Catherine Creek

Growth of the 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.

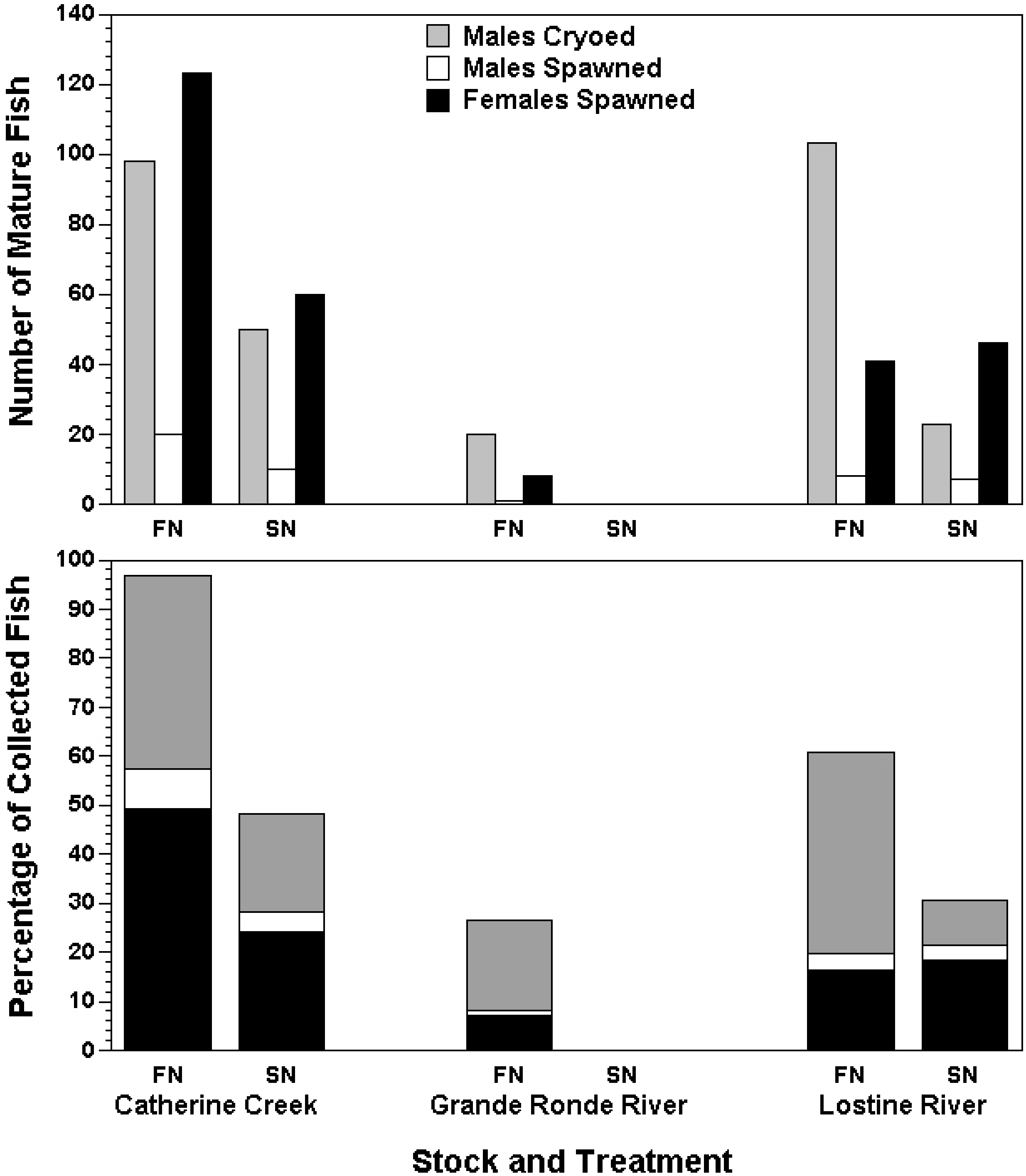


Figure 6. Number and percentage of 1994 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and raised under Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to contribute gametes (spawn or have semen cryopreserved).

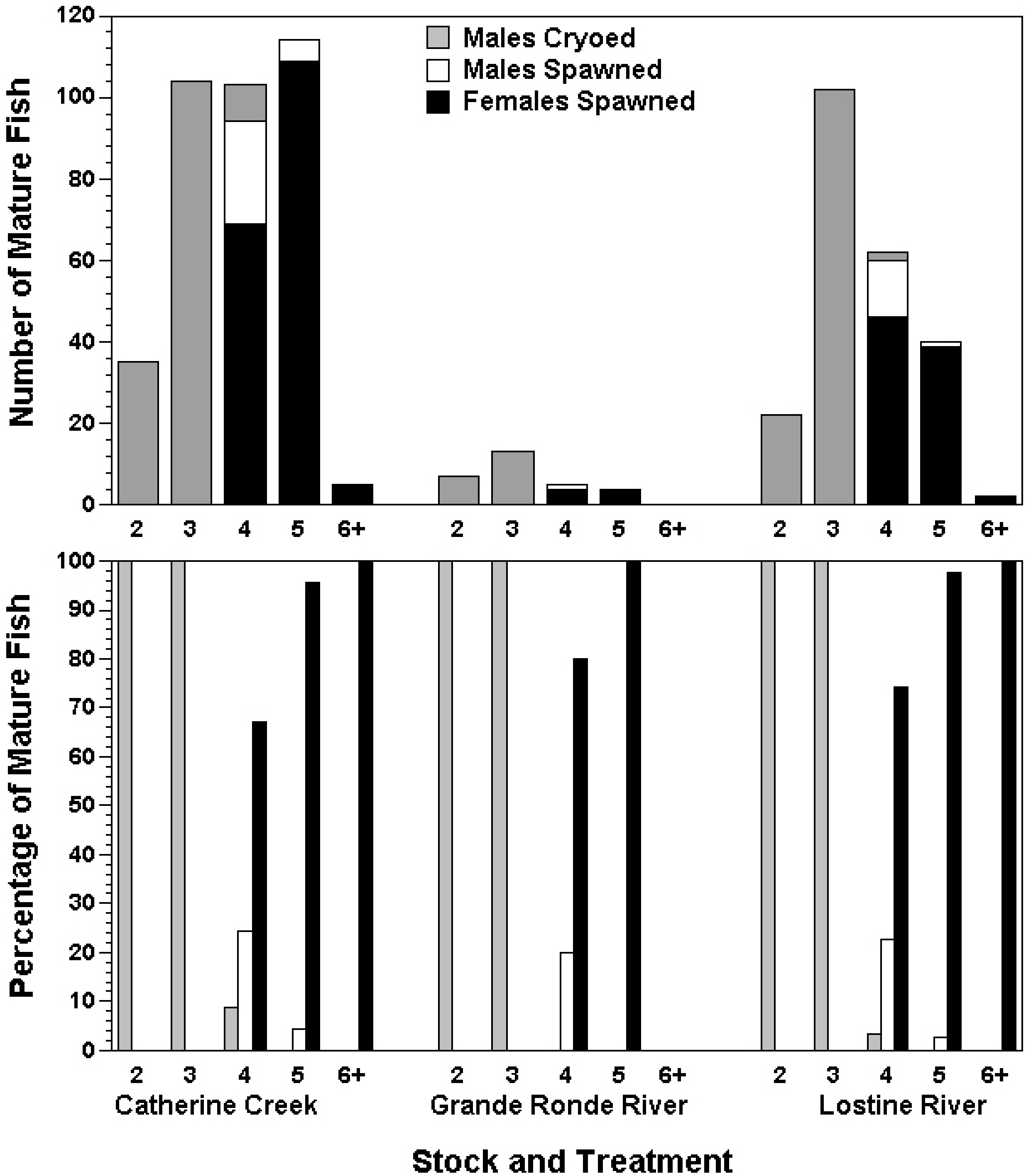


Figure 7. Number and percentage of 1994 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and that matured to contribute gametes (spawn or have semen cryopreserved) at ages 2-6.

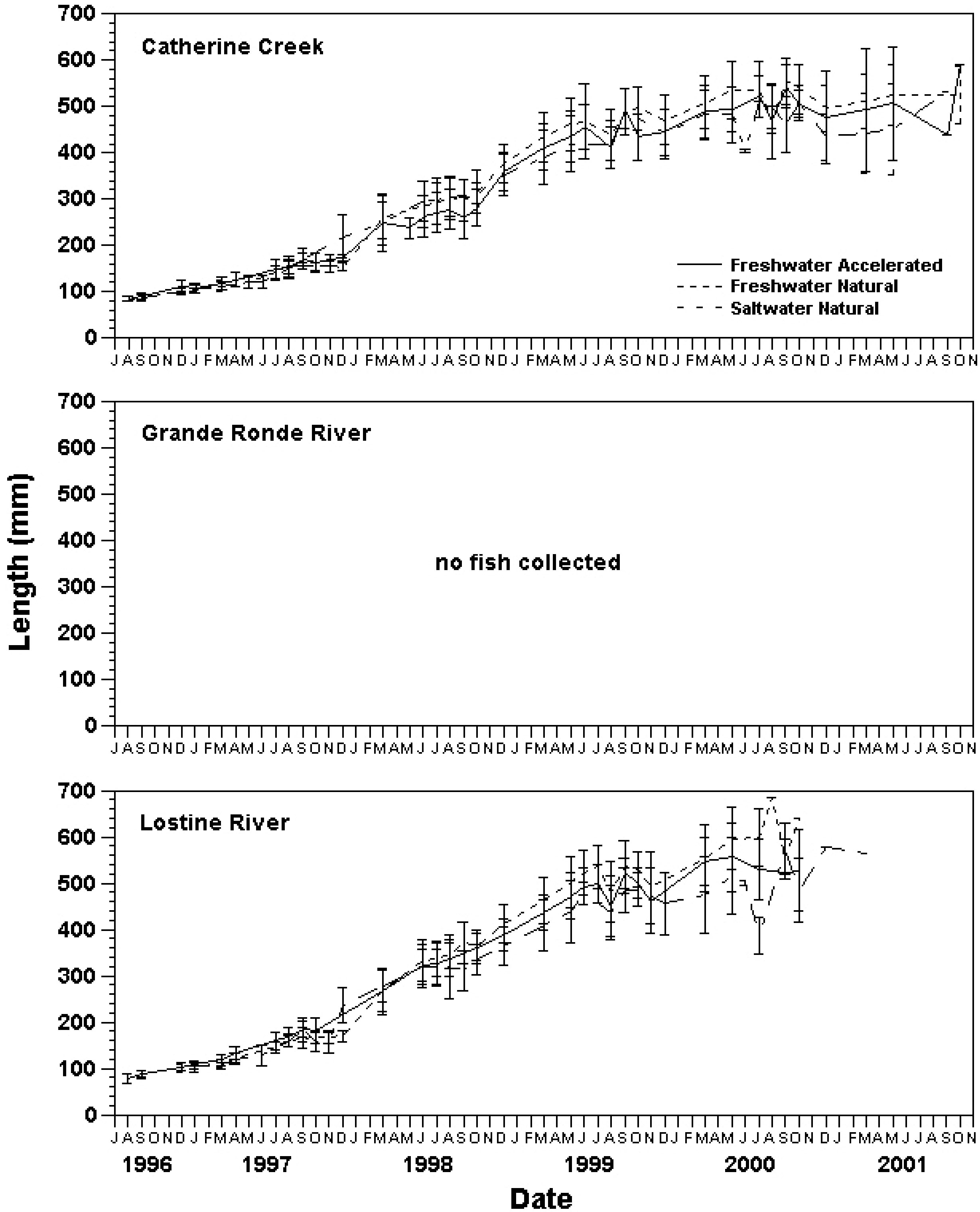


Figure 8. Mean length (±1 SD) of 1995 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1996-2001.

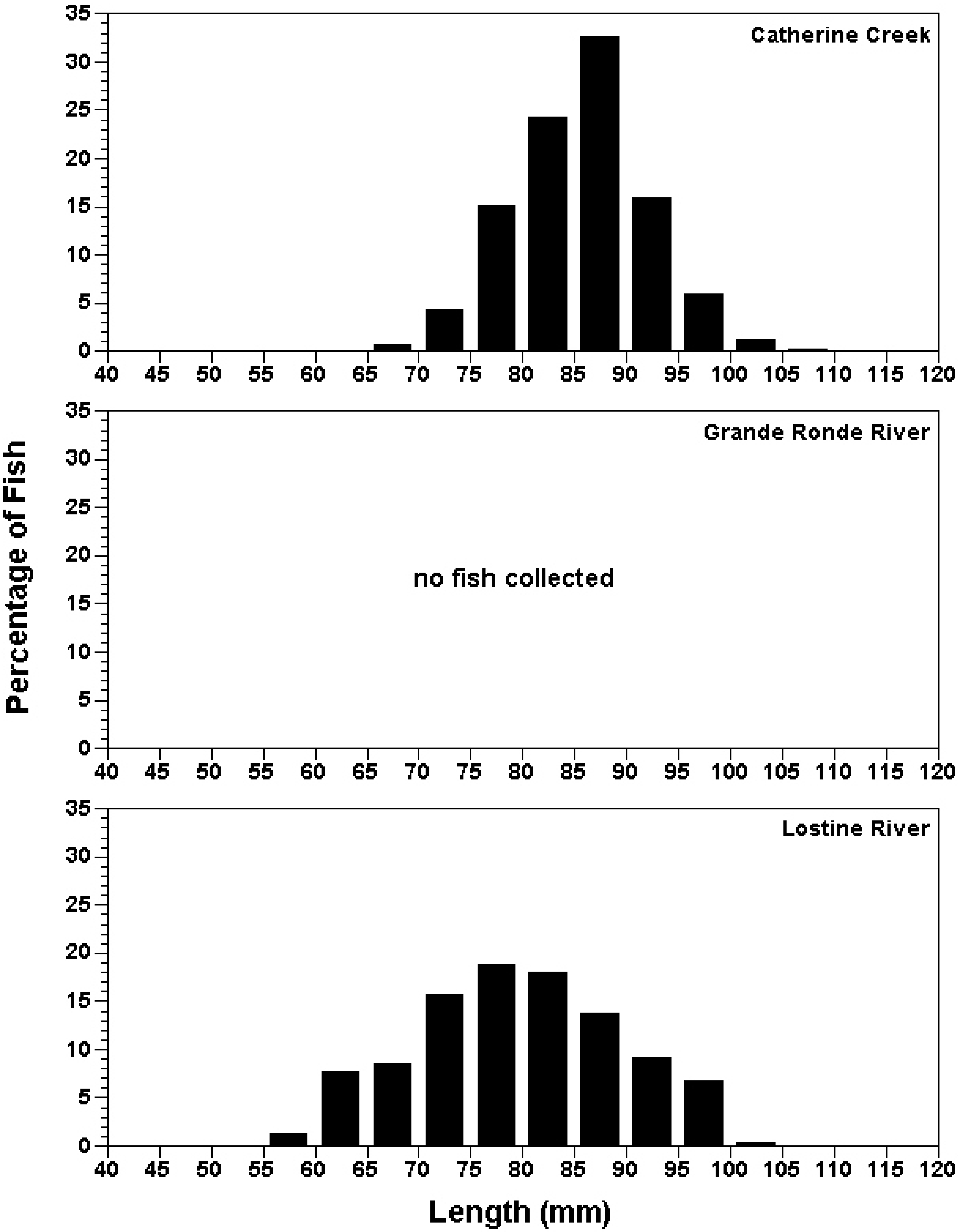


Figure 9. Length frequency distribution for 1995 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1996.

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

The Freshwater Natural Lostine River salmon were consistently larger and the Saltwater Natural fish consistently smaller than the other treatment groups (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.

<u>Mortality</u>

Causes of mortality varied among stocks, 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 (25.4% and 26.5%, respectively) were the largest causes of nonspawning mortality in the Catherine Creek and Lostine River stocks.

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 (41.6%), 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% and 59.0% of the age 4 and 5 fish, respectively, while BKD killed 21.0% and 36.0% of the same fish.

<u>Survival to Spawning and Age of Maturity</u>

A total of 513 of 981 parr collected (52.3%) survived to contribute gametes. Within treatments, 36.4% of the Freshwater Accelerated fish spawned, 74.0% of the Freshwater Natural fish and 45.9% 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

Table 9. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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 1 Catherine Creek mortality which has not yet been examined for cause of death.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
CC	242	48.7	23	4.6	126	25.4	12	2.4	9	1.8	85	17.1
GR	none collected											
LR	271	56.6	24	5.0	127	26.5	10	2.1	9	1.9	38	7.9
Treatment												
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	83	25.5
Age												
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	4	4.0
6	7	77.8	0	0.0	1	11.1	0	0.0	0	0.0	1	11.1
Total	513	52.6	47	4.8	253	25.9	22	2.3	18	1.8	122	12.5

were males from which their semen was cryopreserved. At age 3, 39.6% of the maturing 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 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 were collected from 2-4 September between RK 296-325. Water temperature ranged from 9.0-11.5˚ C. Lostine River parr 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 in any stocks.

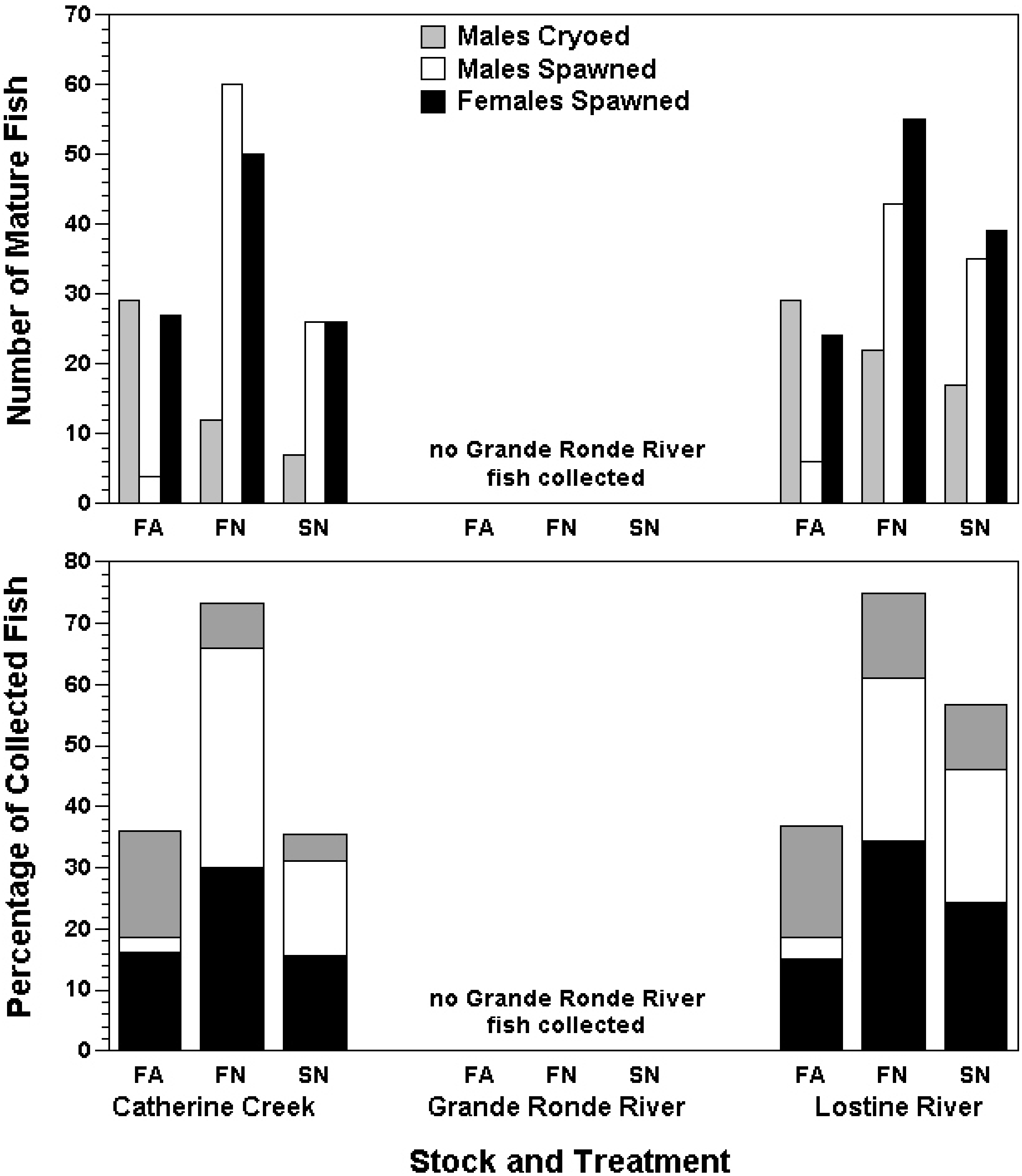


Figure 10. Number and percentage of 1995 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

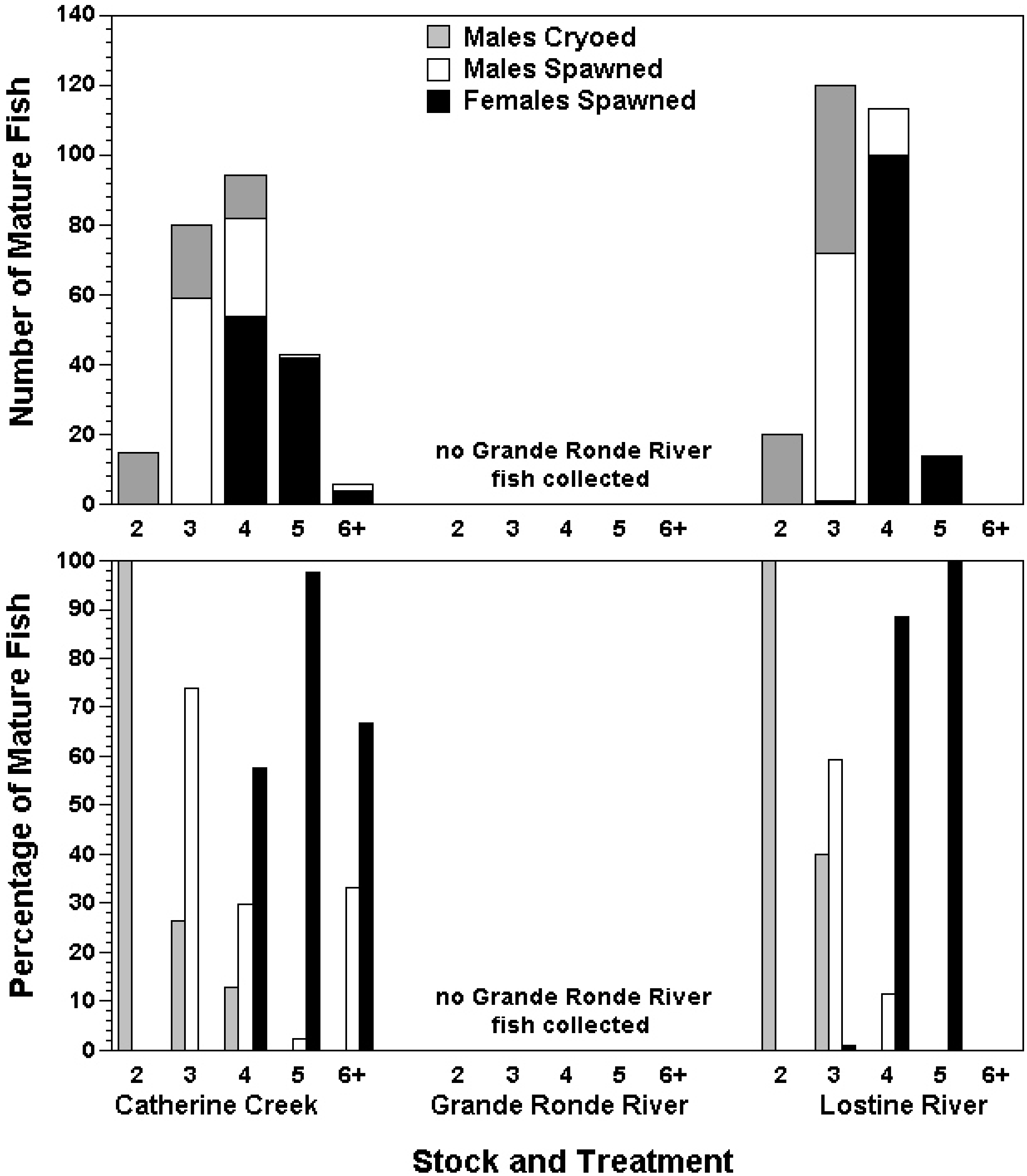


Figure 11. Number and percentage of 1995 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and that matured to spawn or have semen cryopreserved at ages 2-5.

<u>Size at Collection</u>

Size at collection of chinook salmon parr varied with stock (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.

<u>Growth</u>

The fish in the Saltwater Natural groups were consistently smaller than the other treatment groups in the Catherine Creek and Lostine River stocks (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 the fish reared under the Freshwater Accelerated growth regime was 204.9 mm and 123.67 g in 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 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.

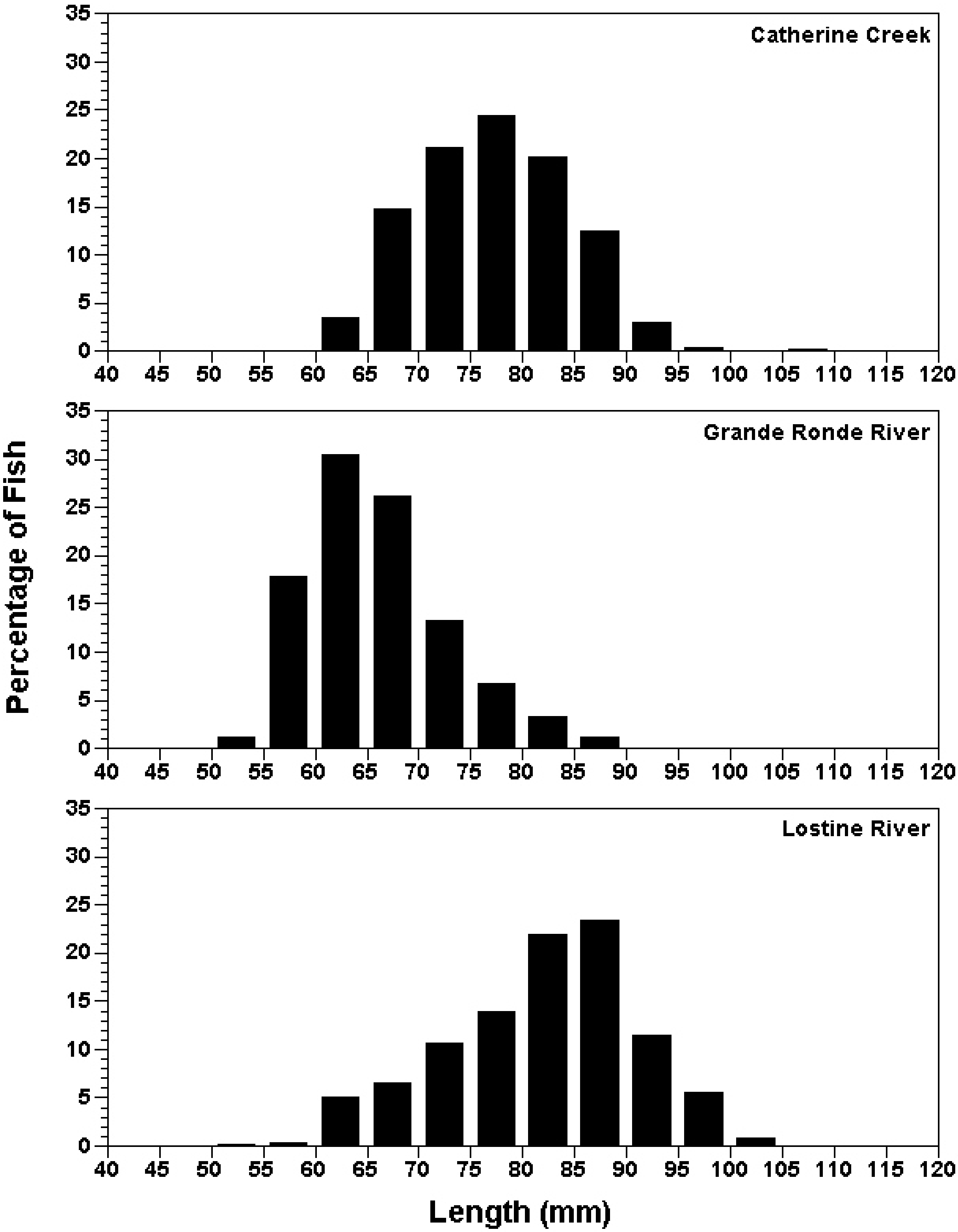


Figure 12. Length frequency distribution for 1996 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1997.

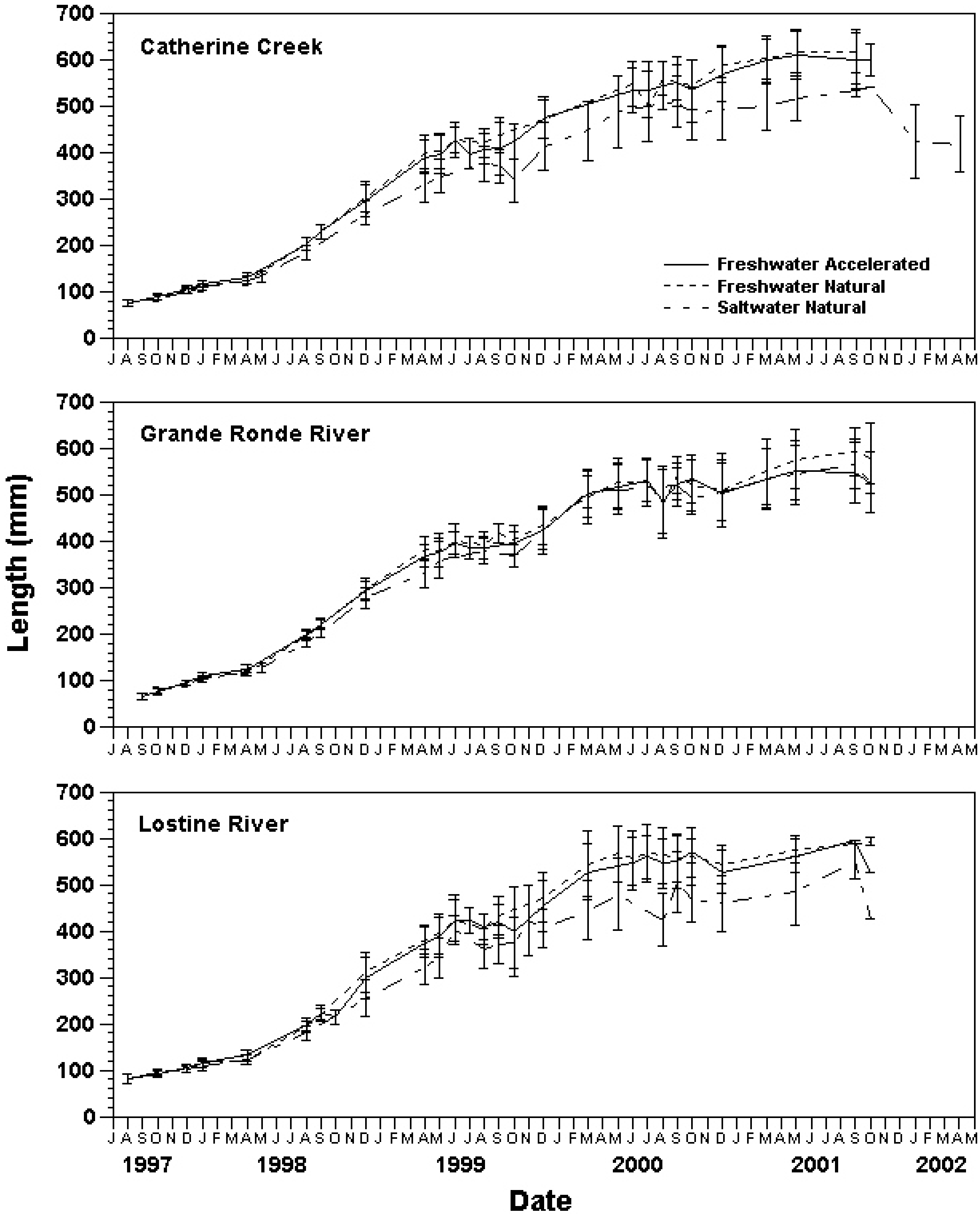


Figure 13. Mean length (±1 SD) of 1996 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1997-2002.

Mortality

All 1996 cohort fish have died, due to maturation and spawning or some other cause. Causes of mortality varied among stocks, treatments and ages (Table 10). Overall, 67.4% of the fish survived to gamete collection. 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% in 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 2-6were 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% 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). At age 2, 5.5% (of the mature fish) matured: 50% were spawned and 50% cryopreserved. At age 3, 40.1% of the maturing 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 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. Grande Ronde River

Table 10. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
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
Treatment												
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	82	16.5
Age												
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	0	0.0
Total	1012	67.6	12	0.8	233	15.6	26	1.7	70	4.7	142	9.5

fish were collected from 8-10 September between RK 296-325. Water temperature ranged from 7.0-13.0˚ C. 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 stocks.

Size at Collection

Size at collection of chinook salmon parr varied with stock (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 similar sized. 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.

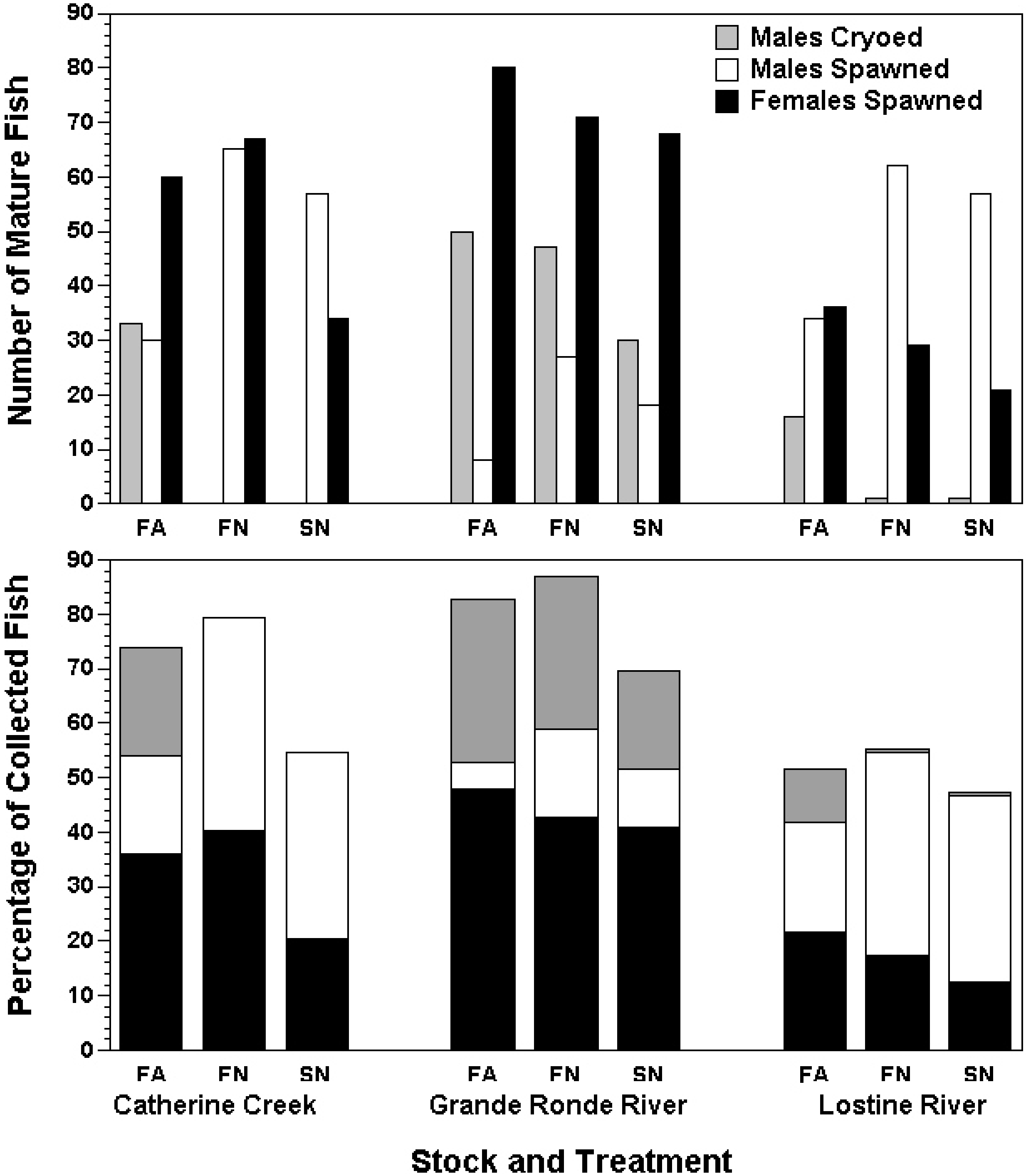


Figure 14. Number and percentage of 1996 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

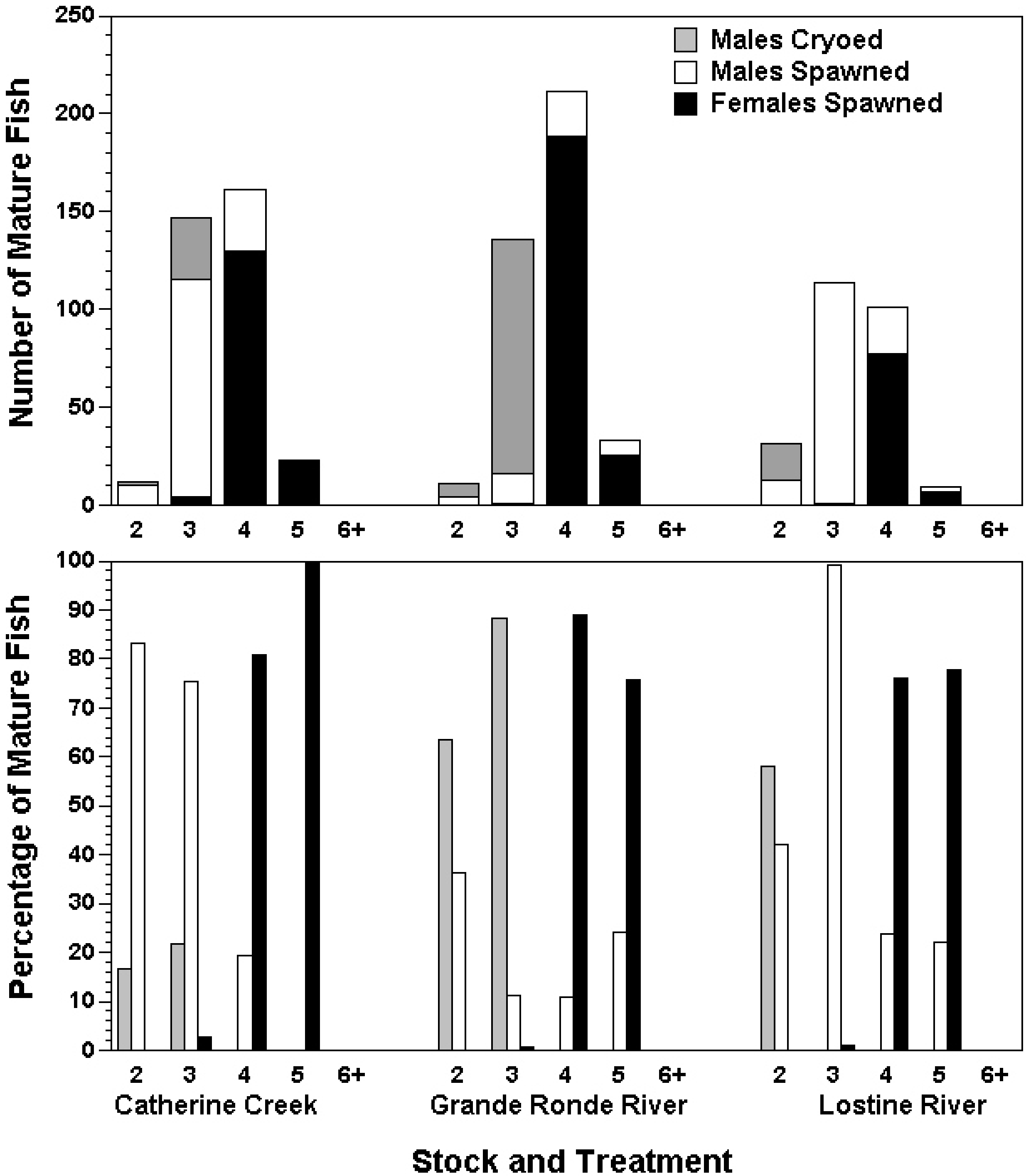


Figure 15. Number and percentage of 1996 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and that matured to spawn or have semen cryopreserved at ages 2-4.

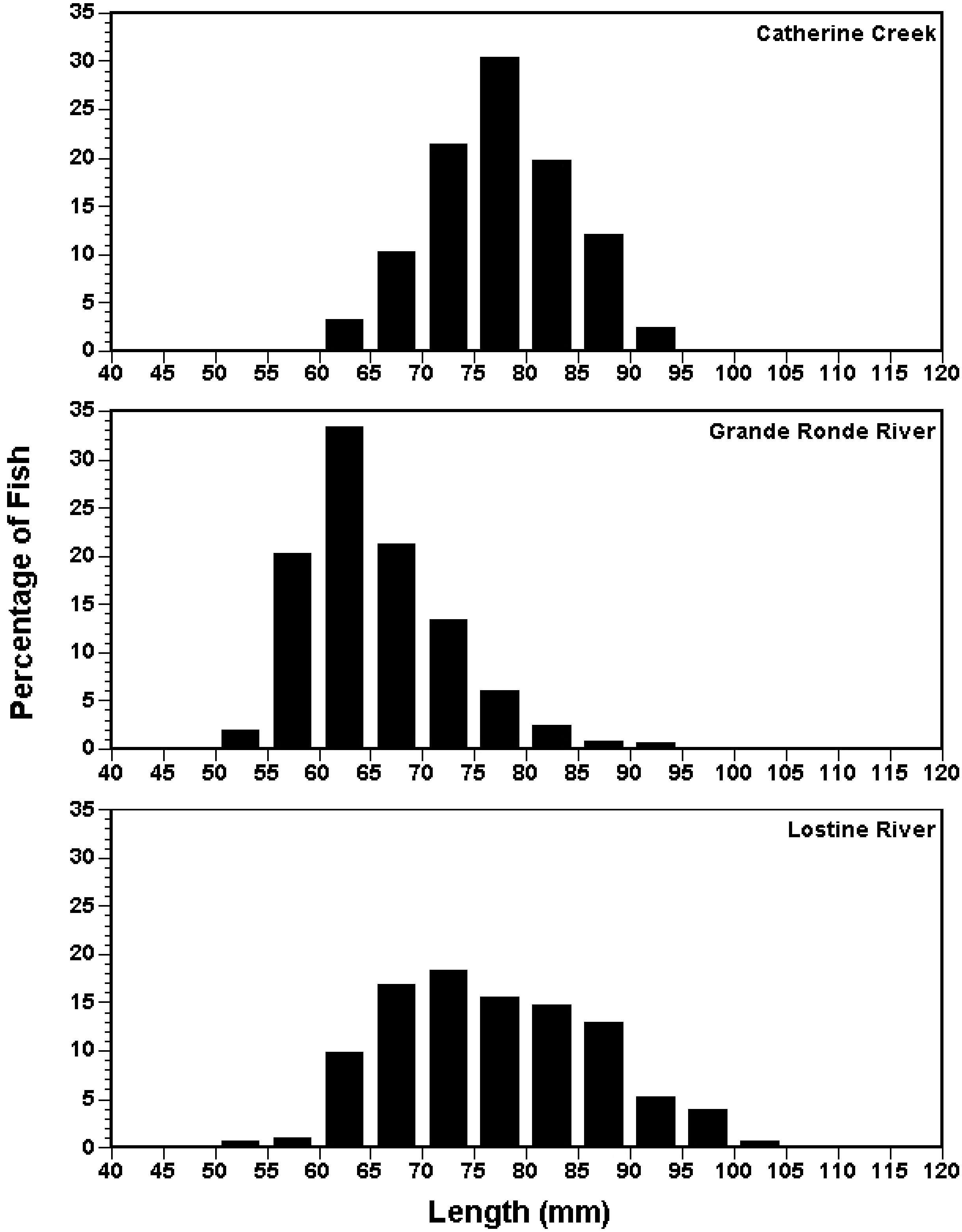


Figure 16. Length frequency distribution for 1997 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1998.

Growth

Growth of all stocks was similar, as was growth of each treatment within each stock (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 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.

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 stocks, treatments and ages (Table 11). Gametes were collected from 72.1% of the fish collected: 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 only 66.8% of the Saltwater Natural mortalities (Table 7). Bacterial kidney disease was the cause of 19.6% of the Saltwater Natural mortalities, 16.9% of the Freshwater Accelerated and 15.1% of the

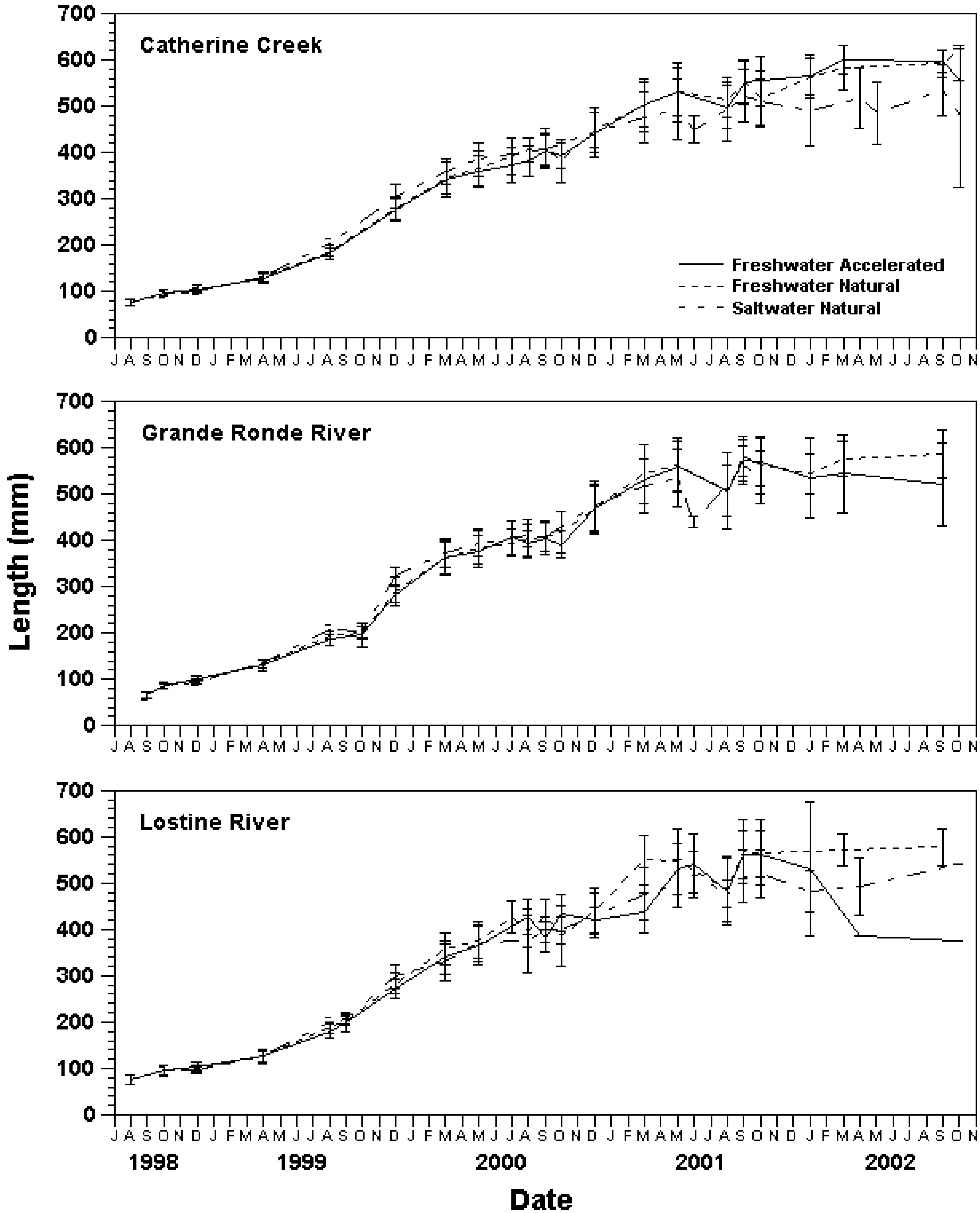


Figure 17. Mean length (±1 SD) of 1997 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1998-2002.

Table 11. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
CC	323	64.9	12	2.4	124	24.9	8	1.6	6	1.2	25	5.0
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
Treatment												
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	42	8.6
Age												
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	39	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	0	0.0
Total	1082	72.4	23	1.5	260	17.4	31	2.1	17	1.1	77	5.2

Freshwater Natural mortalities. 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.

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 moralities, 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 spawned, 75.4% of the Freshwater Natural fish have spawned and 65.6% of the Saltwater Natural fish have spawned (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 mature fish, 10.7% matured at age 2 and all were males: 60.7% were spawned and 39.3% were 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

<u>Collections</u>

The 1998 cohort was collected from Catherine Creek from 16-18 August 1999 between river kilometers (RK) 29.8-52.0. There were six collection-related mortalities. Grande Ronde River fish were collected from 30 August-1 September between RK 296-325. Lostine River parr 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.

<u>Size at Collection</u>

Size at collection of chinook salmon parr varied with stock (Figure 20). All stocks 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.

<u>Growth</u>

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 stocks (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 9713.81 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).

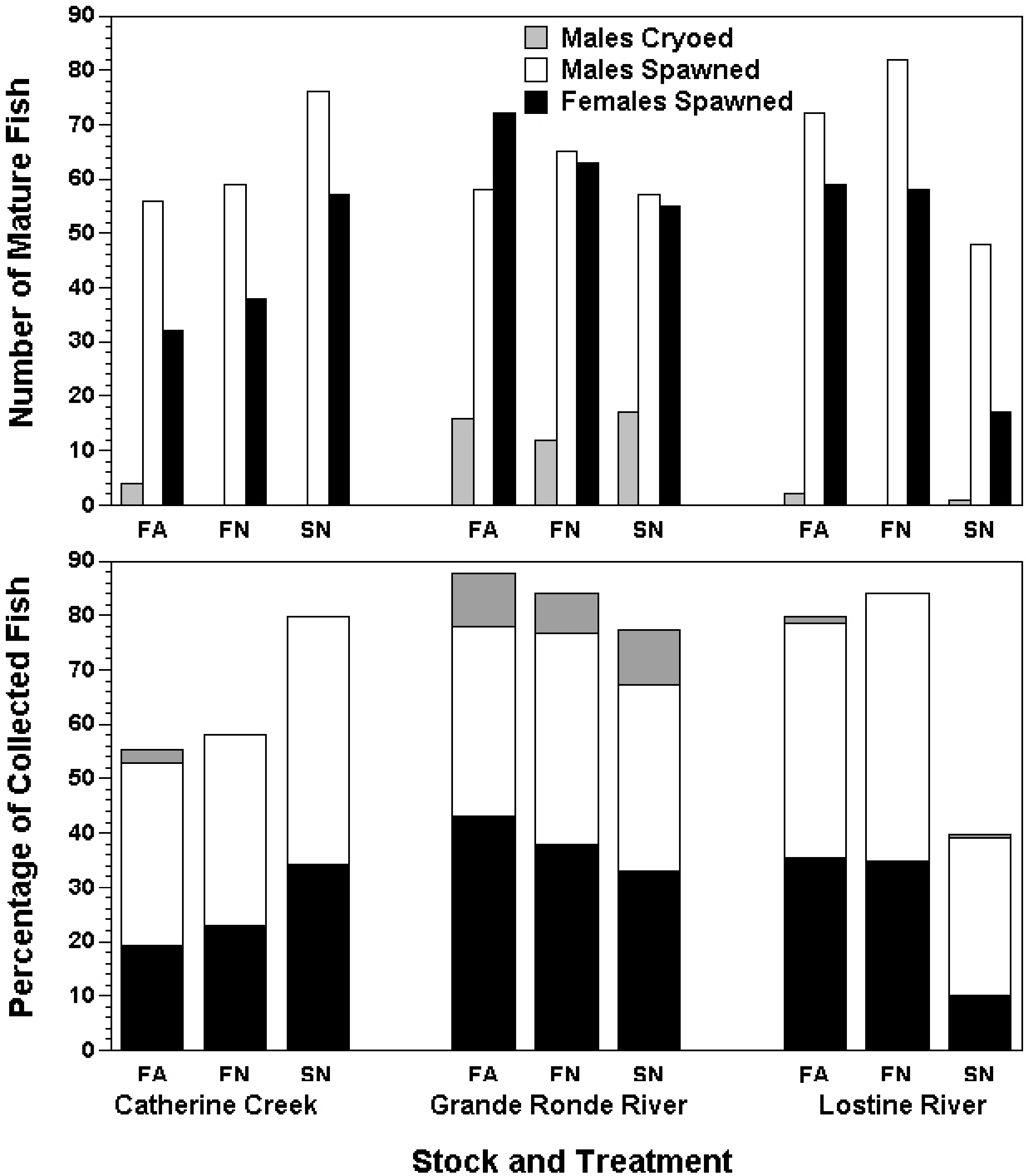


Figure 18. Number and percentage of 1997 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

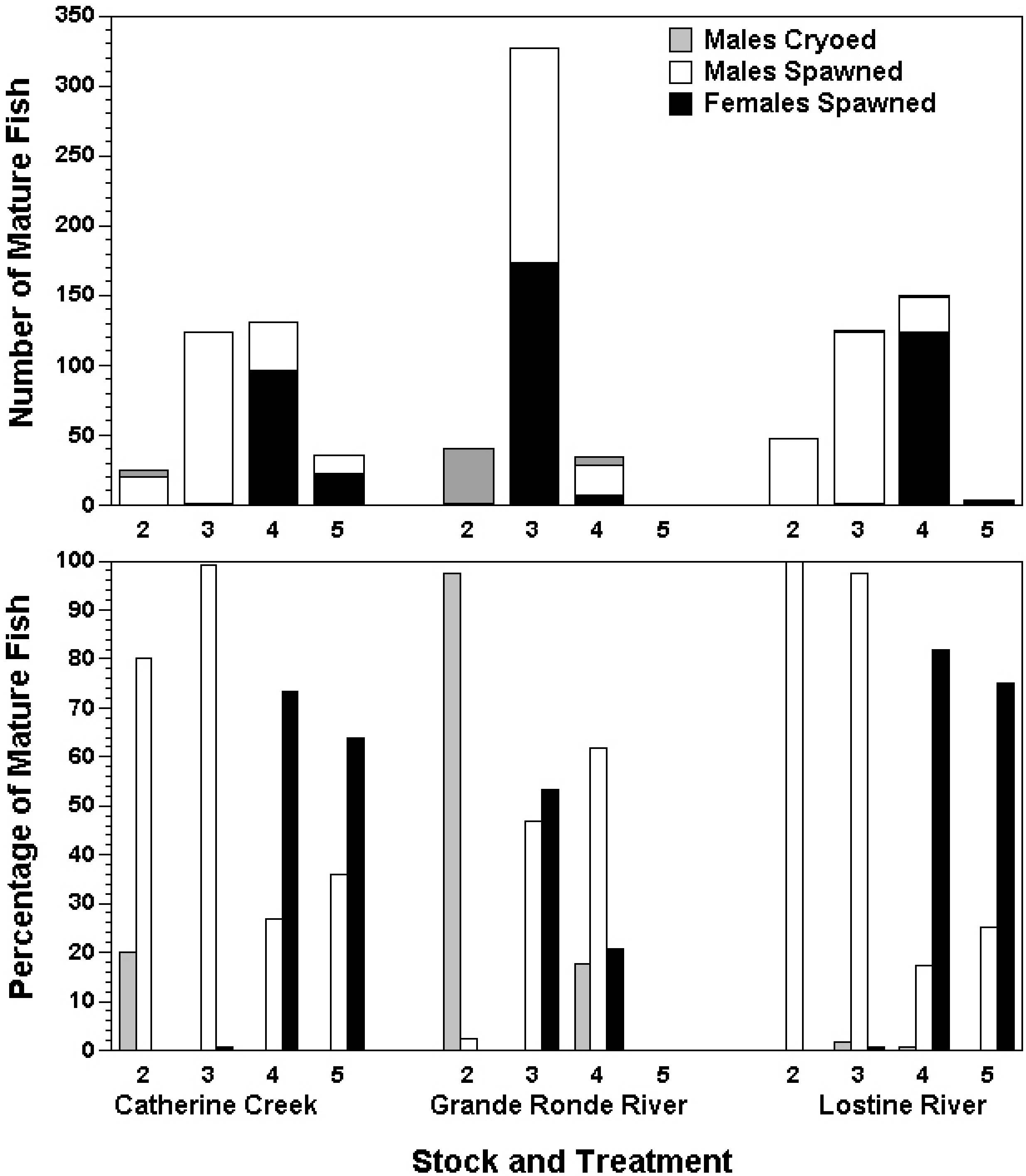


Figure 19. Number and percentage of 1997 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and that matured to spawn or have semen cryopreserved at ages 2-3.

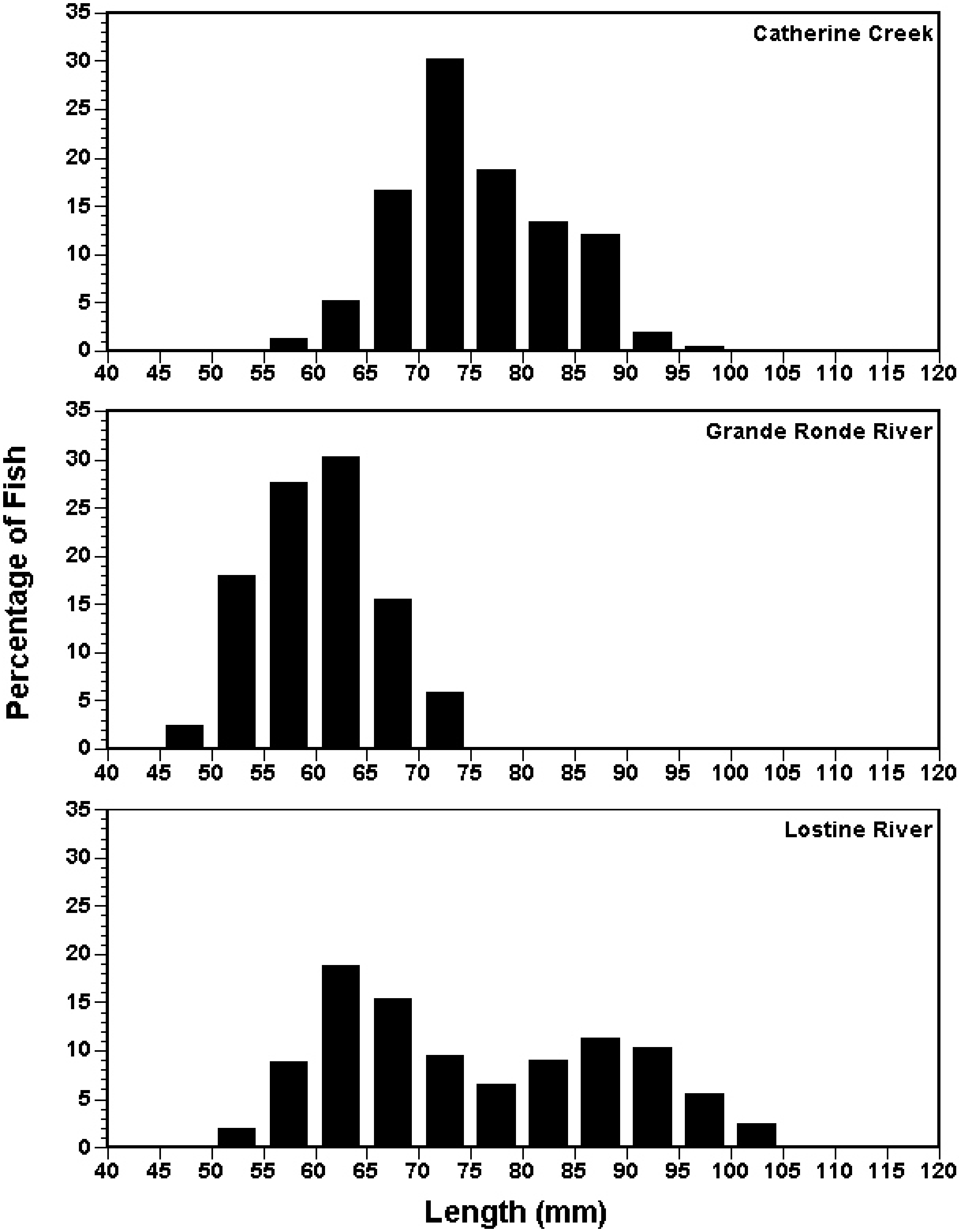


Figure 20. Length frequency distribution for 1998 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 1999.

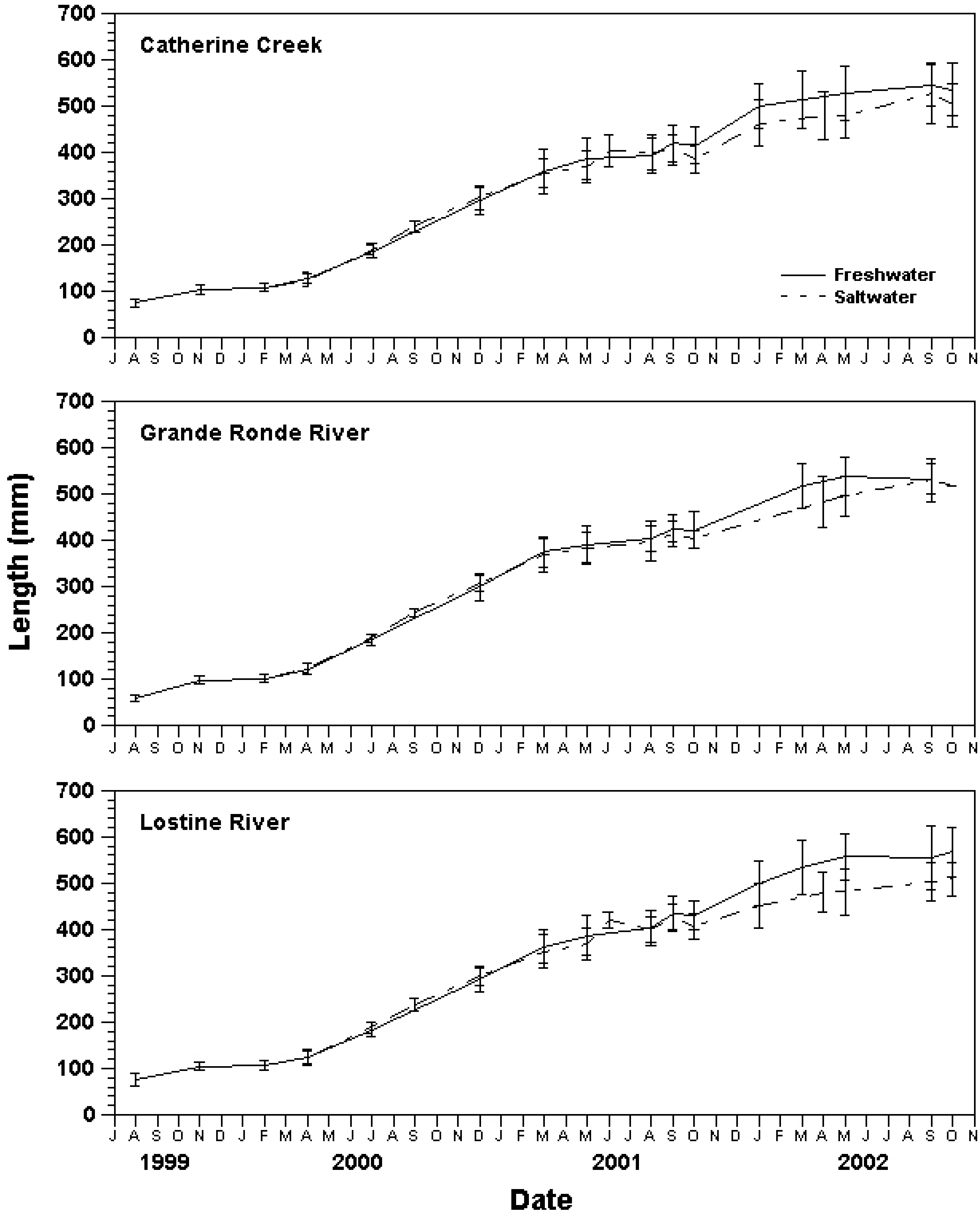


Figure 21. Mean length (±1 SD) of 1998 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 1999-2002.

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 stocks, treatments and ages and 56 mortalities (23 CC; 22 GR; 11 LR) fish 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 stocks, 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 stock and cohort. Another 14.1% died from unknown causes, possibly related to this BKD outbreak In the Lostine River stock, additional sources of mortality were more evenly distributed between BKD (8.7%), unknown causes (4.1%), 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%) 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.

Table 8. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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 11 Lostine River mortalities which have not yet been examined for cause of death.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
CC	325	69.6	11	2.4	63	13.5	24	5.1	6	1.3	15	3.2
GR	221	45.3	4	0.8	165	33.8	9	1.8	20	4.1	47	9.6
LR	368	78.5	7	1.5	41	8.7	16	3.4	18	3.8	8	1.7
Treatment												
FW	626	66.3	8	0.8	201	21.3	45	4.8	27	2.9	21	2.2
SW	288	61.7	7	1.5	67	14.3	4	0.9	17	3.6	44	9.4
Age												
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	0	0.0
Total	914	64.2	22	1.5	269	18.9	49	3.4	44	3.1	70	4.9

1999 Cohort

Collections

The 1999 cohort was collected from Catherine Creek from 14-17 August 2000 between river kilometers (RK) 29.8-52.0. There were three collection-related mortalities. Grande Ronde River fish were collected from 7-10 August between RK 286-325. 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 Grande Ronde or Lostine river stocks.

Size at Collection

All chinook salmon parr were collected in August and size at collection varied with stock (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. We were unable to capture any Grande Ronde River parr in 2000, so there will not be 1999 cohort Grande Ronde River fish in the Captive Broodstock Program.

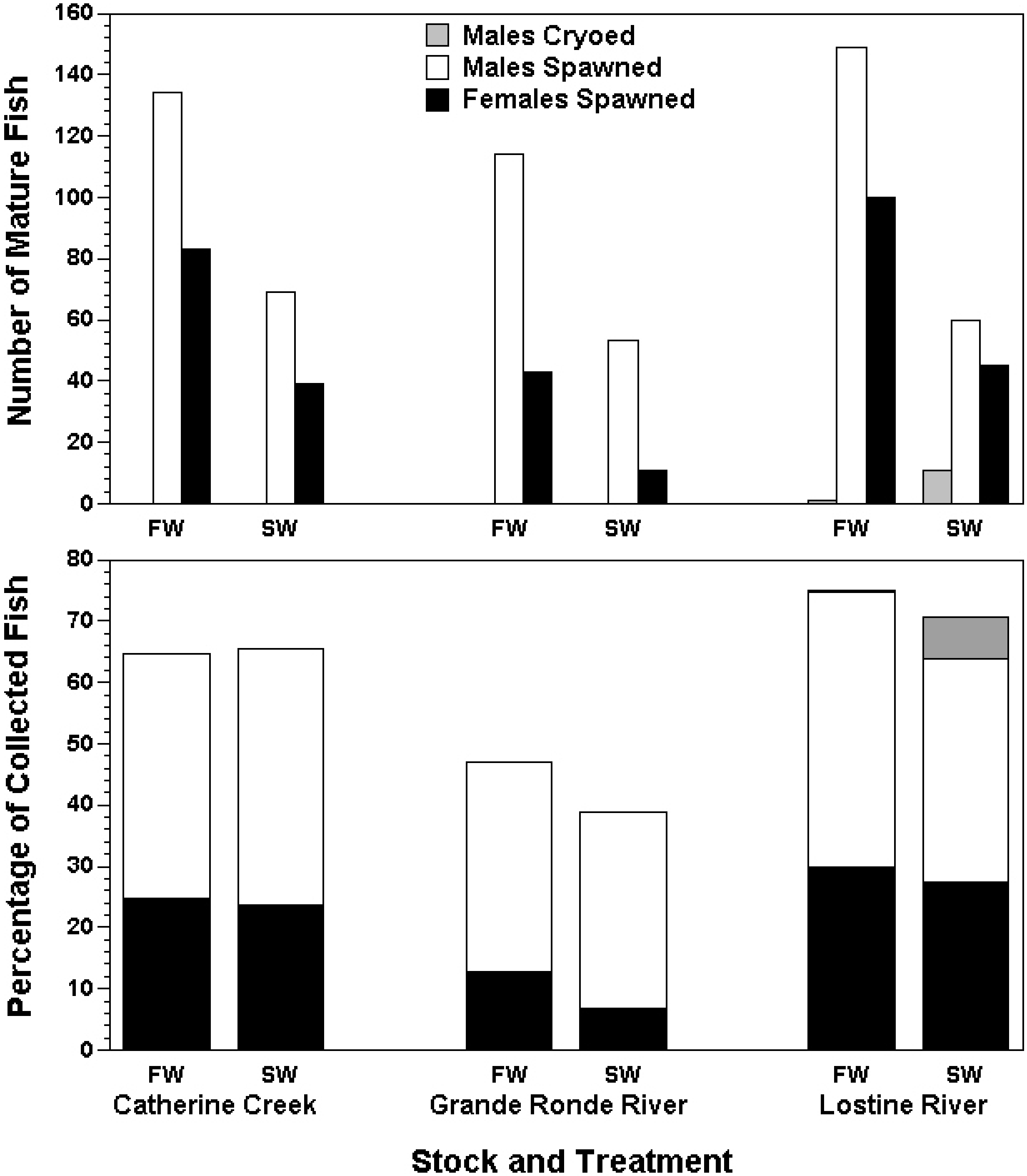


Figure 22. Number and percentage of 1998 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

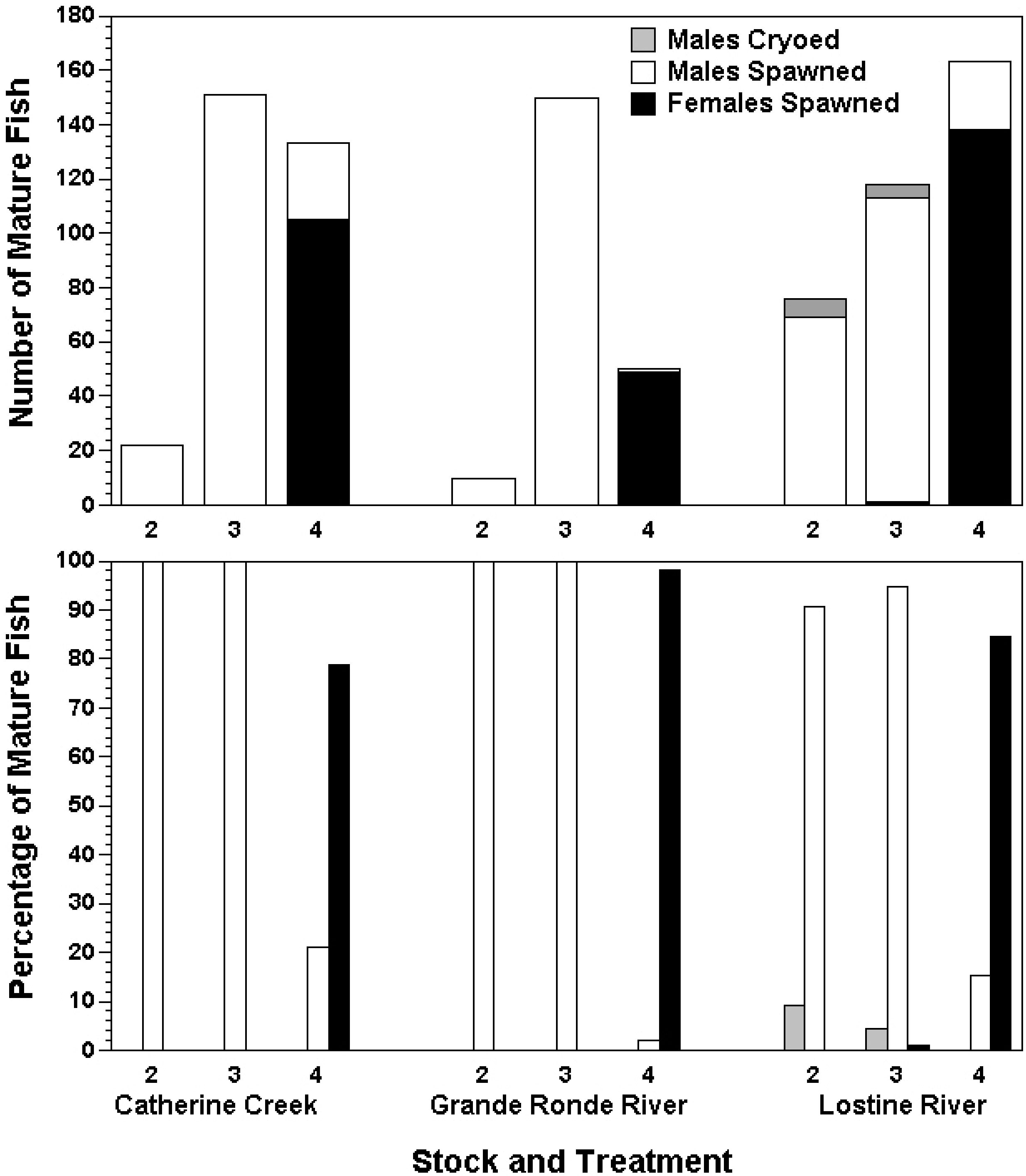


Figure 23. Number and percentage of 1998 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and that matured to spawn or have semen cryopreserved at age 2.

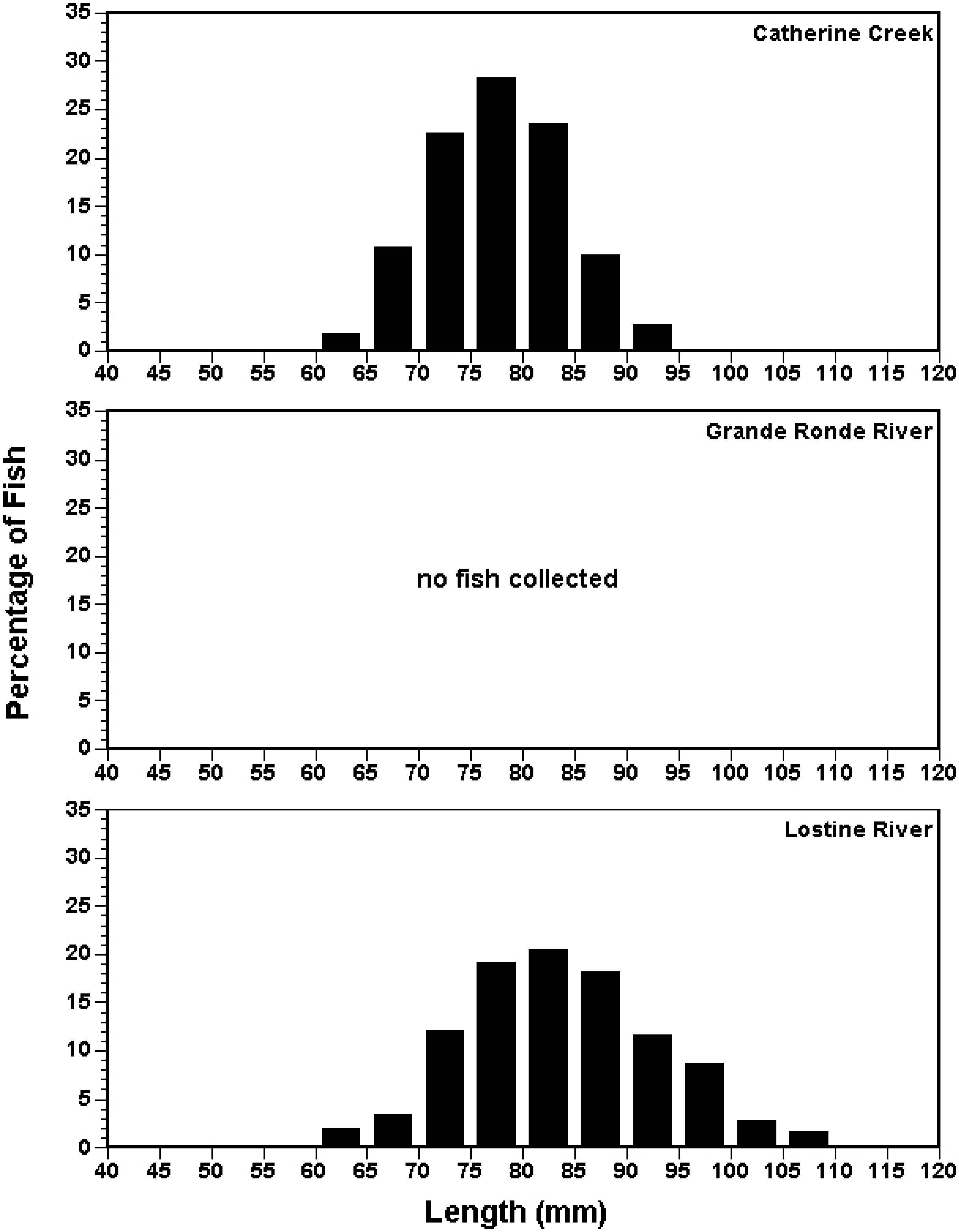


Figure 24. Length frequency distribution for 1999 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 2000.

<u>Growth</u>

The 1999 cohort was the first to completely conform to the three treatments of this study. The problems with the water/chilling system 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 salmon varied between the accelerated and natural growth 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 growth 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 they 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 growth regime 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 growth regime 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 Accelerated group grew faster than the natural growth groups (Figure 25). Following transfer to saltwater, the Saltwater Natural group slowed it's growth, while size of the two freshwater groups were nearly identical. Mean fork length and weight of the Freshwater Accelerated growth 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 growth regime 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 growth regime 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.

<u>Mortality</u>

Approximately half of the 1999 cohort fish have died, leaving 252 Catherine Creek and 255 Lostine River reamining. Causes of mortality varied among stocks, treatments and ages (Table 13). Gamete collection was 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

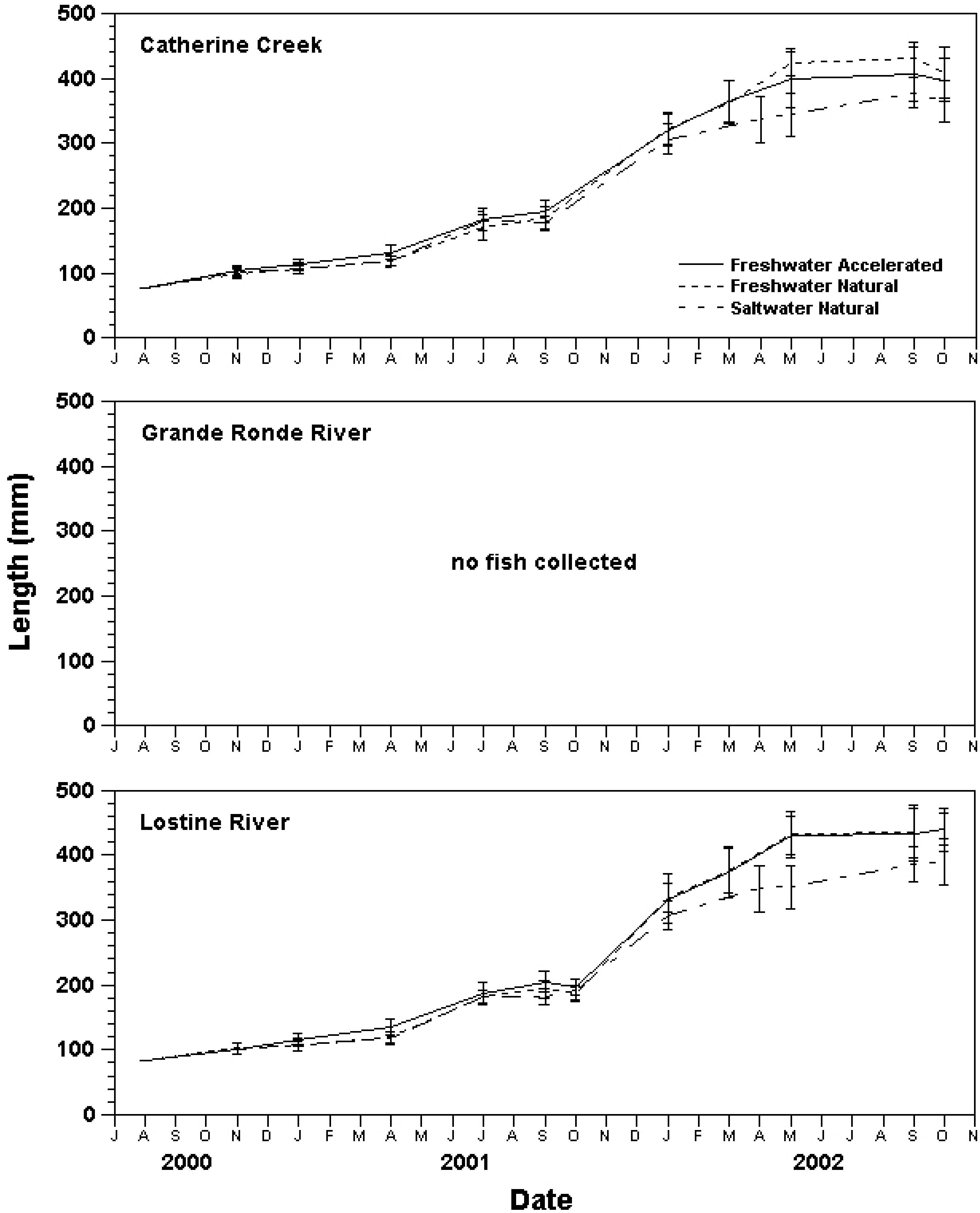


Figure 25. Mean length (±1 SD) of 1999 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural and Saltwater Natural growth regimes, 2000-2002.

Table 13. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
CC	113	45.0	45	17.9	8	3.2	61	24.3	5	2.0	7	2.8
GR						none collected						
LR	172	70.2	7	2.9	7	2.9	32	13.1	13	5.3	7	2.9
Treatment												
FA	102	60.4	28	16.6	3	1.8	24	14.2	6	3.6	4	2.4
FN	90	58.4	23	14.9	5	3.2	25	16.2	4	2.6	5	3.2
SN	91	53.8	1	0.6	7	4.1	43	25.4	8	4.7	5	3.0
Age												
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	0	0.0
Total	285	57.5	52	10.5	15	3.0	93	18.8	18	3.6	14	2.8

Lostine River stock, 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%) causes was 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

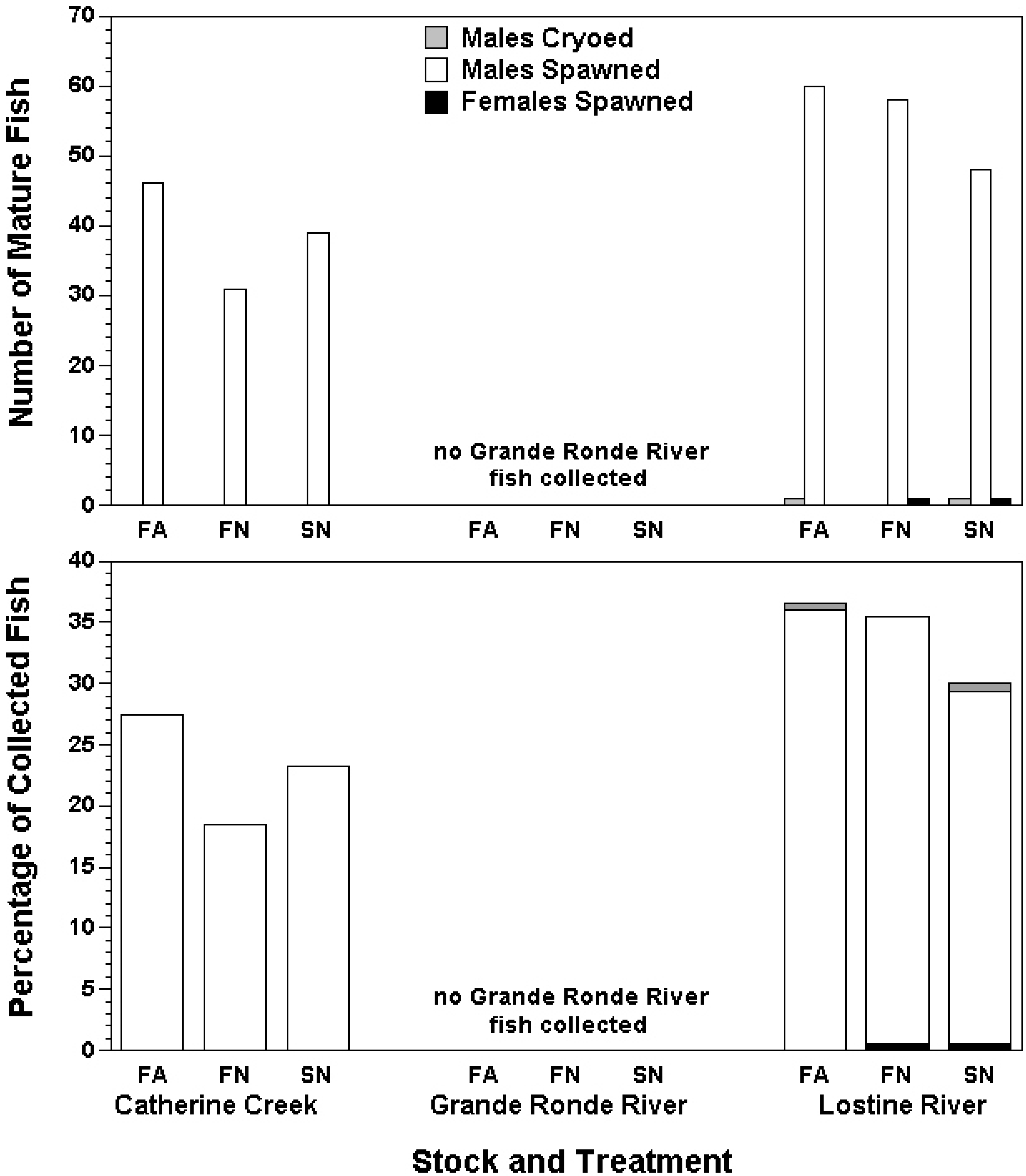


Figure 26. Number and percentage of 1999 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) growth regimes that matured to spawn or have semen cryopreserved.

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.

2000 Cohort

<u>Collections</u>

The 2000 cohort was collected from Catherine Creek from 6-9 August 2001 between river kilometers (RK) 32.2-52.0. Water temperature ranged from 12.0-17.0˚ C. Grande Ronde River fish 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 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 parr but not in the Catherine Creek or Grande Ronde River stocks.

<u>Size at Collection</u>

Size at collection varied with stock (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.

<u>Growth</u>

The 2000 cohort was divided into four treatment groups; two pre-smolt treatments and two post-smolt treatments. One half of the fish from each stock is being 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. The Saltwater Natural and Saltwater Accelerated groups will be reared to maturity at Manchester Marine Lab while the Freshwater Natural and Freshwater Accelerated groups will be reared Bonneville Fish Hatchery. These fish have been in captivity for 17 months and all stocks are showing similar growth patterns (Figure 29). The accelerated pre-smolt groups grew faster to smoltification but the natural growth groups grew faster following smoltification, resulting in similar-sized fish 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

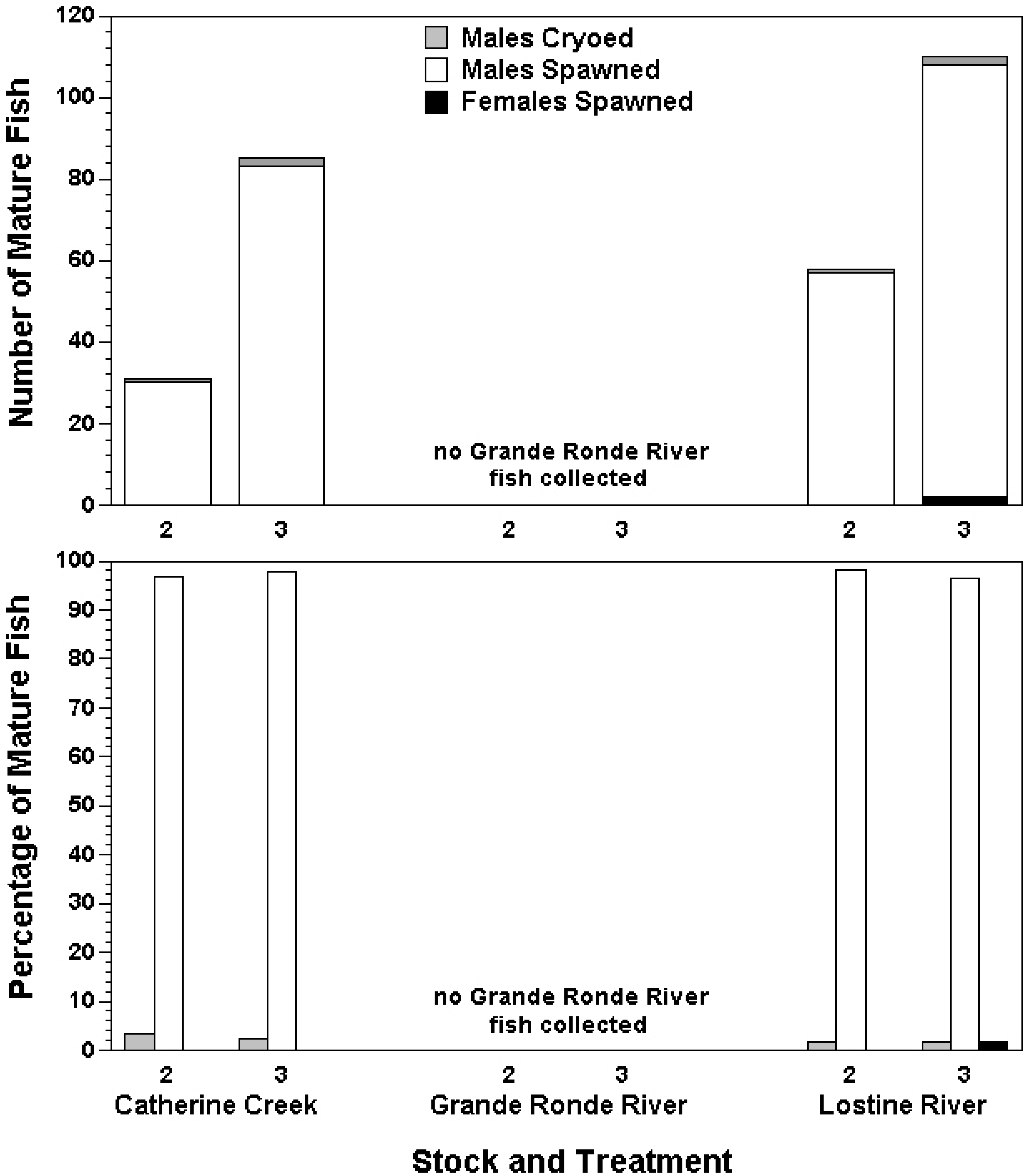


Figure 27. Number and percentage of 1999 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and that matured to spawn or have semen cryopreserved at age 2.

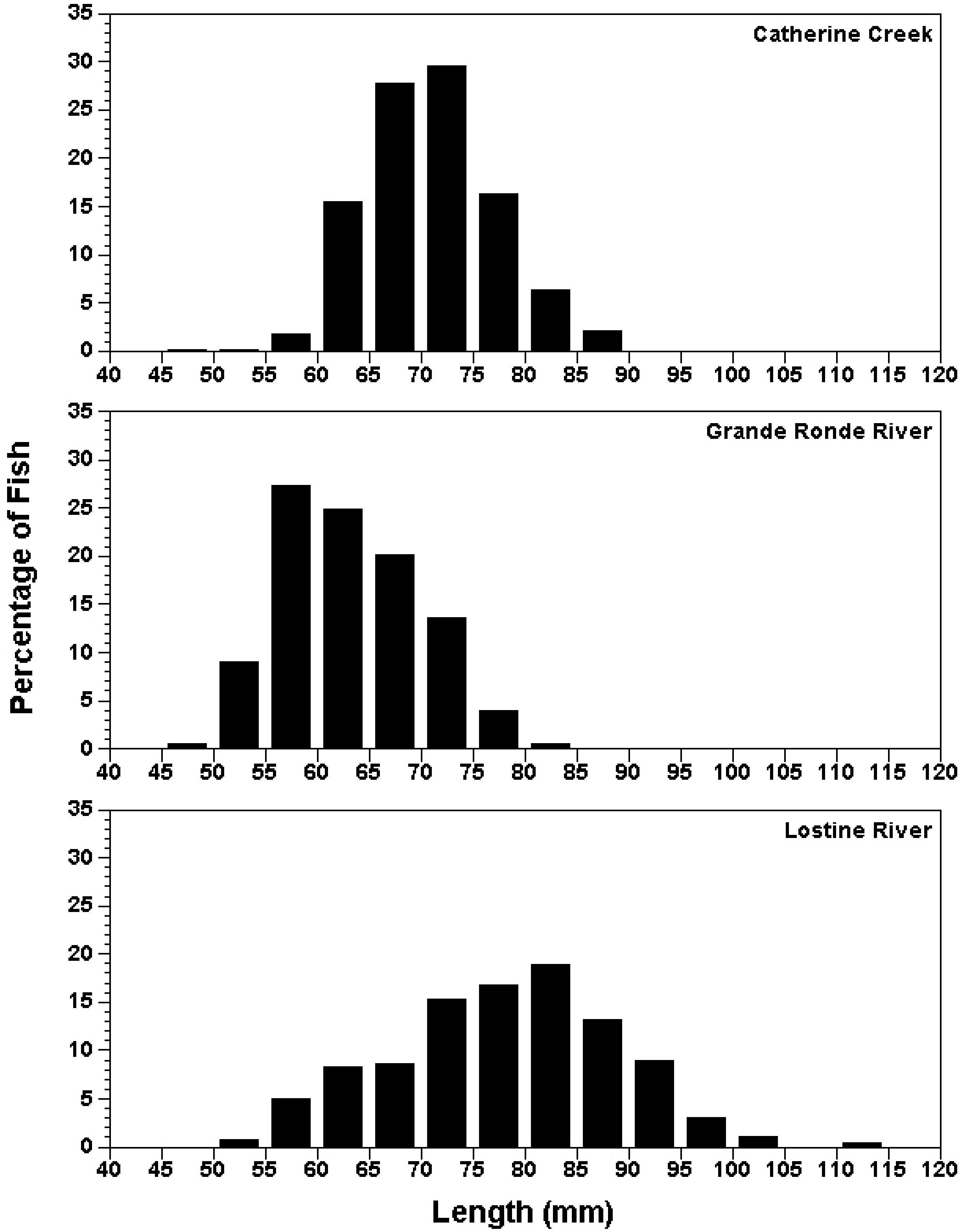


Figure 28. Length frequency distribution for 2000 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 2001.

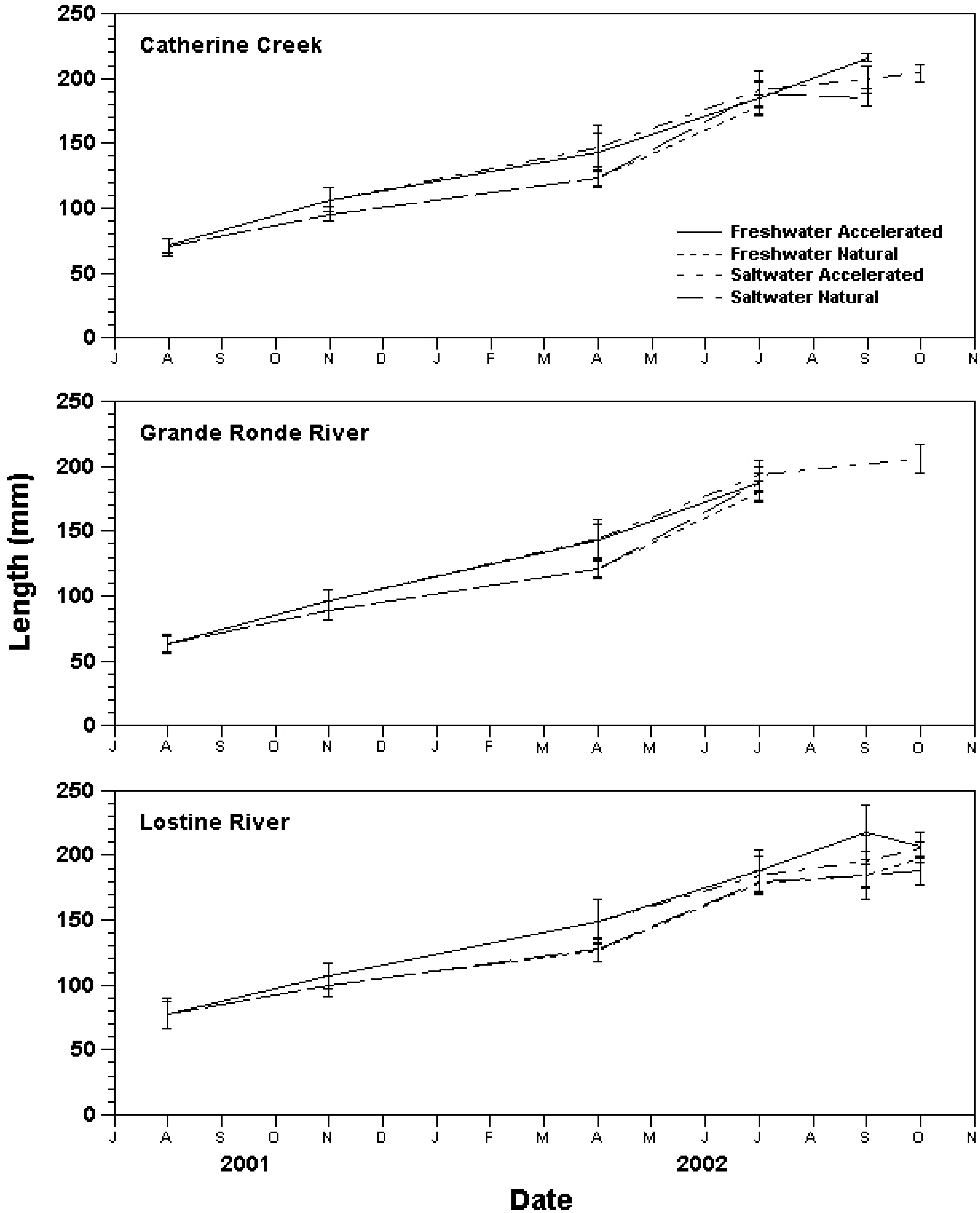


Figure 29. Mean length (±1 SD) of 2000 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Freshwater Accelerated, Freshwater Natural, Saltwater Accelerated and Saltwater Natural growth regimes, 2001-2002.

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 and 11.97 g in November. They were substantially larger than the Natural growth group, at 142.5 mm and 36.23 g, in April 2002. However, by July 2002 (approximately two months following transfer) the Natural growth groups had caught up with the Accelerated fish: 180.5 mm and 77.15 g and 186.7 mm and 86.67 g for the Freshwater Natural and Freshwater Accelerated fish, respectively, and 187.5 mm and 87.65 g and 192.8 mm and 95.34 g for the 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 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.

<u>Mortality</u>

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 comprised 38.6%, 15.4% and 36.8% of the Catherine Creek, Grande Ronde River and Lostine River stock mortality, respectively. Non-spawning mortality in the Catherine Creek was caused mostly by unknown causes (43.2%).

Table 14. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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 27 Lostine River mortalities which have not yet been examined for cause of death.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
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	33	28.9
Treatment												
FA	21	50.0	0	0.0	4	9.5	1	2.4	2	4.8	14	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
Age												
1	0	0.0	13	43.3	2	6.7	5	16.7	4	13.3	6	20.0
2	65	64.4	0	0.0	16	15.8	5	5.0	13	12.9	2	2.0
Total	65	33.0	13	6.6	18	9.1	10	5.1	17	8.6	8	4.1

Grande Ronde River fish died mostly from unknown (56.4%) and operational (12.8%) causes. stocks, with only 10 and 11 fish, respectively, having died. In Lostine River fish, unknown causes caused 28.9% and BKD caused 14.0% of the mortalities. Note that "unknown" mortalities include 17 Catherine Creek, 22 Grande Ronde River and 27 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 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% were cryopreserved.

2001 Cohort

Collections

The 2001 cohort was collected from Catherine Creek (n=500) 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 (n=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. The Lostine River parr (n=500) 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 stock.

Size at Collection

Size at collection varied with stock (Figure 30). Lostine River parr had a mean fork length of 70.5 mm and weight of 4.54 g. Catherine Creek parr had a mean fork length of 70.9 mm and weight of 4.38 g. Grande Ronde River parr were the smallest, at 67.9 mm and 3.78 g.

Growth

The 2001 cohort will be divided into four treatment groups; two pre-smolt treatments and two post-smolt treatments. One half of the fish from each stock 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 stocks (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.

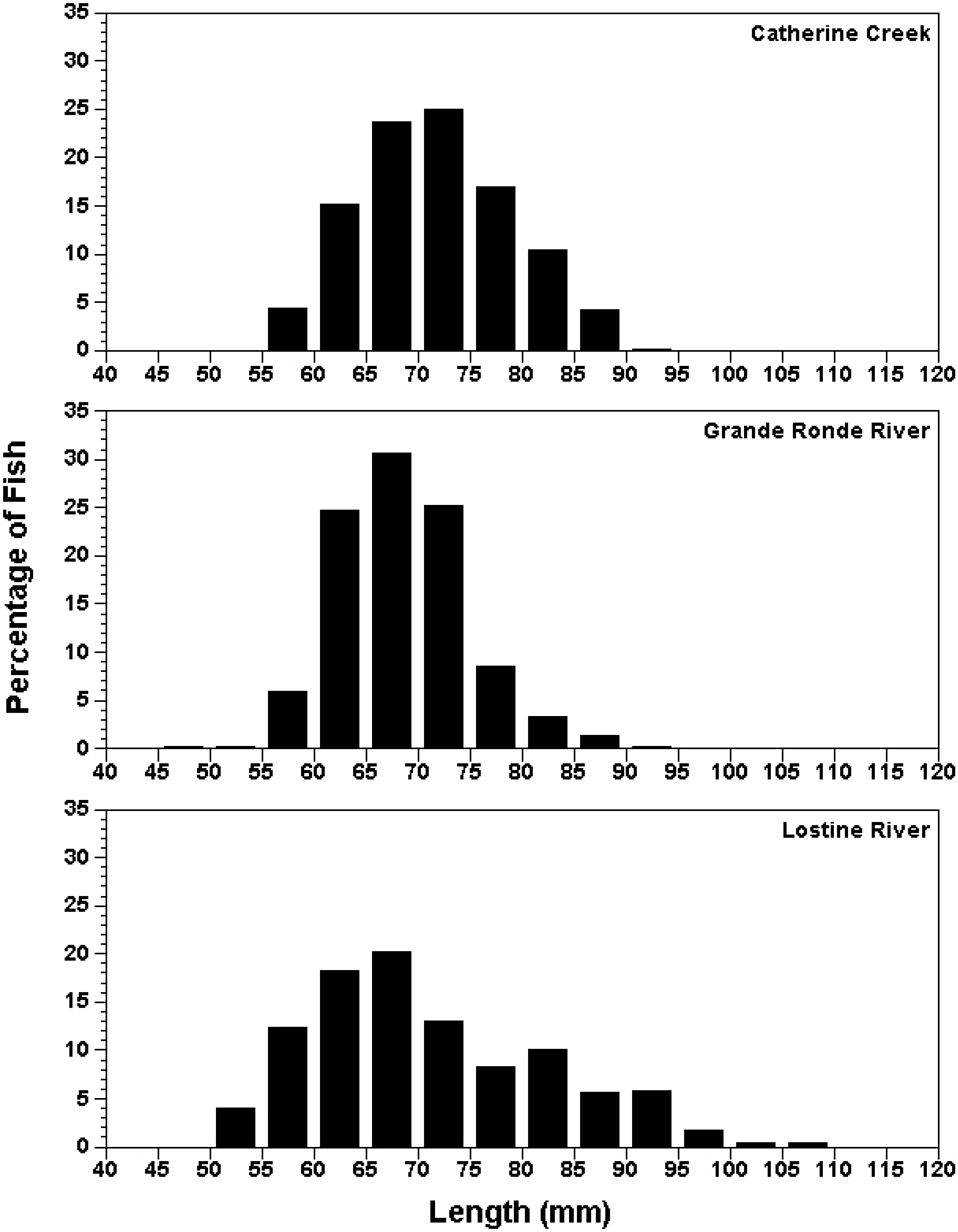


Figure 30. Length frequency distribution for 2001 cohort spring chinook salmon parr collected for captive broodstock from Catherine Creek, Grande Ronde River and Lostine River, 2002.

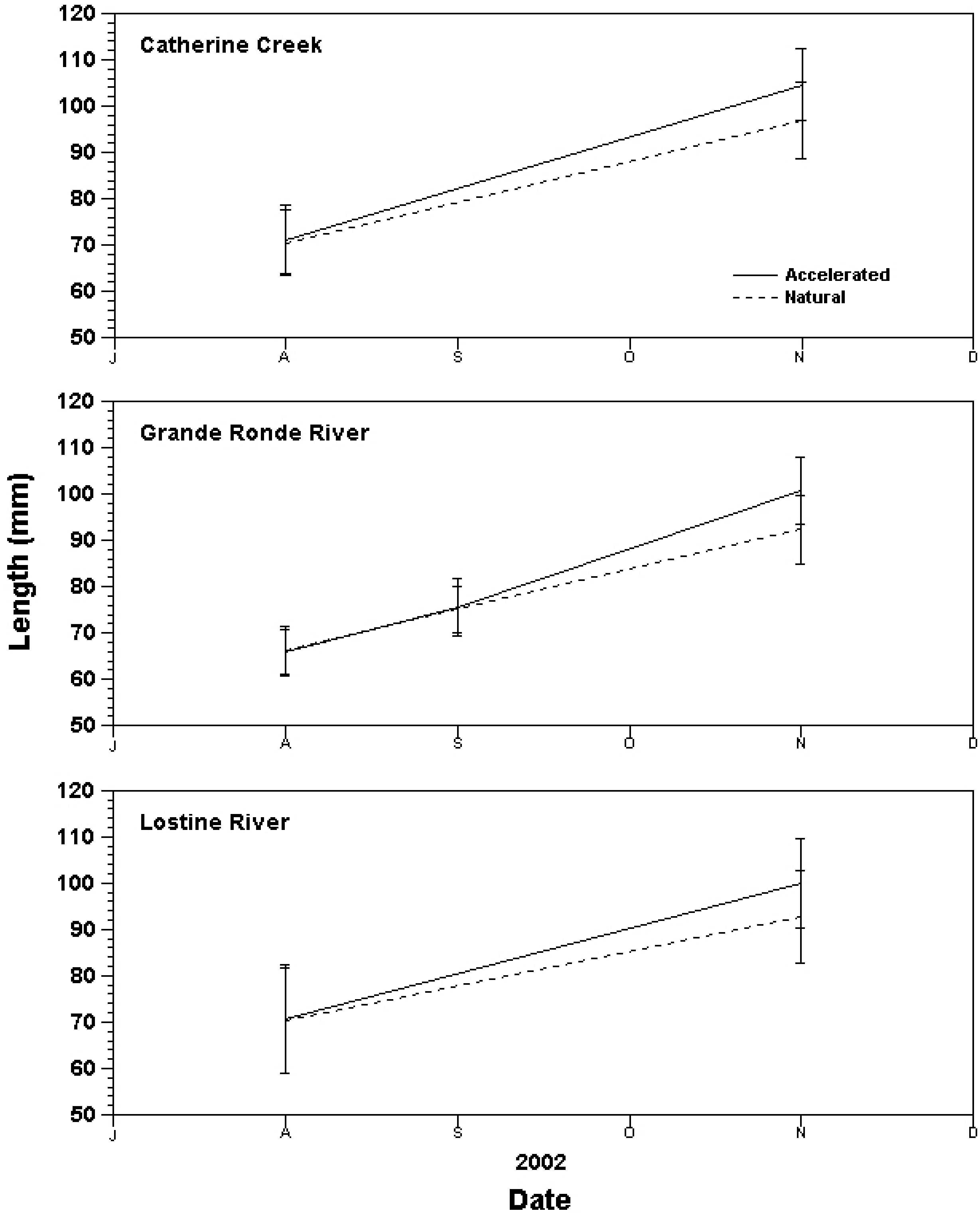


Figure 31. Mean length (±1 SD) of 2001 cohort spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River raised under Accelerated and Natural pre-smolt growth regimes, 2002.

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 14). 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 14). Five natural growth fish died from other diseases, two from unknown causes and one from operational causes.

Spawning

1998

Number of Spawners

A total of 318 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 3-5 and males matured at an earlier age than females (Figure 32). Of the males, 13.5% were age 3, 65.5% were age 4 and 21.0% were age 5. Of the females, 8.4% were age 3, 41.3% age 4 and 50.3% age 5.

Fecundity

Fecundity varied among treatments and age classes of spawning females. Mean fecundity of Freshwater Natural females was 1400.6 eggs, ranging from 141-2318 eggs (Figure 33). Mean Fecundity of Saltwater Natural females was 1313.7 eggs (range: 57-2341 eggs).

Within age classes, fecundity was measured for only one 4-year old female: 1111 eggs (Figure 25). Five-year old females had a mean fecundity of 1354.9 eggs (range: 57-2341 eggs).

Table 14. Number and percentage of mortalities attributed to gamete collection, operational causes, bacterial kidney disease (BKD), other diseases, other causes and unknown causes in each stock (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 Grande Ronde River and 3 Lostine River mortalities which have not yet been examined for cause of death.

	Gametes		Operational		BKD		Other diseases		Other		Unknown	
Variable	N	%	N	%	N	%	N	%	N	%	N	%
Stock												
CC	0	0.0	1	50.0	0	0.0	0	0.0	0	0.0	1	50.0
GR	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	100
LR	0	0.0	0	0.0	0	0.0	6	60.0	0	0.0	4	40.0
Treatment												
XA	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	4	80.0
XN	0	0.0	1	12.5	0	0.0	5	62.5	0	0.0	2	25.0
Age												
1	0	0.0	1	7.7	0	0.0	6	46.2	0	0.0	6	46.2
Total	0	0.0	1	7.7	0	0.0	6	46.2	0	0.0	6	46.2

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, ranging from 0.070-0.248 g.

Eggs were weighed from only one age 4 female: mean egg weight was 0.116 g (Figure 34). Mean egg weight of age 5 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 and/or female. The mean percent eyed eggs from females in the Freshwater Natural treatment group was 41.4% and ranged from 0-97% (Figure 35). In Saltwater Natural females, 66.5% reached the eyed stage, ranging from 0-98%.

Fertility was measured for only one 4-year old female: 75% (Figure 35). In age 5 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 35). 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%.

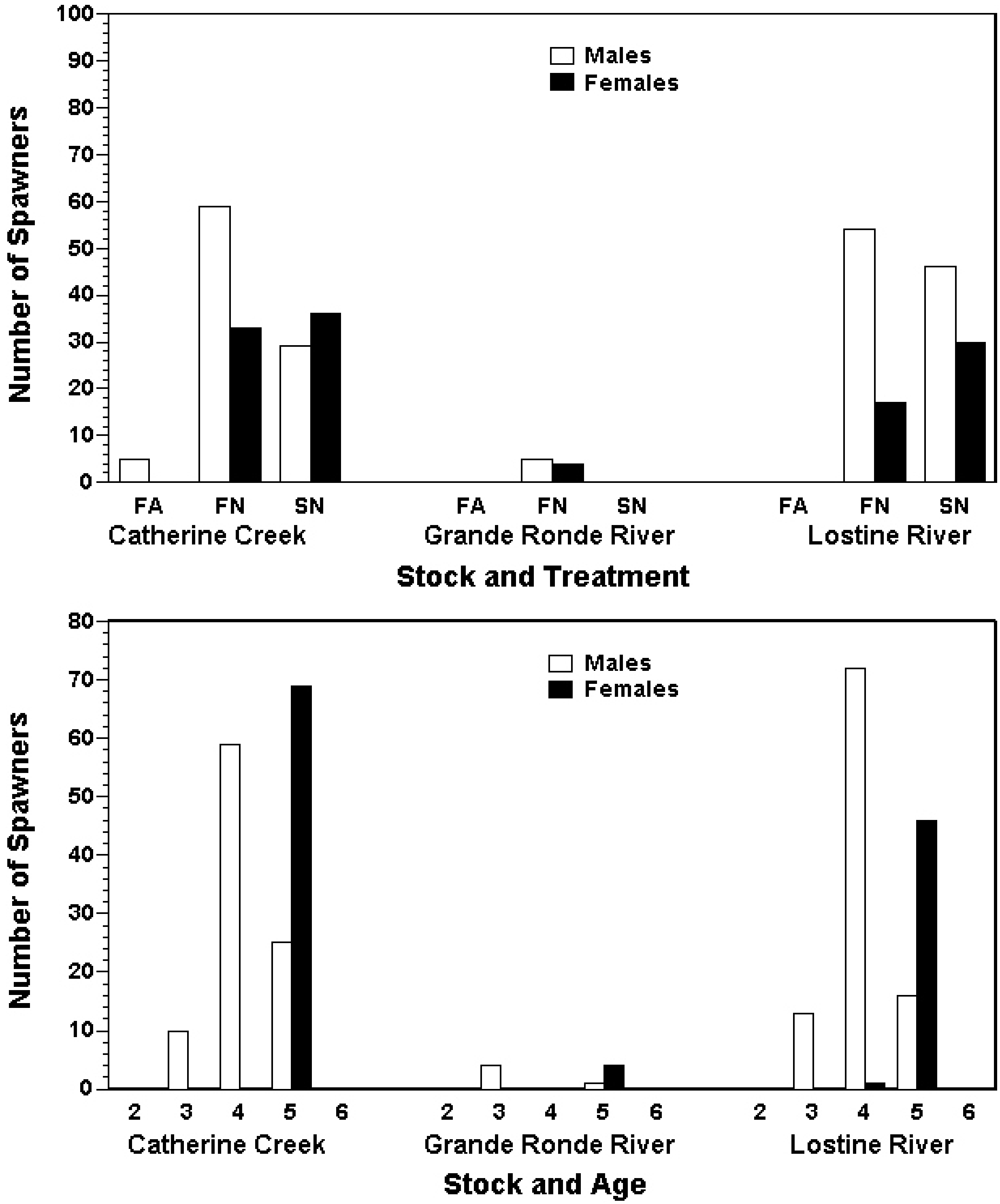


Figure 32. Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River stocks spawned in 1998.

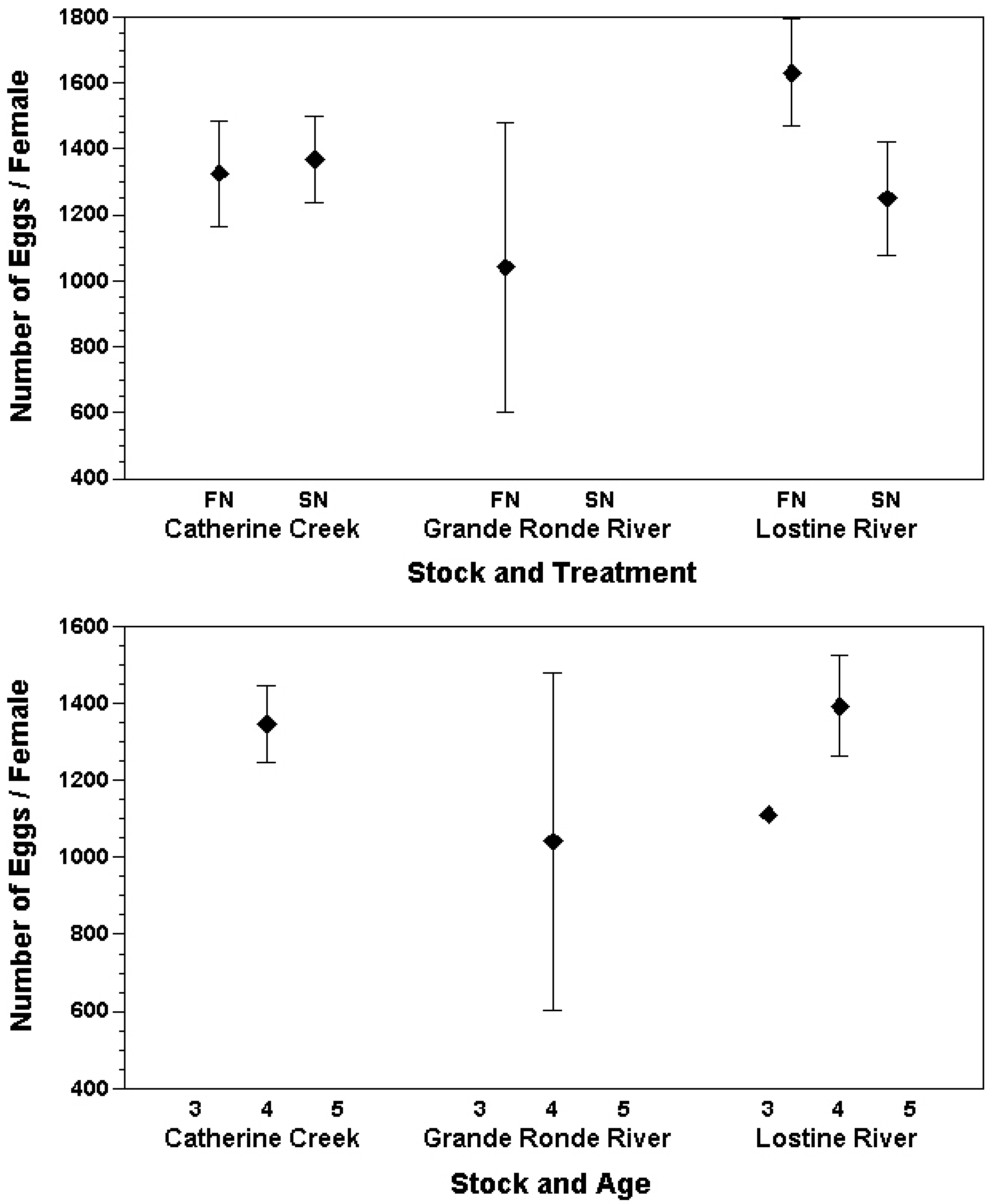


Figure 33. Mean (±95% CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and spawned in 1998 that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4 and 5 (bottom).

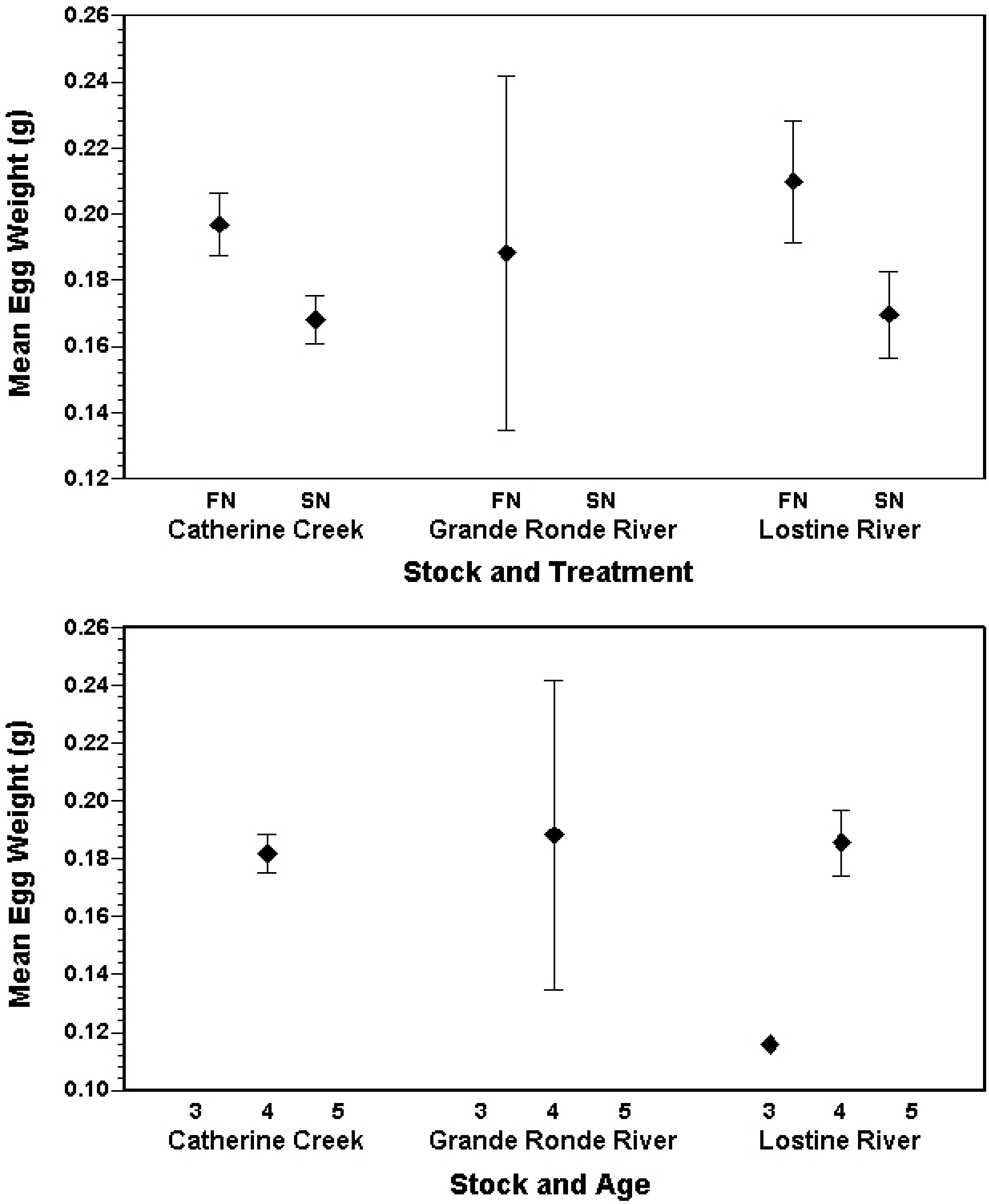


Figure 34. Mean (±95% CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon spawned in 1998 and raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4 and 5 (bottom).

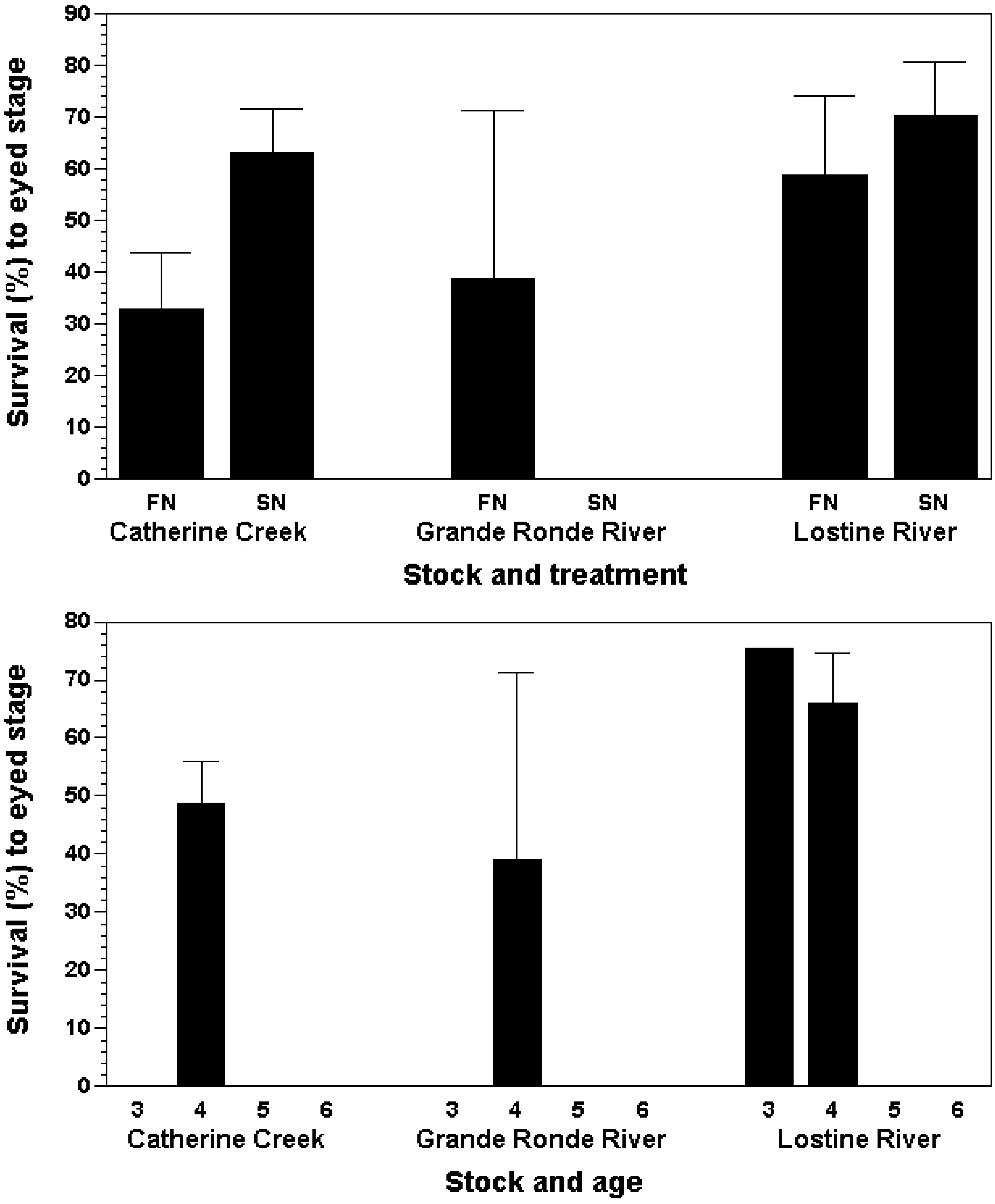


Figure 35. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon females raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and spawned at ages 3, 4, 5 and 6 (bottom) in 1998.

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.

<u>1999</u>

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 304 (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.

The age distribution of 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 1502.9 eggs and ranged from 312-2274 eggs (Figure 38). Mean fecundity of Freshwater Natural females was 1780.8 eggs and ranged from 65-3365 eggs. Mean Fecundity of Saltwater Natural females was 1477.2 eggs and ranged from 150-2802 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 1591.8 eggs and ranged from 307-2796 eggs Five-year old females had a mean fecundity of 1743.8 eggs, ranging from 65-3365 eggs.

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%.

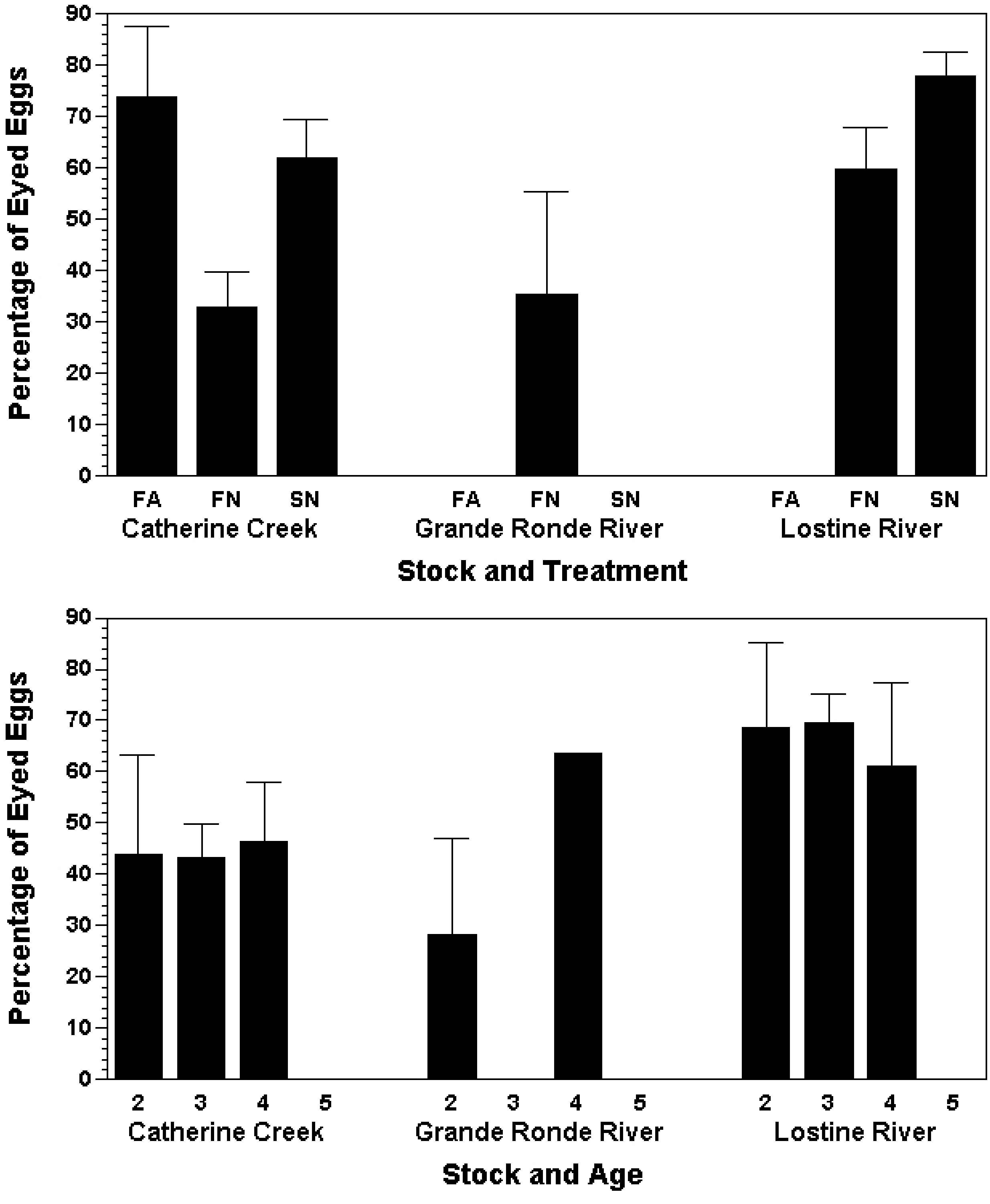


Figure 36. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and spawned at ages 2, 3, 4 and 5 (bottom) in 1998.

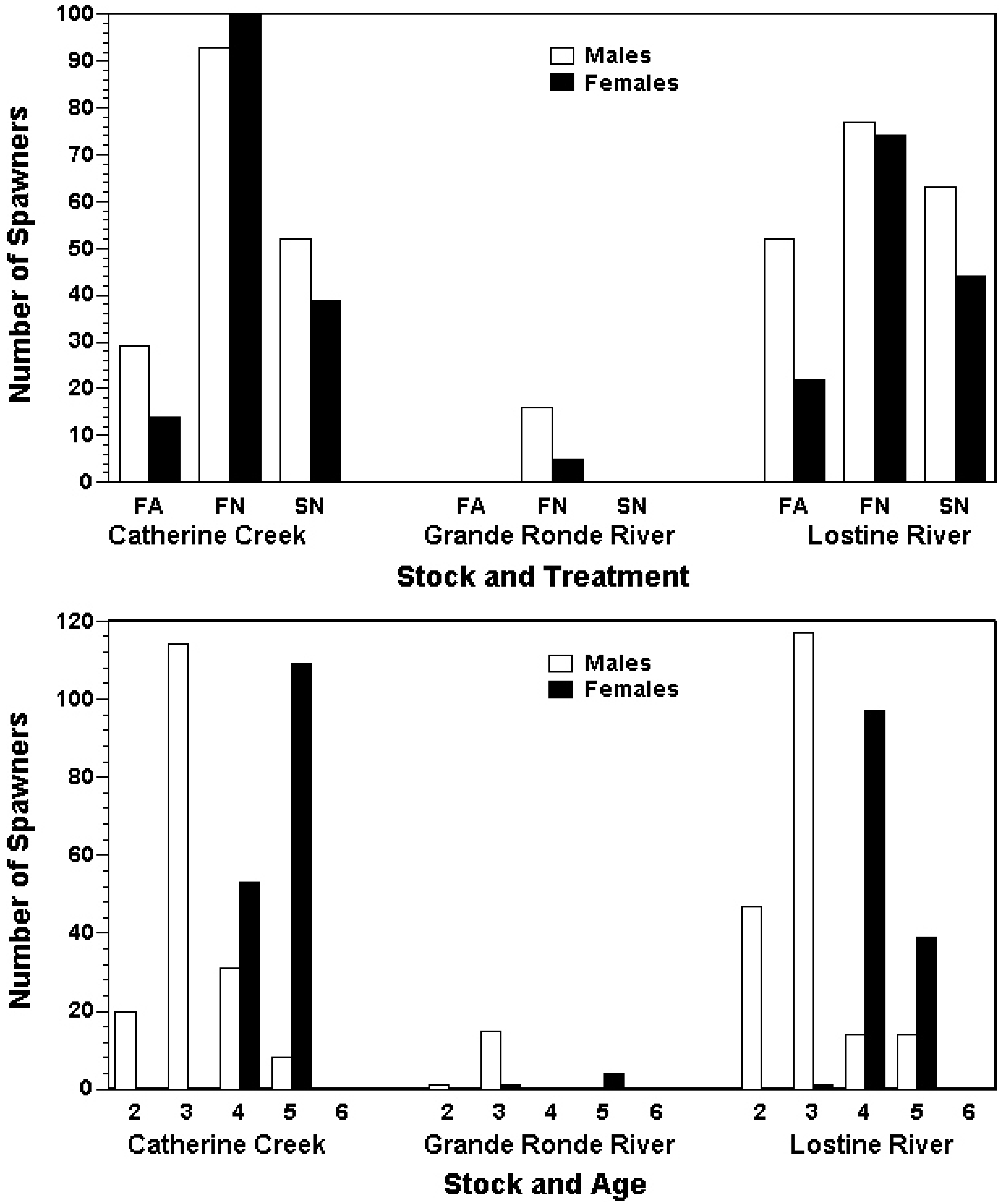


Figure 37. Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River stocks spawned in 1999.

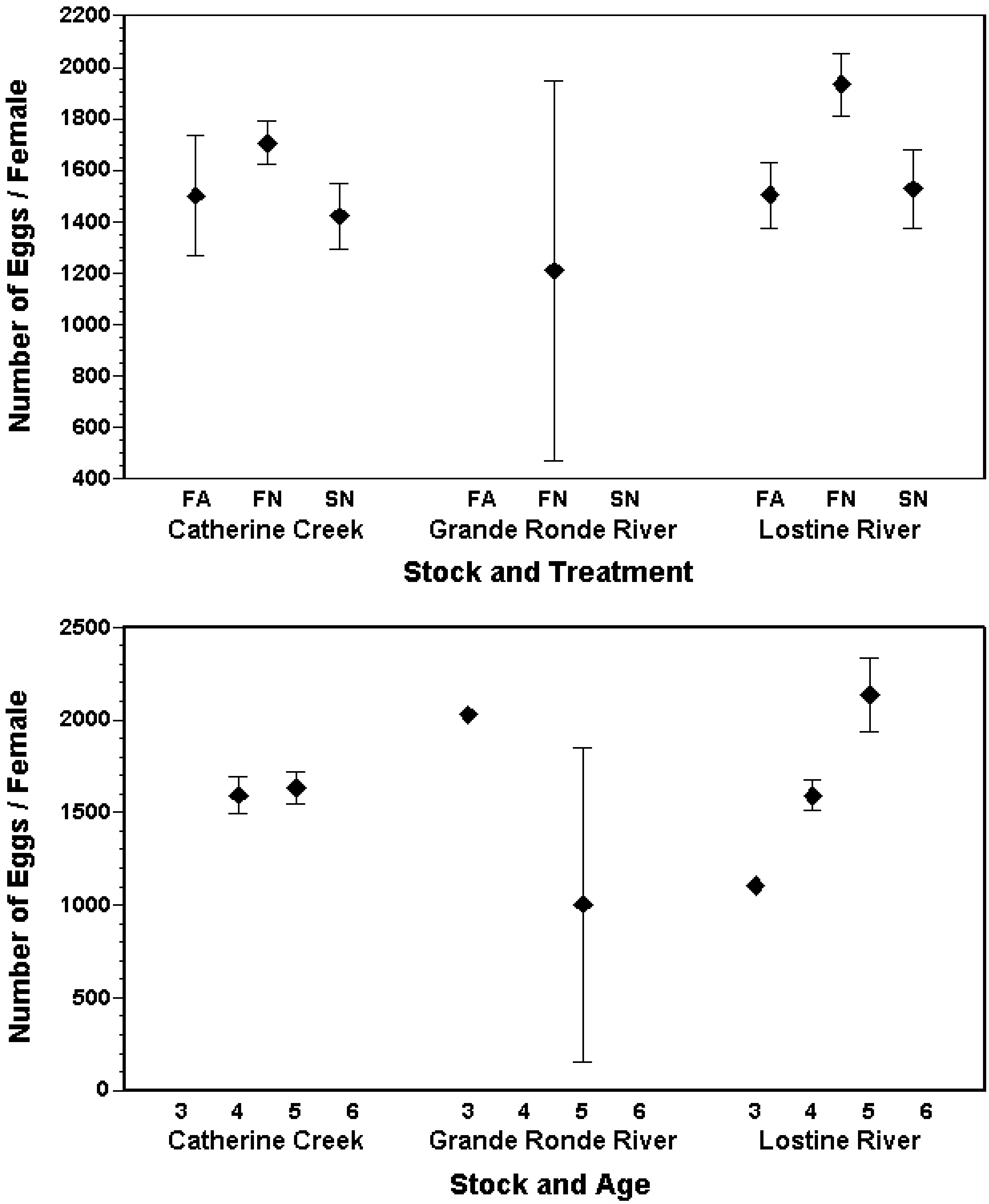


Figure 38. Mean (±95% CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and spawned in 1999 that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

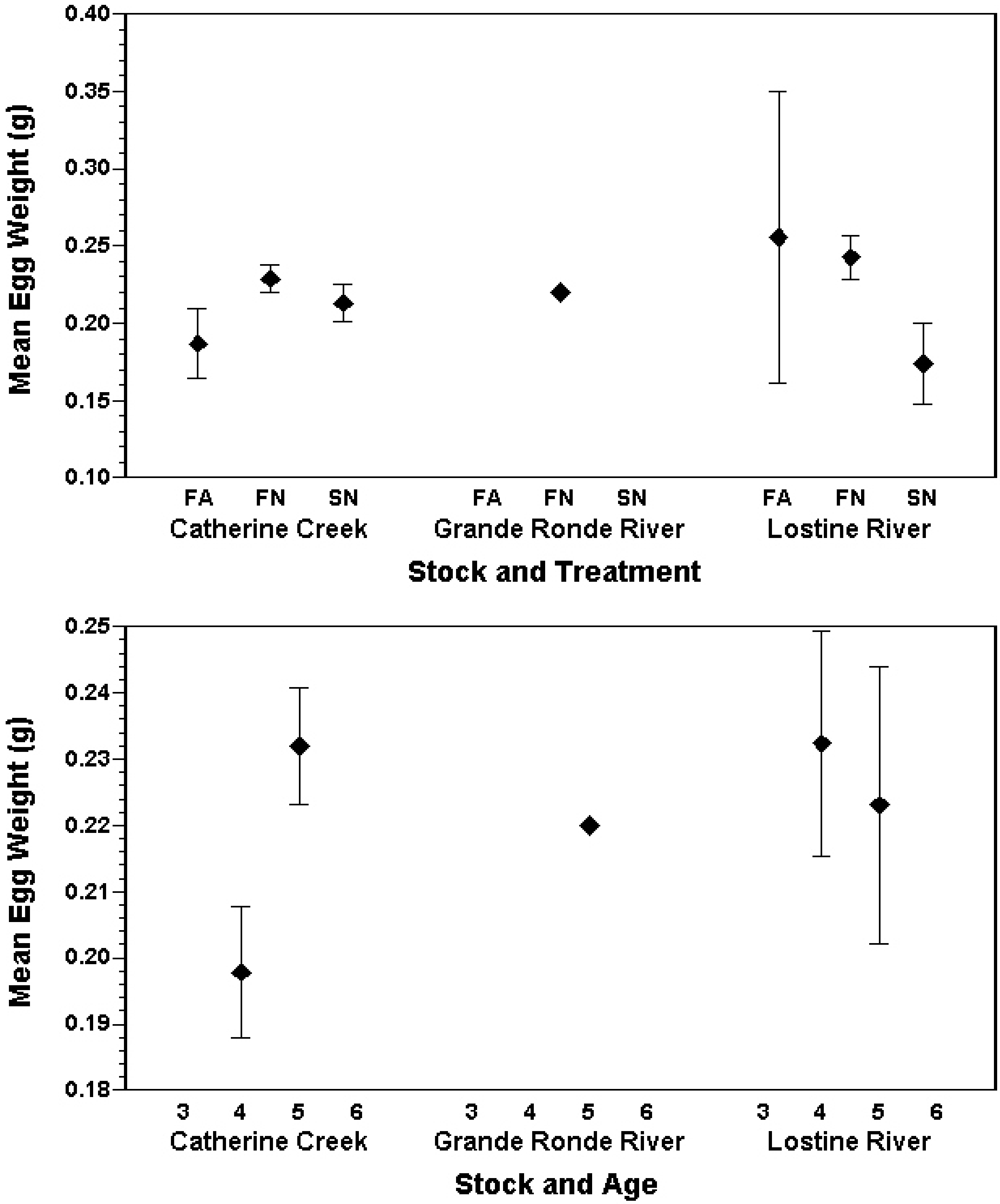


Figure 39. Mean (±95% CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon spawned in 1999 and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

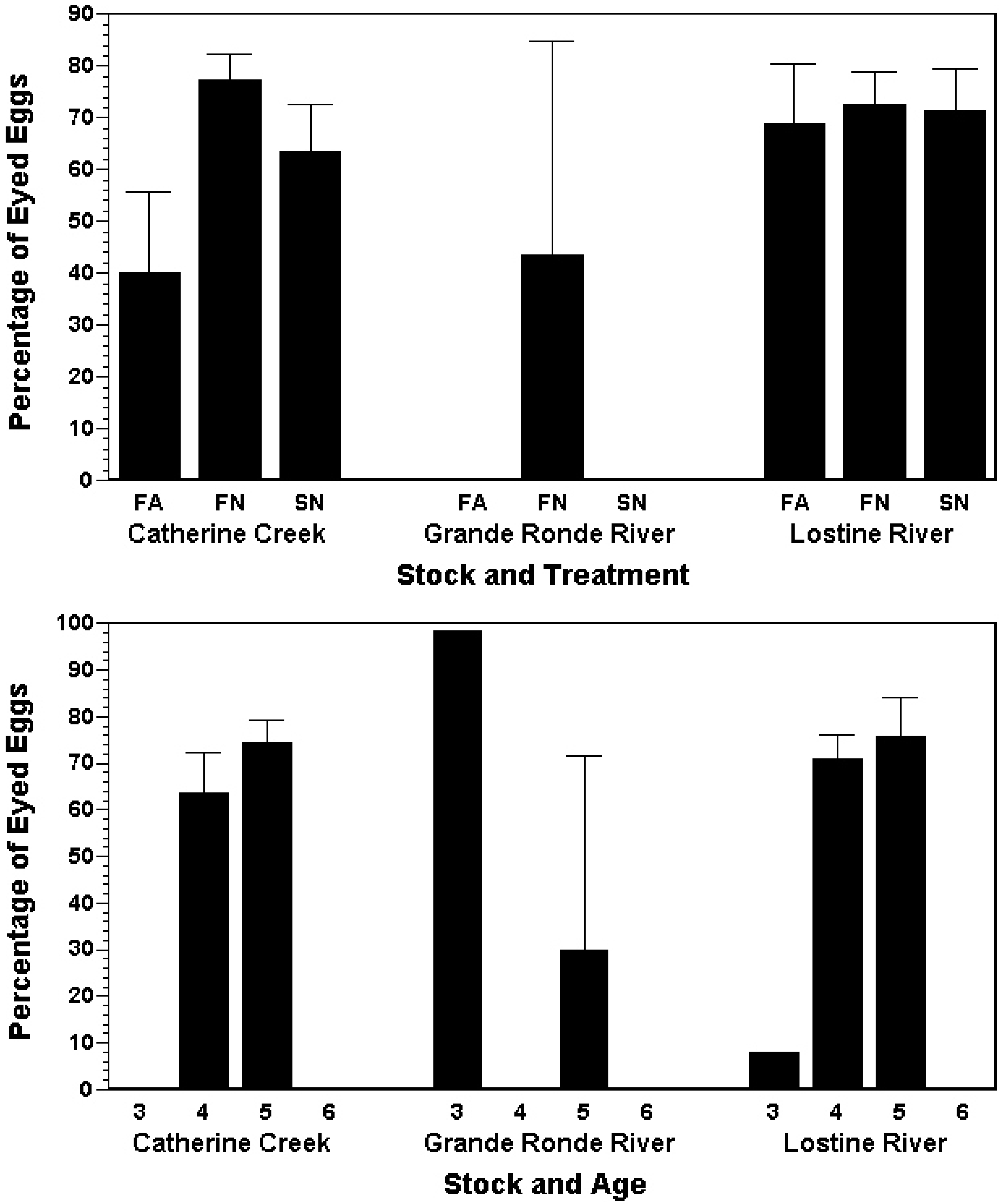


Figure 40. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) from spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) Catherine Creek, Grande Ronde River and Lostine River spawned in 1999.

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%.

<u>2000</u>

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 456 (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 35). 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.

The age distribution of spawners 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). 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 and ranged from 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.

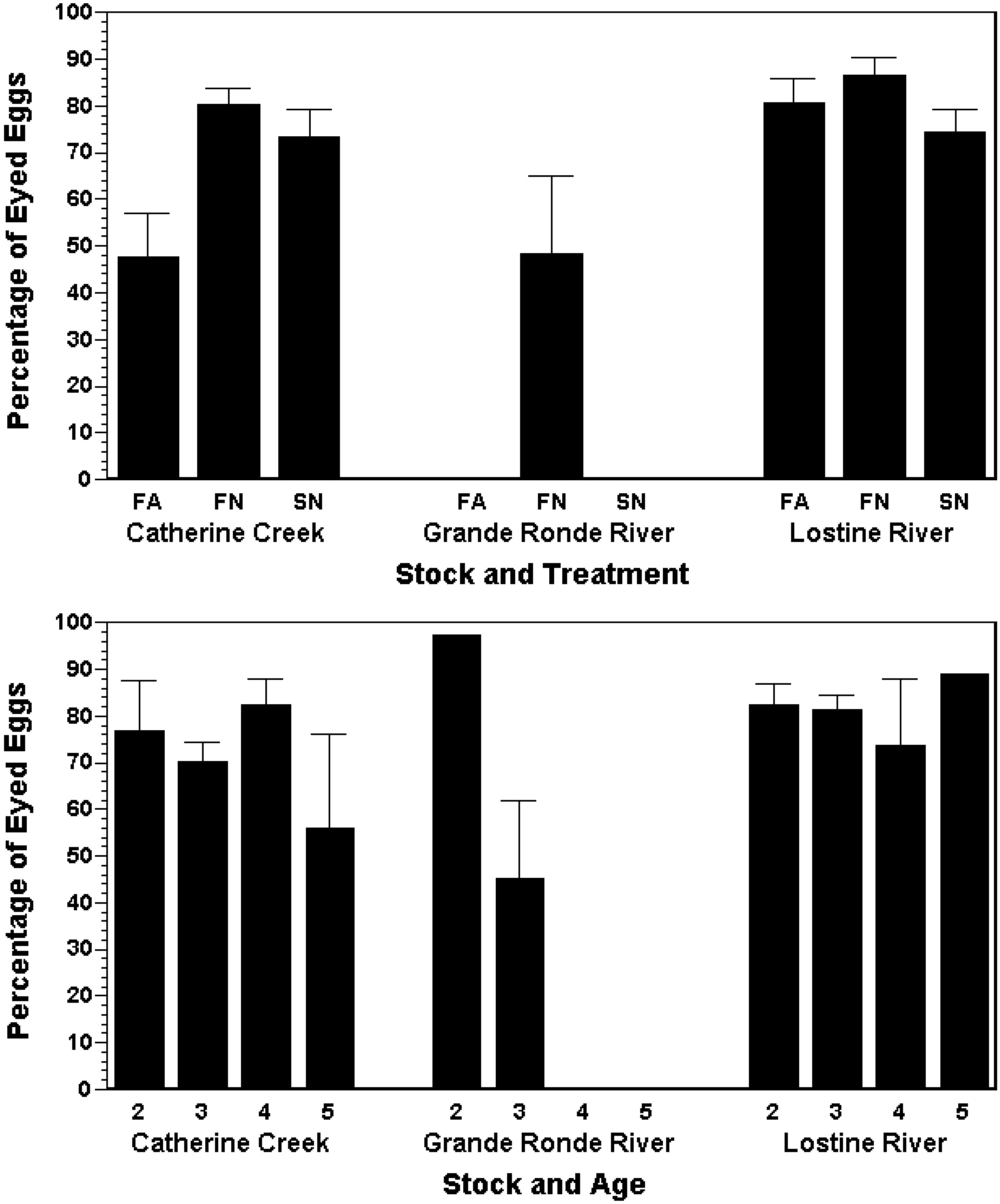


Figure 41. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 2, 3, 4 and 5 year old (bottom) in 1999.

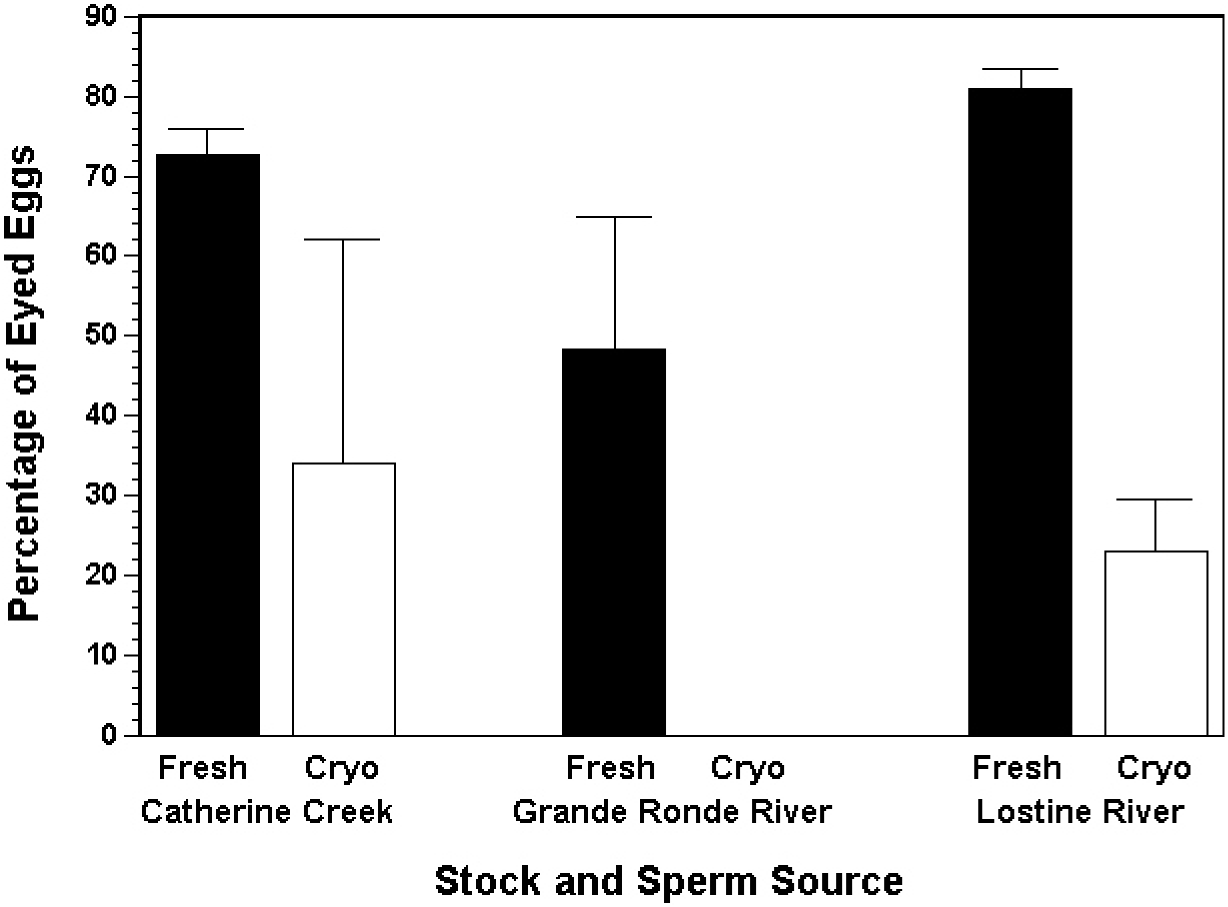


Figure 42. Mean (±95% CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males, 1999.

Mean 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.

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, 54.4% (range: 3- 88%) of the eggs reached the eyed stage.

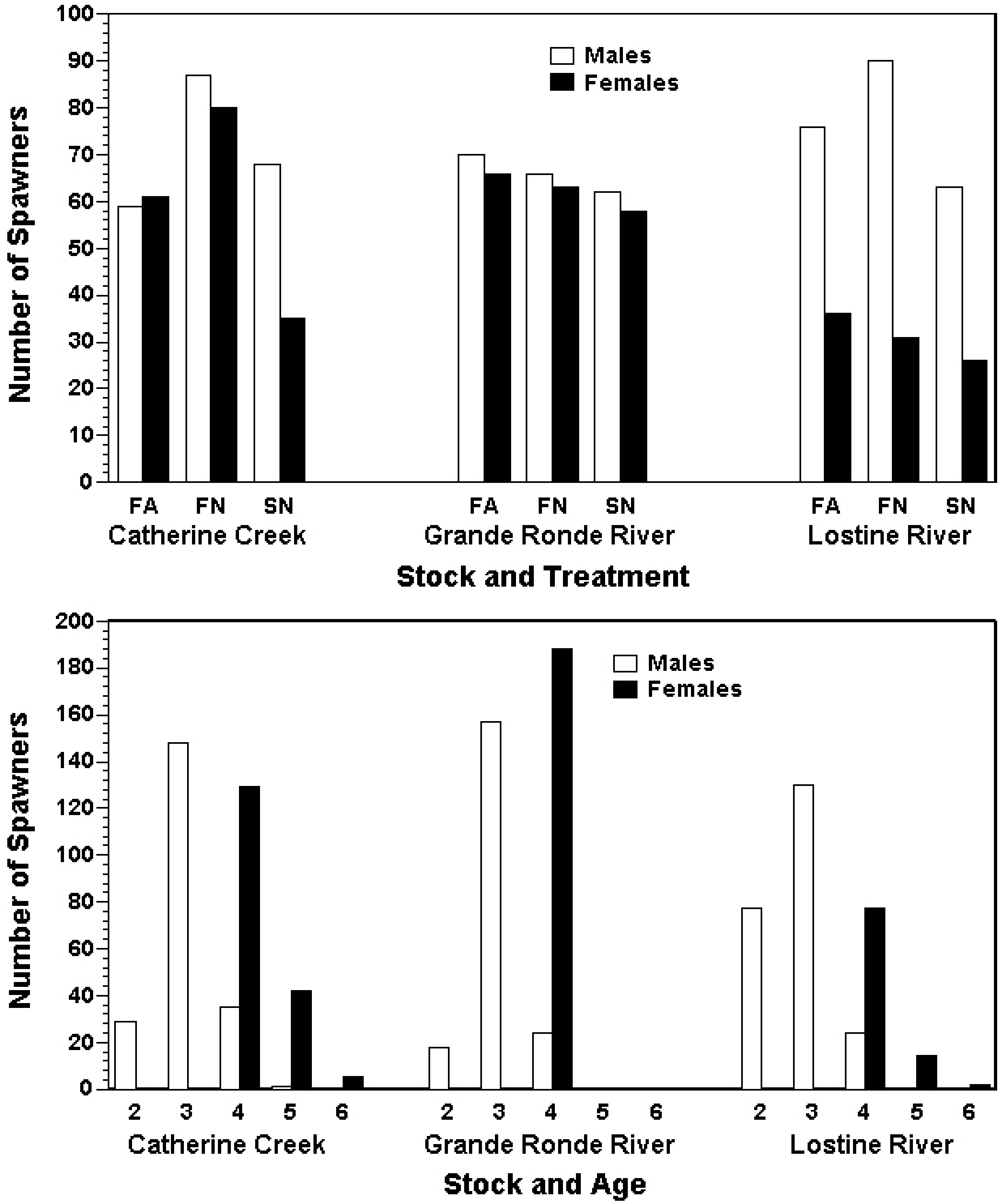


Figure 43. Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River stocks spawned in 2000.

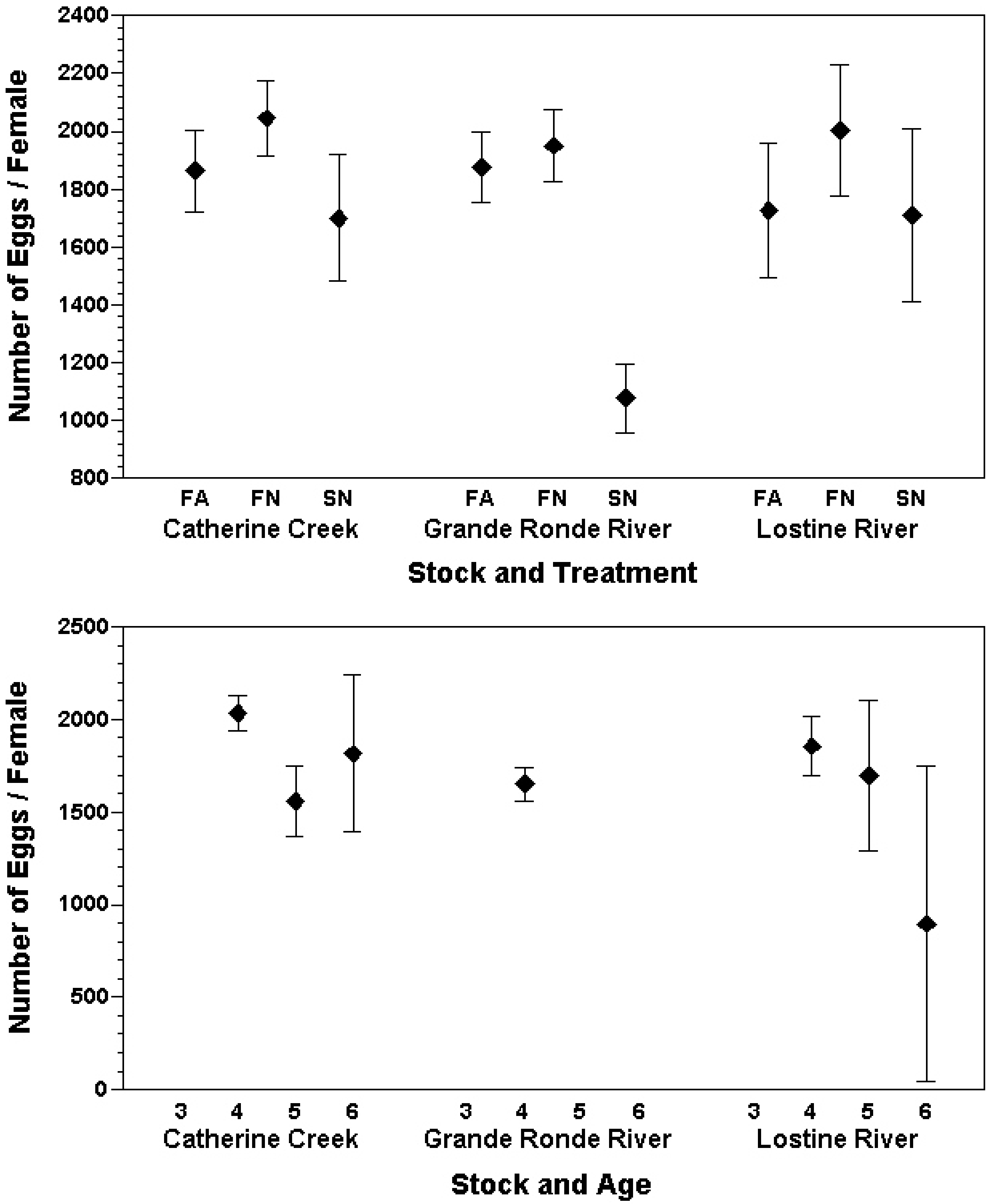


Figure 44. Mean (±95% CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and spawned in 2000 that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

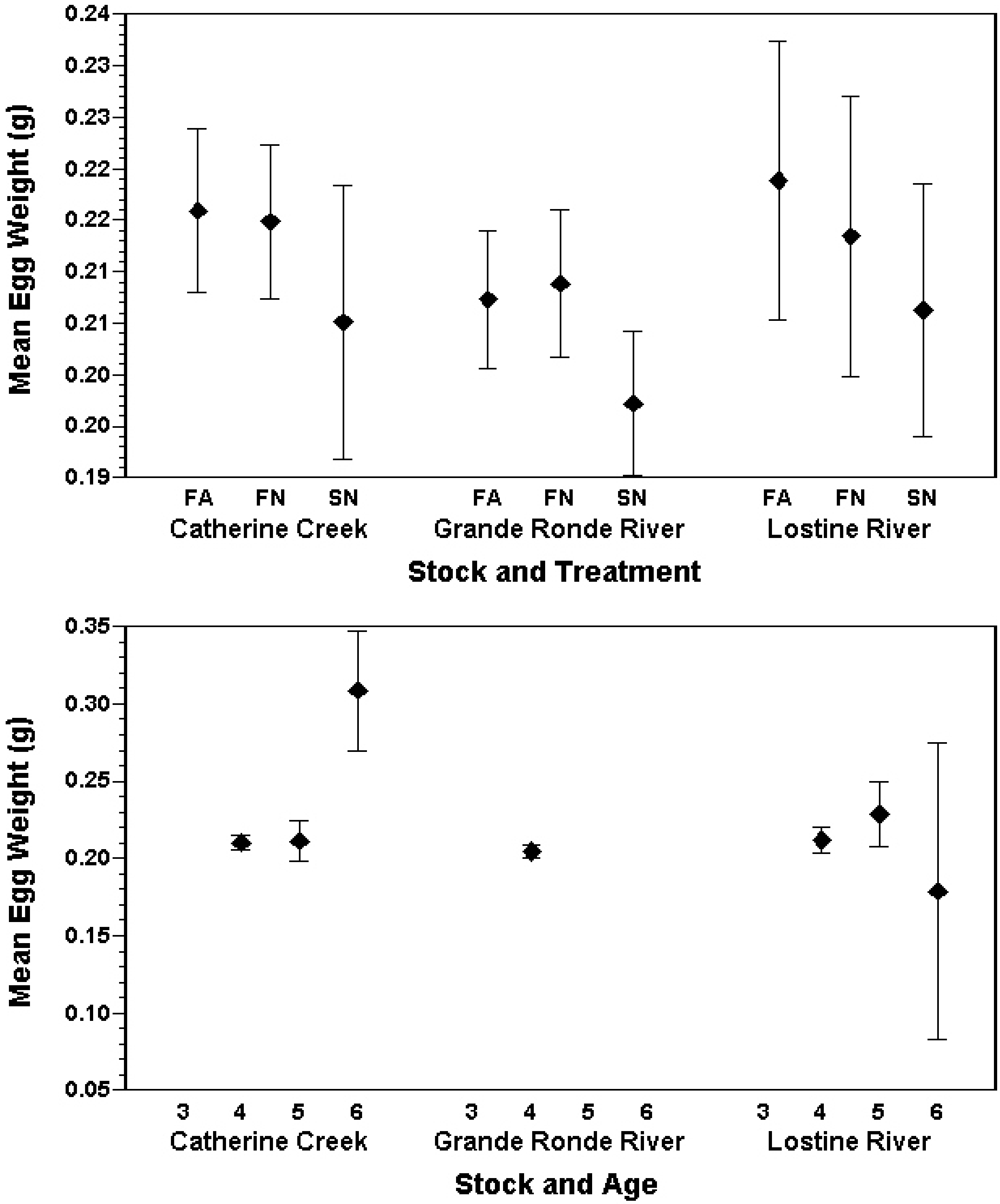


Figure 45. Mean (±95% CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon spawned in 2000 and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

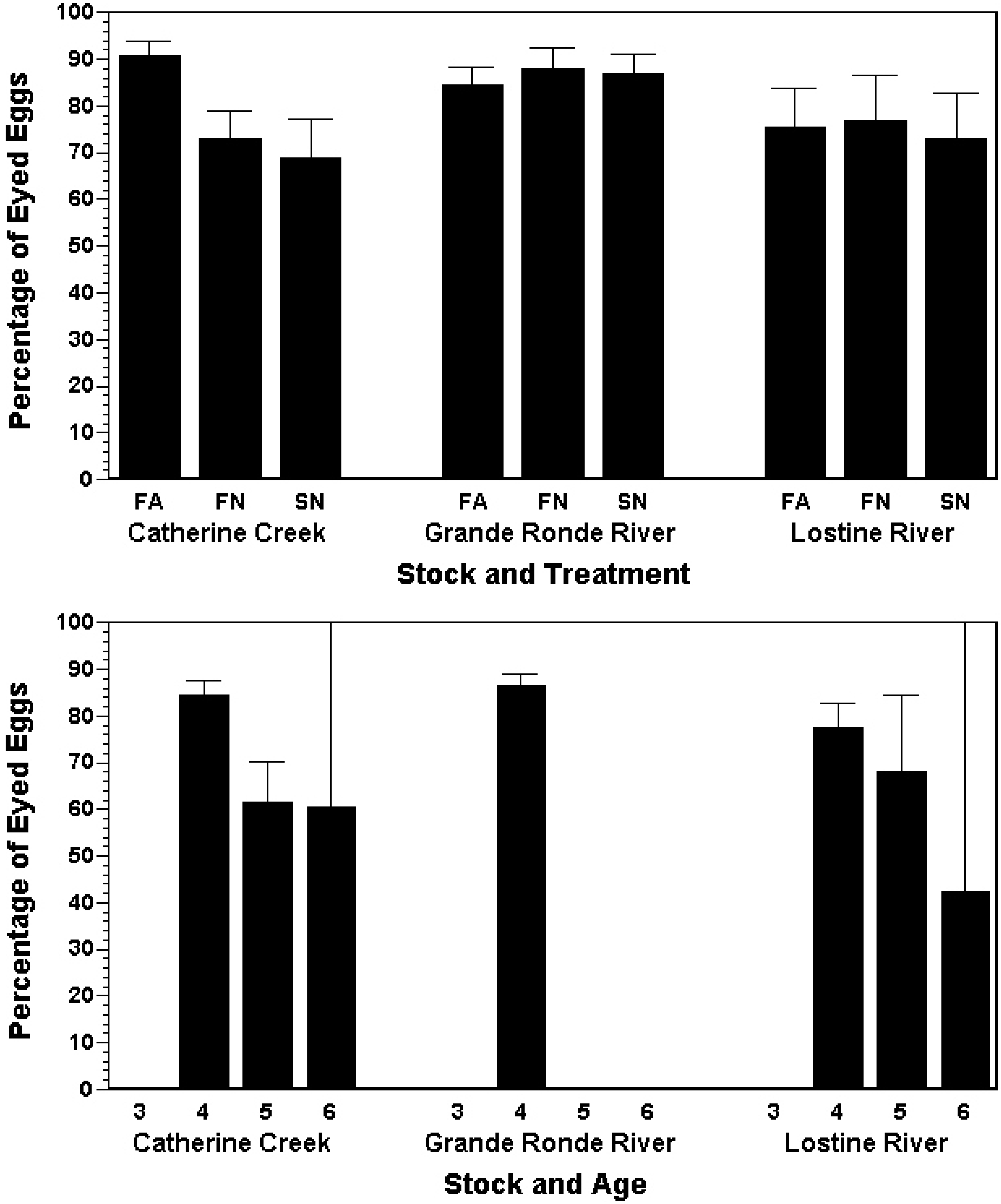


Figure 46. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) from spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) Catherine Creek, Grande Ronde River and Lostine River spawned in 2000.

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%.

<u>2001</u>

Number of Spawners

A total of 1110 fish matured and contributed gametes (were spawned or had semen cryopreserved) in 2001: 631 (56.8%) males and 479 (43.2%) 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 (Figure 35). 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.

The age distribution of spawners in 1999 included ages 2-6 and males matured at an earlier age 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 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.

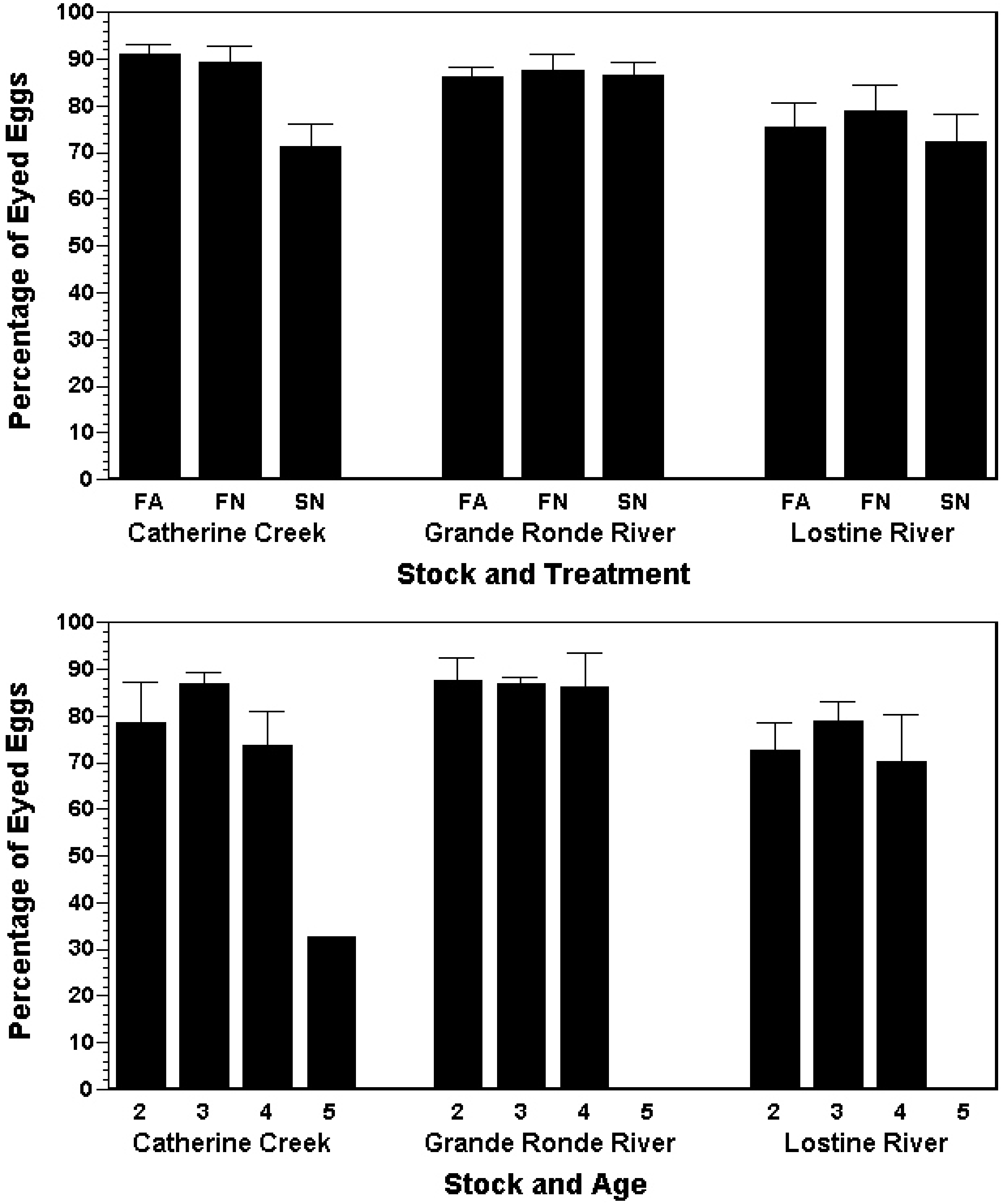


Figure 47. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) in 2000.

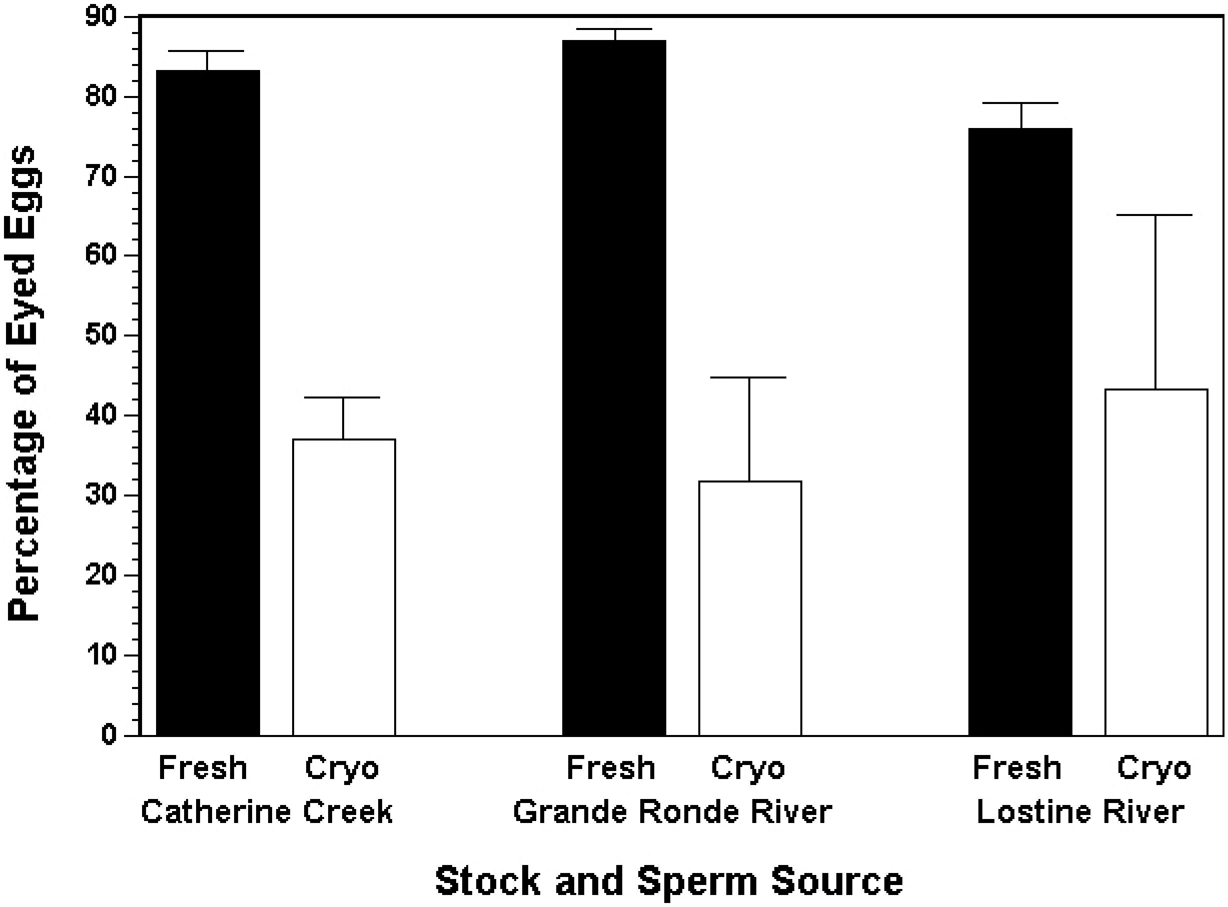


Figure 48. Mean (±95% CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males, 2000.

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%.

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-

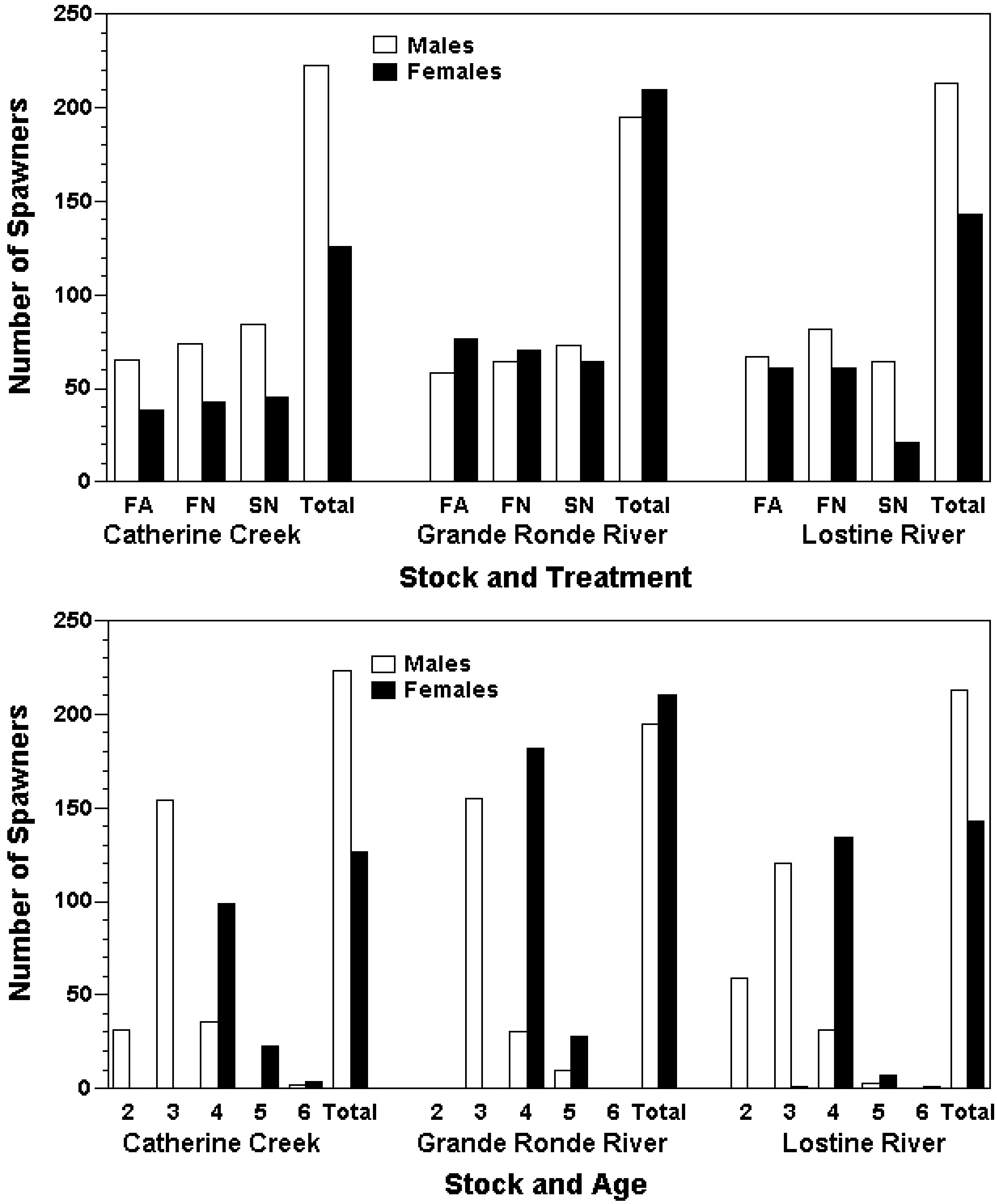
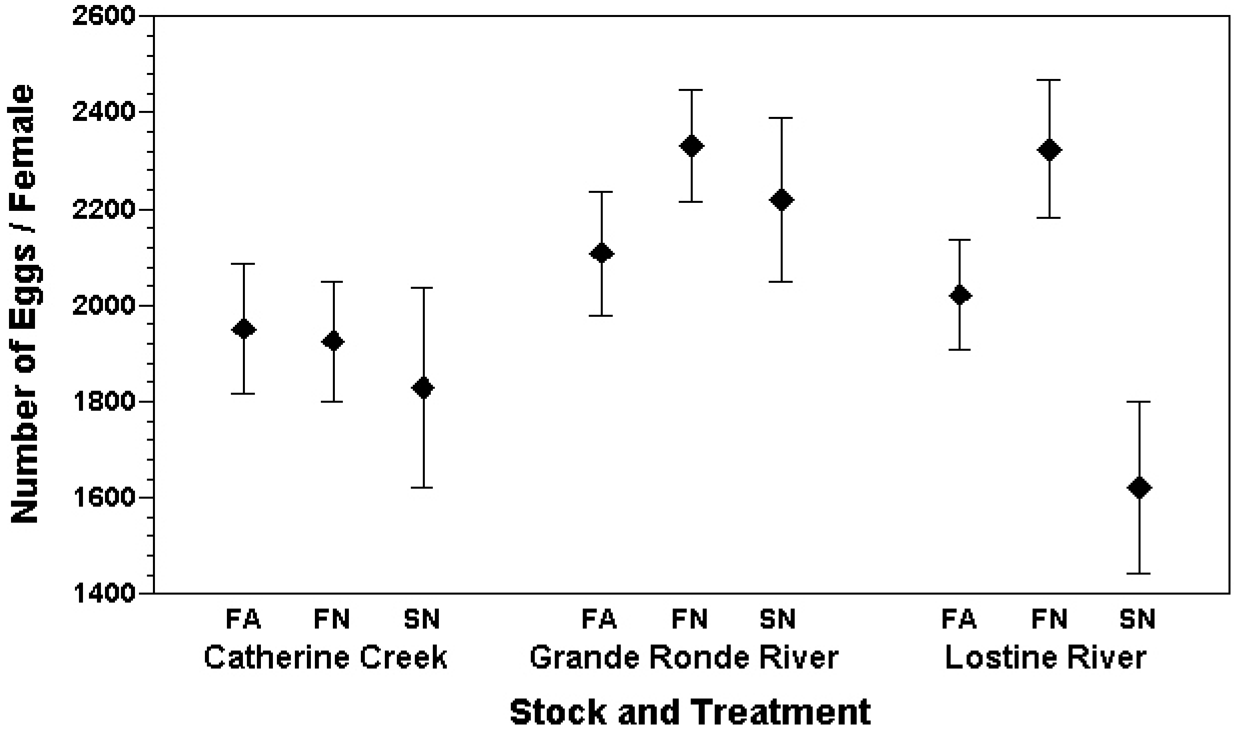


Figure 49. Number of males and females from Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River stocks spawned in 2001.



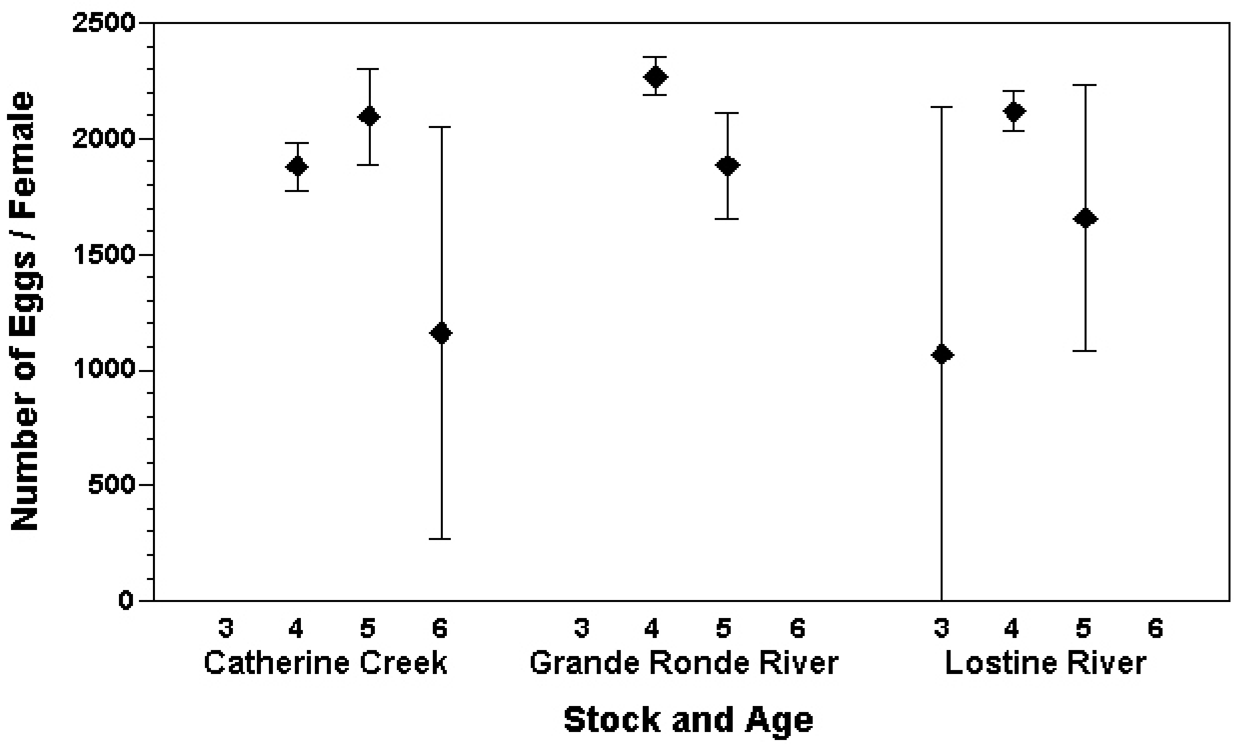


Figure 50. Mean (±95% CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and spawned in 2001 that were raised under Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

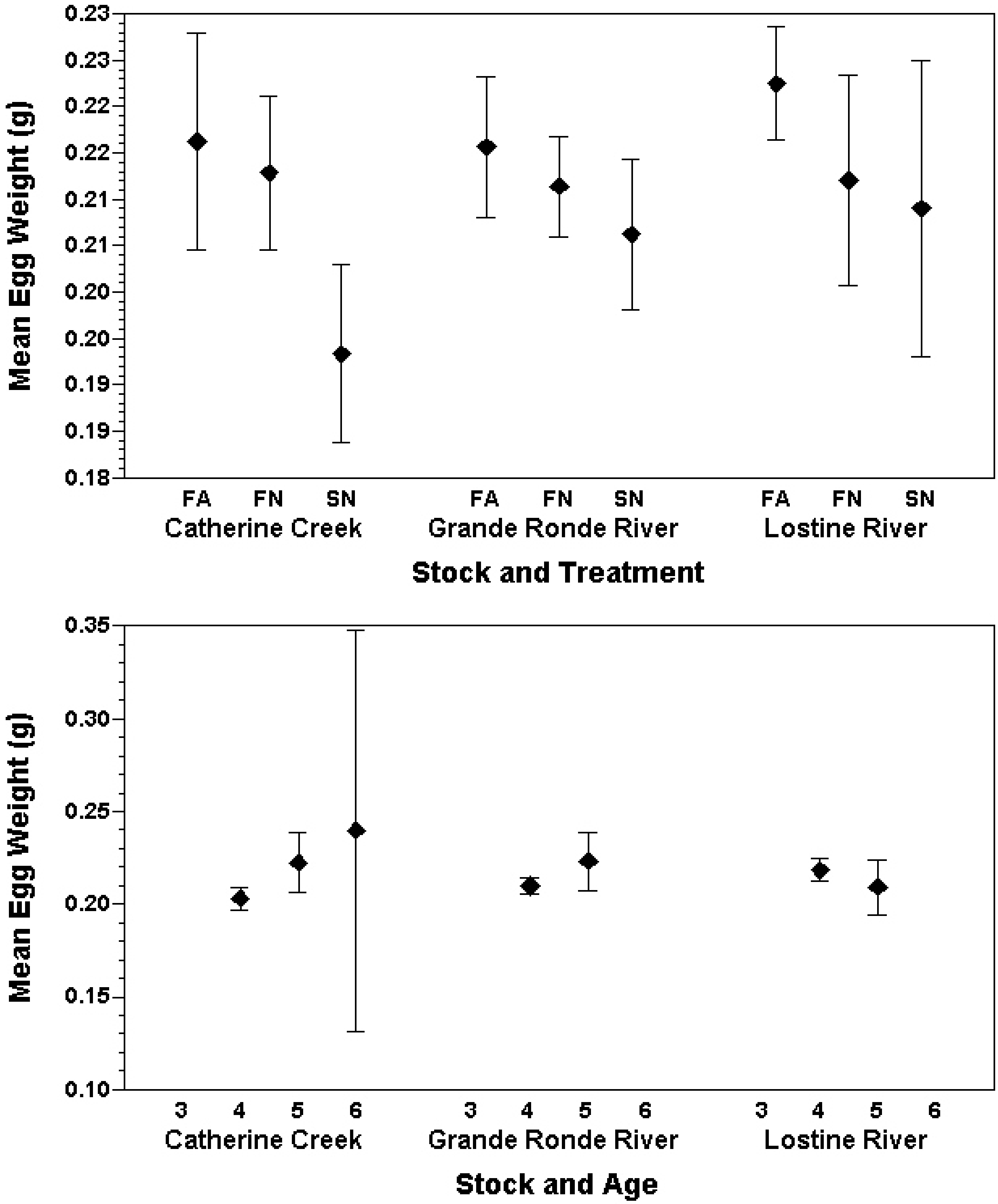


Figure 51. Mean (±95% CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon spawned in 2001 and raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

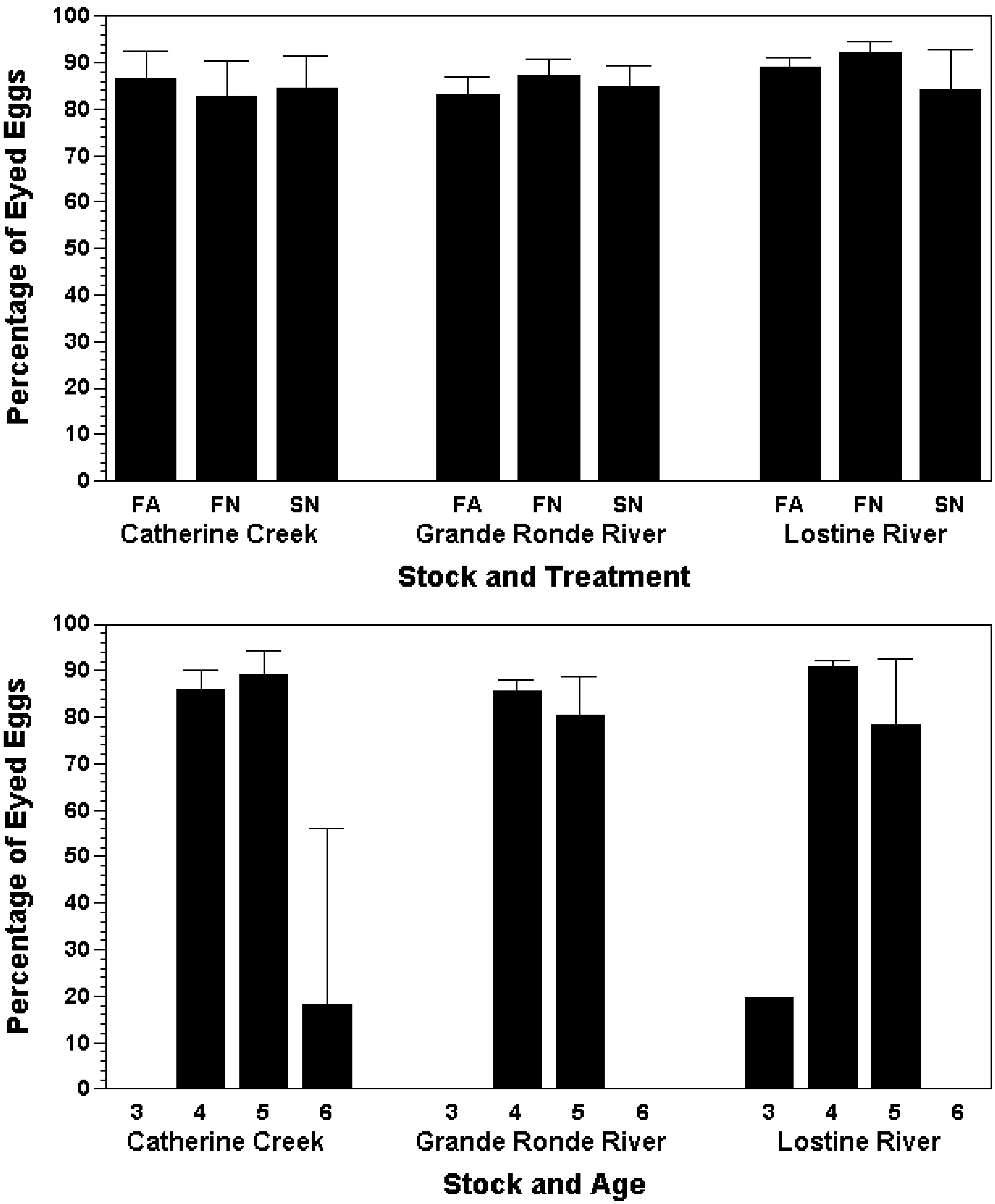


Figure 52. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year olds (bottom) spawned in 2001.

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 freshwater to saltwater at a time more similar to when wild fish of these stocks would be entering freshwater. We also ceased feeding the maturing fish earlier (after sorting for maturity). Our hope was that this would induce the fish to spawn earlier - closer to the time that wild fish of these stocks 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. 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 and spawn. 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.

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

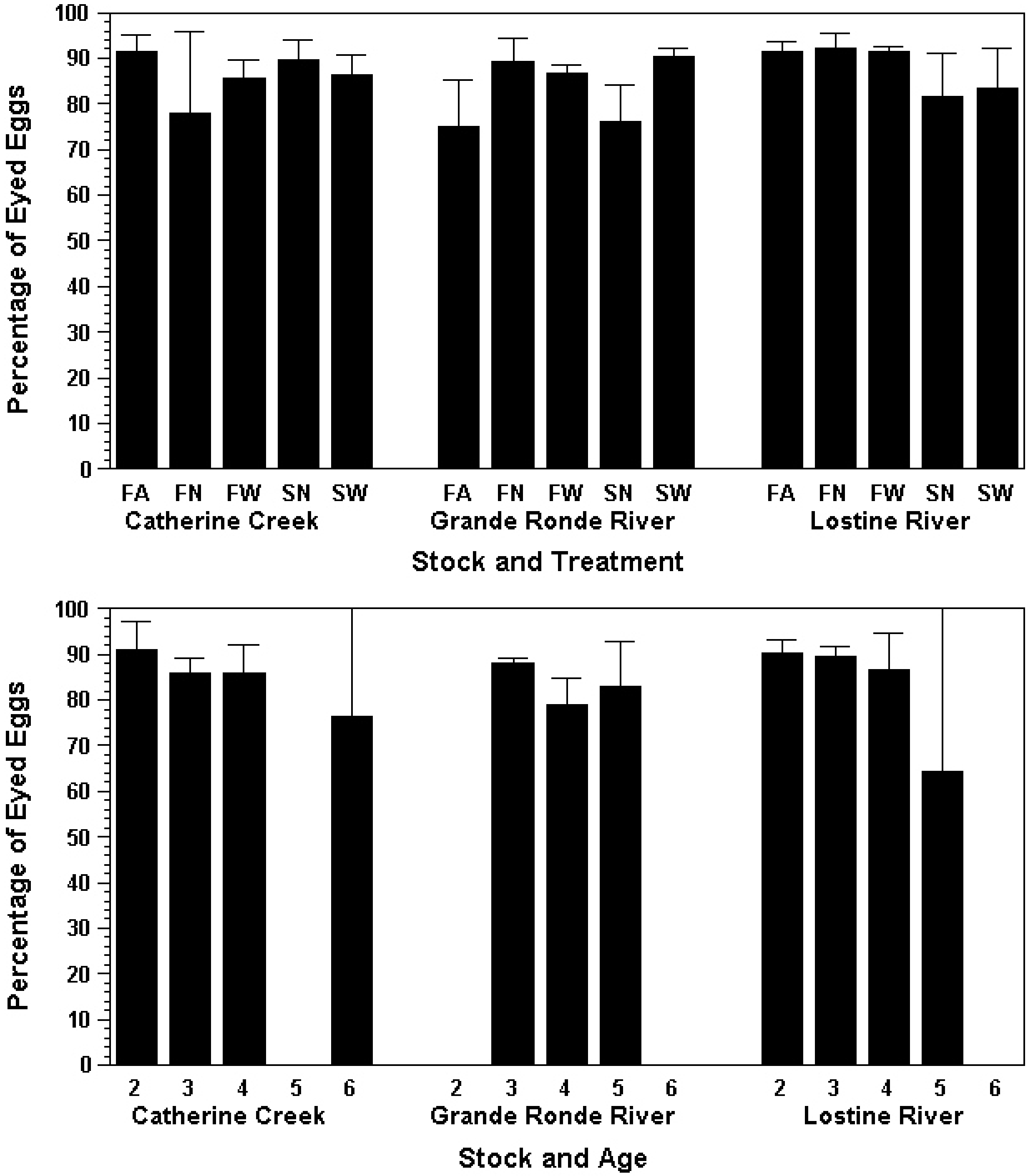


Figure 53. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN), Freshwater (FW; 1998 cohort, in 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.

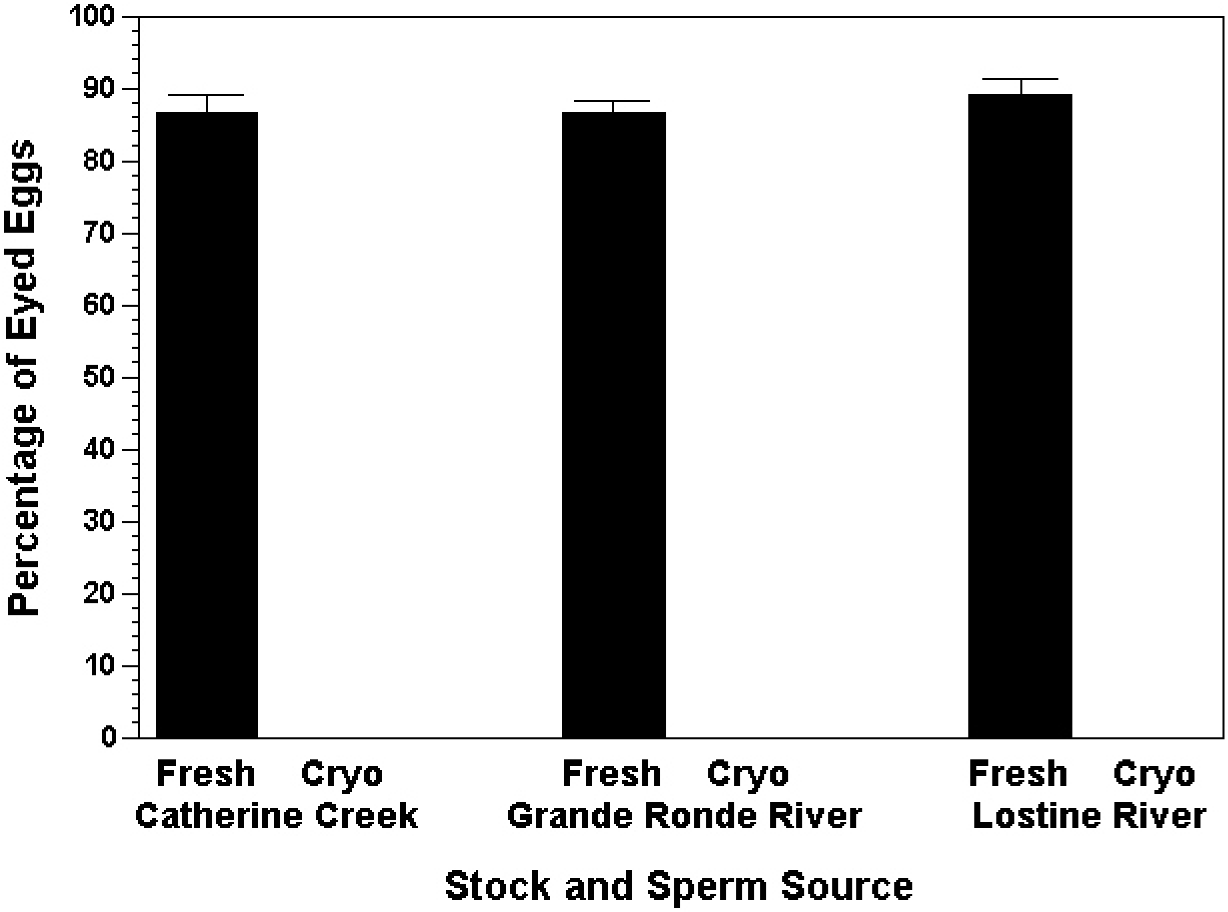


Figure 54. Mean (±95% CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males in 2001. Note: no cryopreserved semen was used for spawning in 2001.

treatment groups, 420 (63.5%) were from the Freshwater groups: 170 (40.0%) were males and 250 (60.0%) were females (Figure 55). Two hundred forty-one (36.5%) were from the Saltwater groups: 102 (42.3%) males and 139 (57.7%) females.

The 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 Saltwater 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

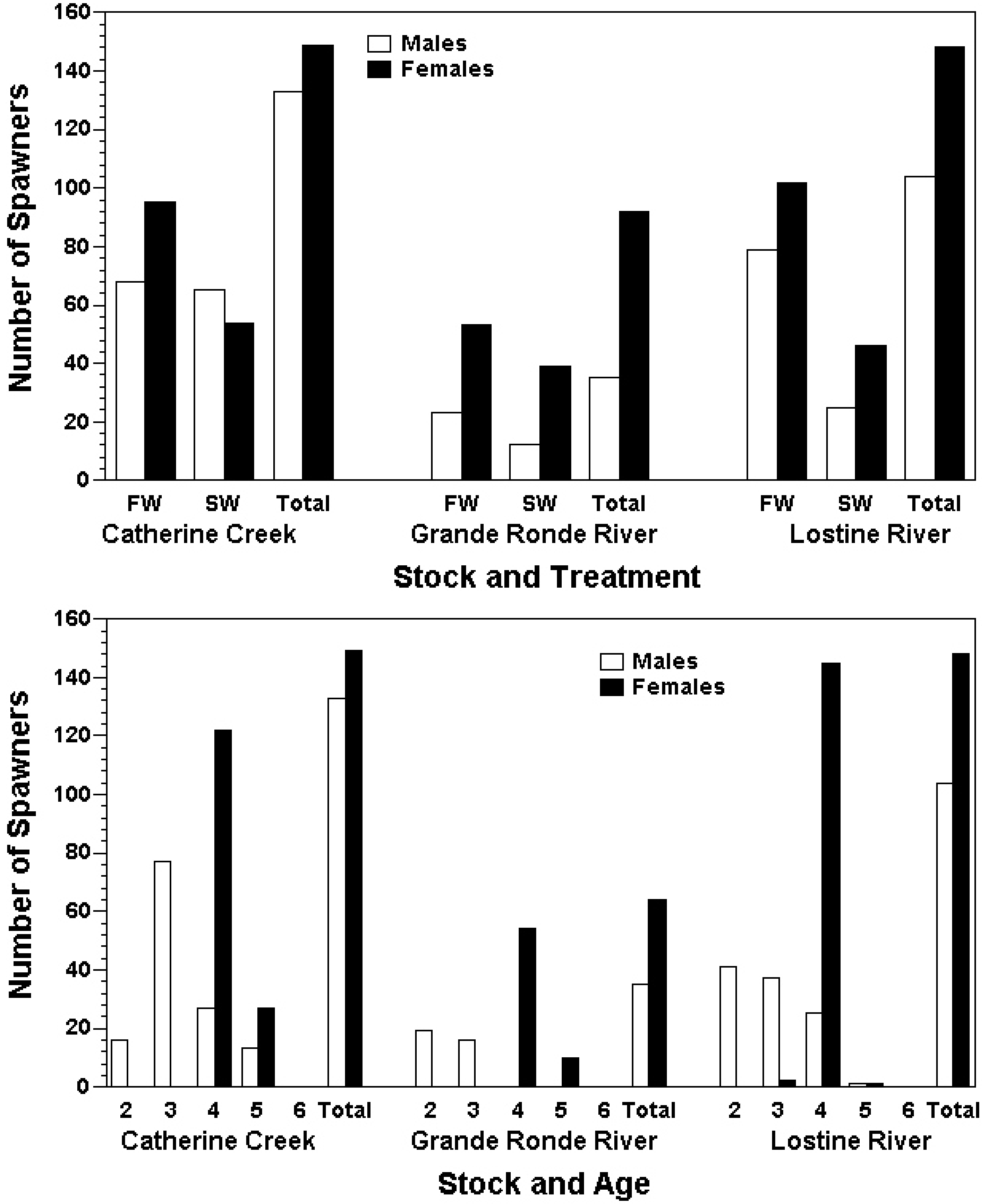


Figure 55. Number of males and females from Freshwater (FW) and Saltwater Natural (SW) treatment groups (top) and number of males and females of ages 2, 3, 4, 5 and 6 (bottom) of Catherine Creek, Grande Ronde River and Lostine River stocks spawned in 2002.

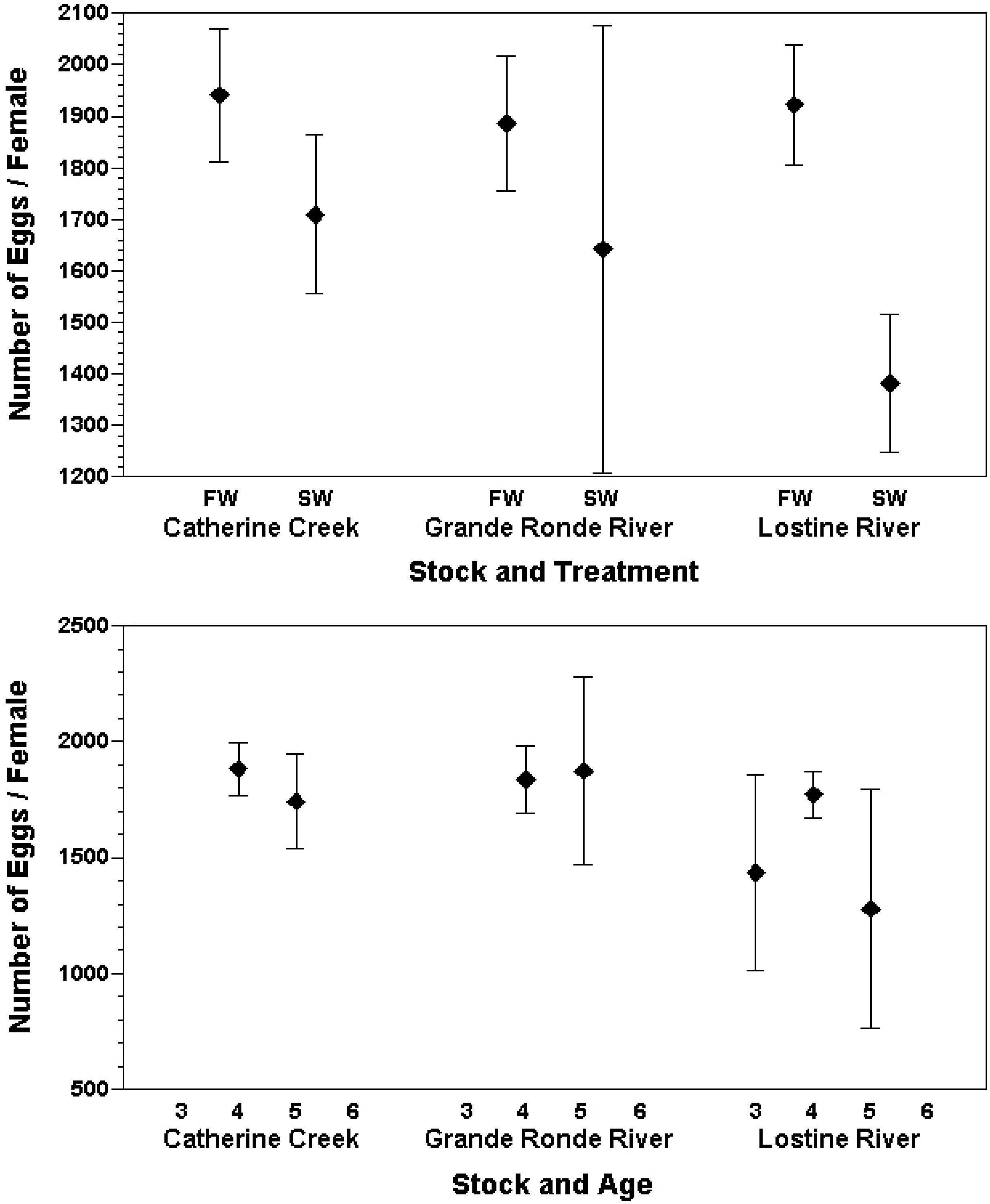


Figure 56. Mean (±95% CI) fecundity of spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River and spawned in 2002 that were raised under Freshwater (FW) and Saltwater (SW) treatments (top) and at ages 3, 4, 5 and 6 (bottom).

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.

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 (Figure 57). 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 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 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 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 60). 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%.

Analyses

Here, we analyze the available data to examine the benchmarks for success that were 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 analyses can give an overview of how well we are achieving the goals of the program.

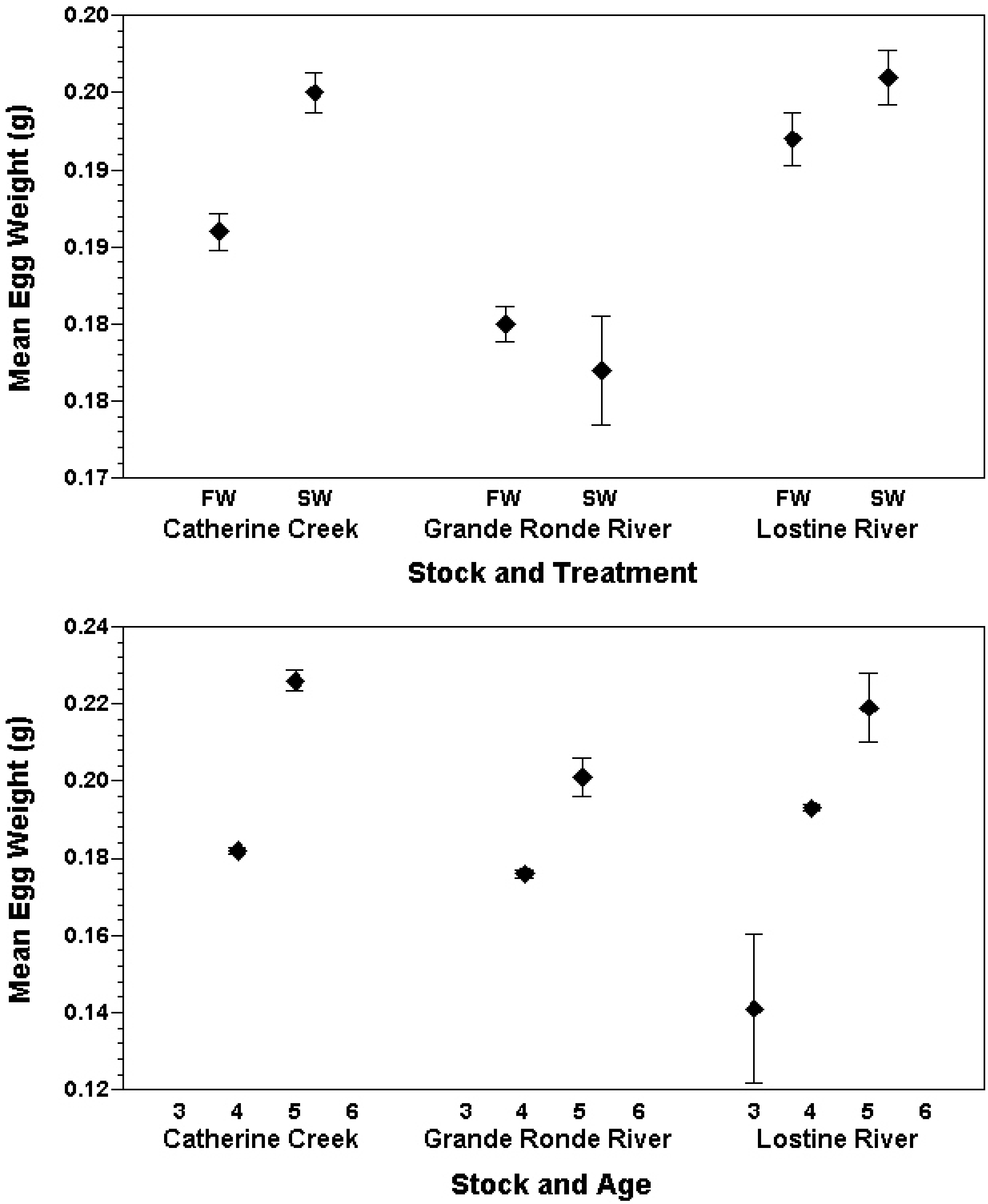


Figure 57. Mean (±95% CI) weight of eggs of Catherine Creek, Grande Ronde River and Lostine River spring chinook salmon spawned in 2002 and raised in freshwater (FW) or saltwater (SW) for post-smolt growth (top) and at ages 3, 4, 5 and 6 (bottom).

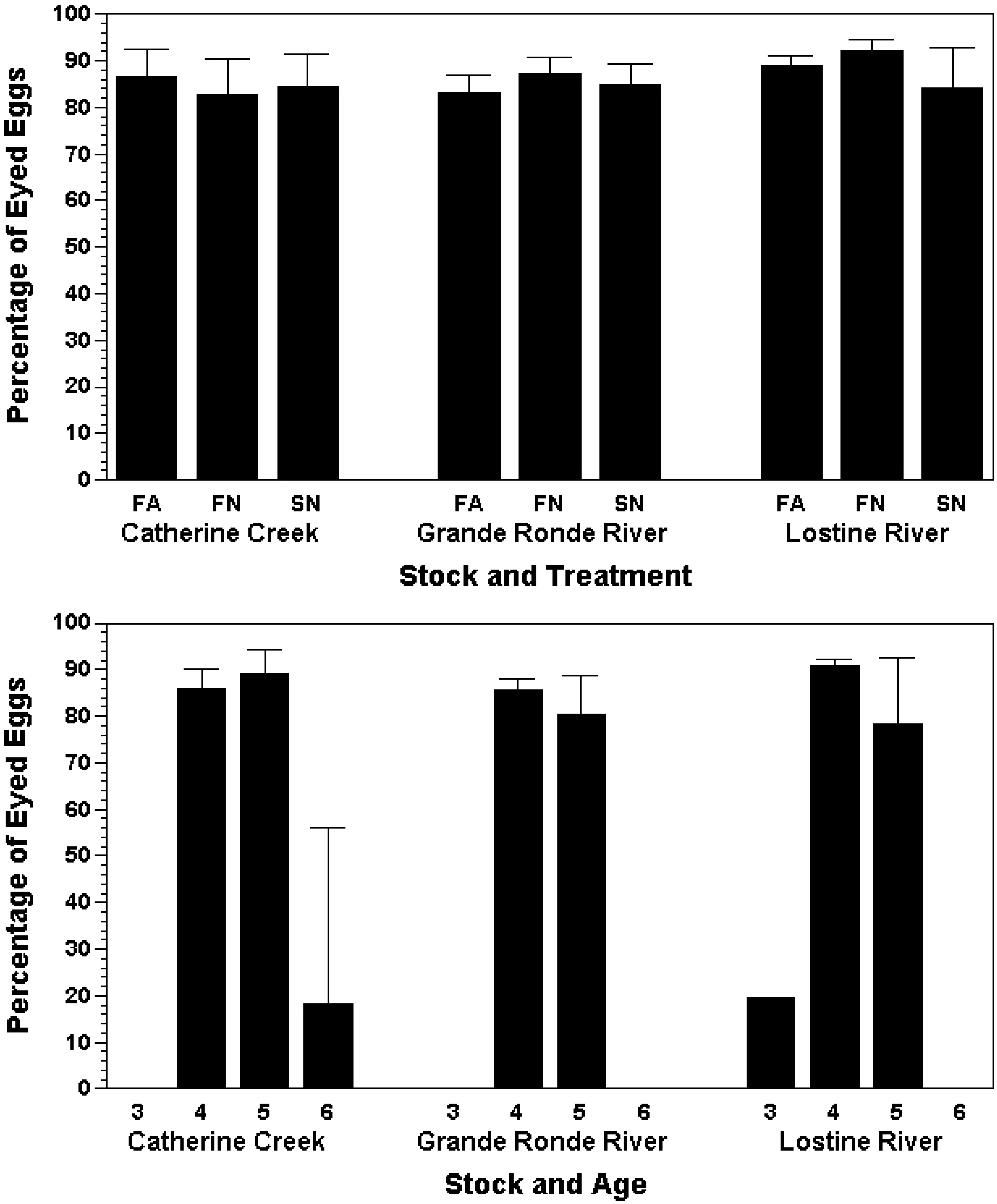


Figure 58. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) from spring chinook salmon females raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) Catherine Creek, Grande Ronde River and Lostine River spawned in 2001.

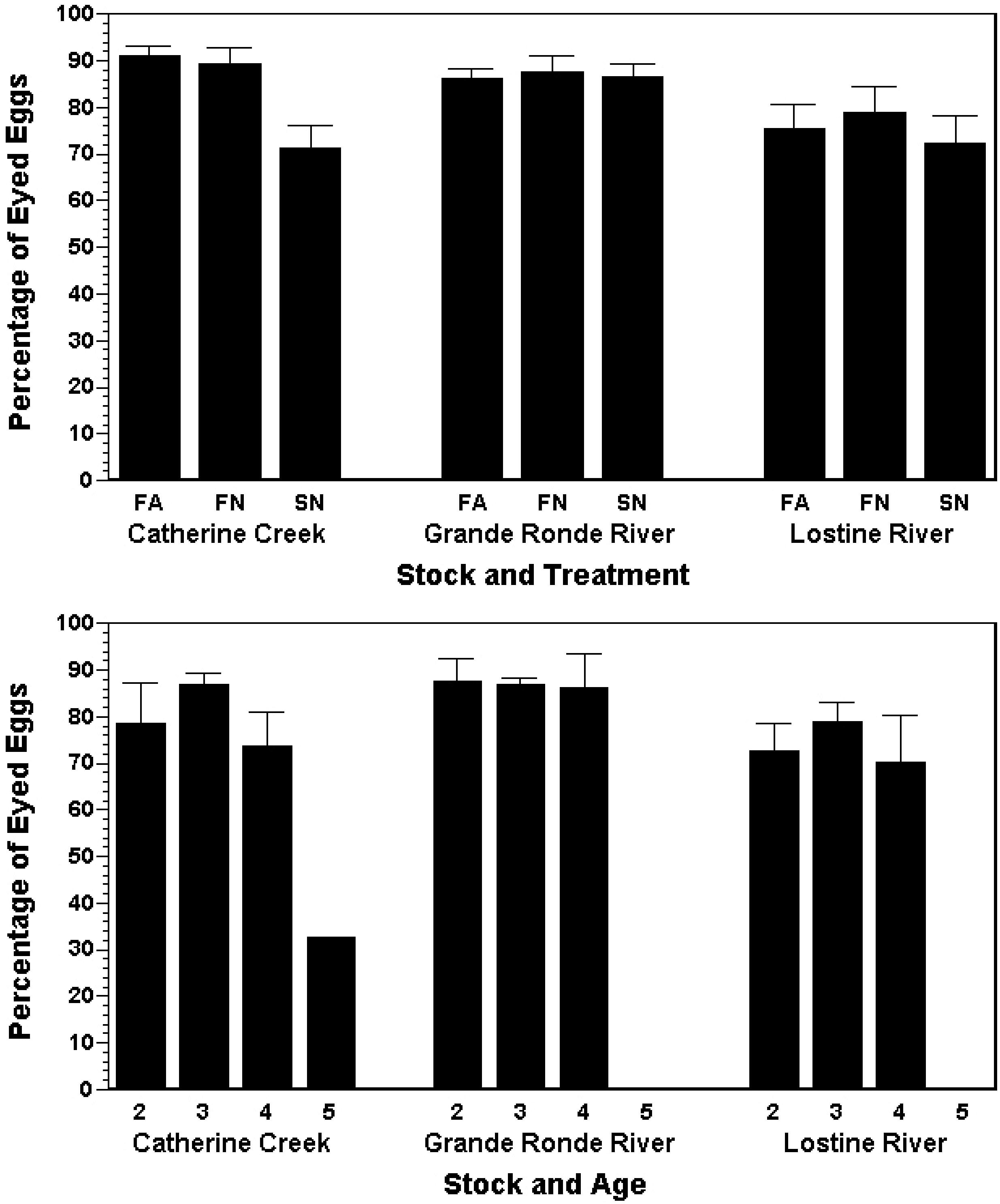


Figure 59. Mean (±95% CI) percentage of fertilized eggs (total eggs that reached eyed stage) fertilized with semen from spring chinook salmon males raised under Freshwater Accelerated (FA), Freshwater Natural (FN) and Saltwater Natural (SN) treatments (top) and from 3, 4, 5 and 6 year old (bottom) in 2000.

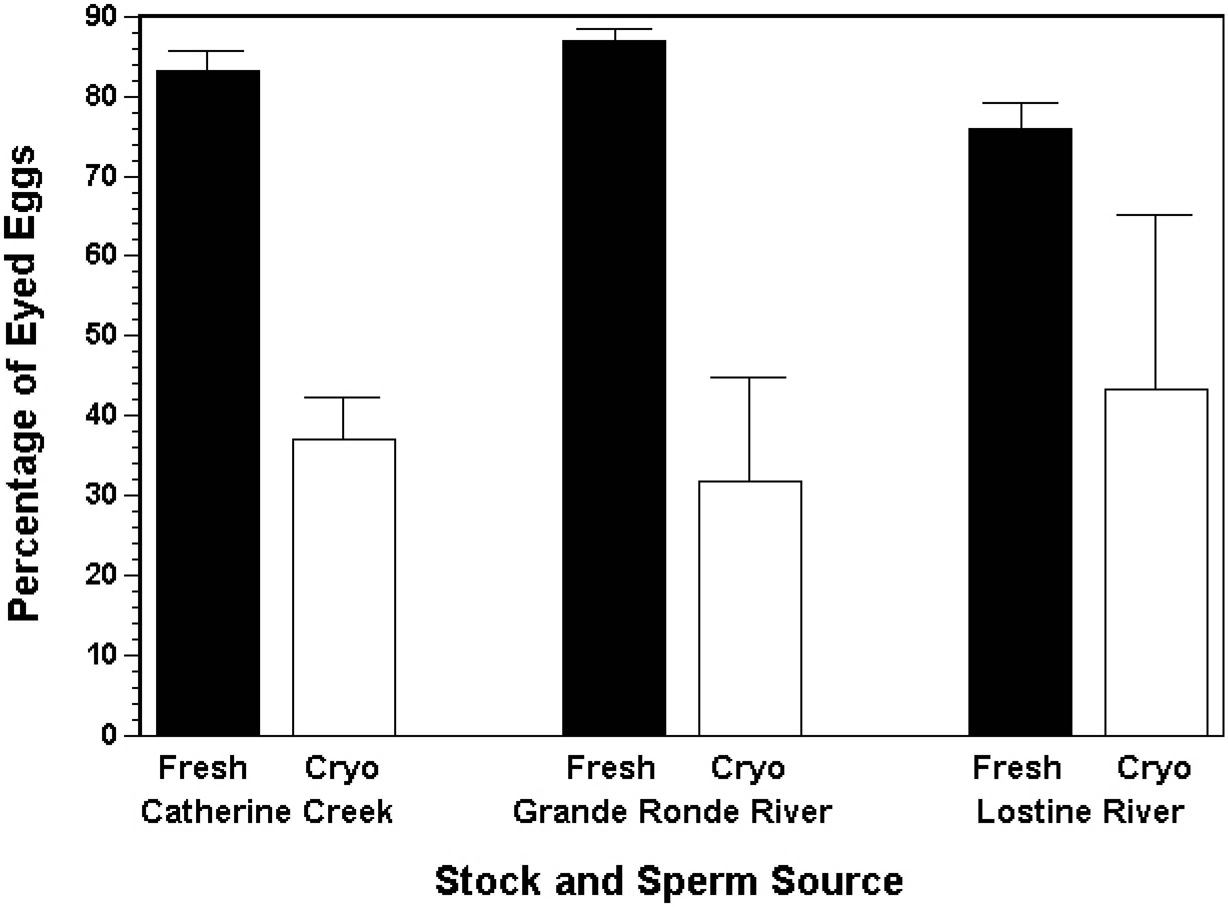


Figure 60. Mean (±95% CI) fertilization rate (percentage of total eggs that reached the eyed stage) of eggs that were fertilized with fresh or cryopreserved semen from Catherine Creek, Grande Ronde River and Lostine River males, 2000.

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.

<u>Parr collection</u>

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) for Lostine River 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 stock and year. 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 vary from each other.

<u>Growth</u>

Parr-to-smolt growth

Due to various problems at Lookingglass Fish Hatchery (chiller failures and water supply problems), we were unable to achieve acceptable pre-smolt treatments (Natural and Accelerated) which allowed us to achieve significantly different growth rates between treatments. Target smolt lengths at inception of this program were 186 mm for the Accelerated growth fish and 128 mm for the Natural growth fish. For the 1994-1998 cohorts, mean length at smoltification ranged from 112-134 mm for the Natural treatments and 126-132 mm for the Accelerated treatments. Although growth did not vary, 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, with the 1999 cohort, 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 between the Natural and Accelerated treatments. Mean length at smoltification ranged from 119-127 mm for the Natural fish and 132-149 mm for the Accelerated fish in the 1999 and 2000 cohorts.

Smolt-to-adult growth

Growth of the post-smolt fish has been slower than anticipated. Fish reared in freshwater grew faster and were larger at maturity for both sexes than those reared in saltwater. Surprisingly, the Natural treatment fish were able to match the size of the Accelerated growth fish within one year of smoltification - this is being seen in the 1999 and 2000 cohorts, as well, which were at significantly different lengths at smoltification. We appear to be seeing fish reaching a critical length of 500-550 mm at maturity, as size of mature males and females (females slightly larger) does not vary between 4, 5 and 6 year-old fish. Those fish that reach this threshold appear to mature, while those that do not reach that length wait for at least another year before maturing.

<u>Survival</u>

Parr-to-smolt survival

Parr-to-smolt survival has generally exceeded the expected rate of 95% (Figure 61). In general, all stocks and treatments survived well - mean survival was 96.9% and ranged from 86.8% (1994 Lostine River) to 99.4 (1997 Grande Ronde River and Lostine River). Mortality at

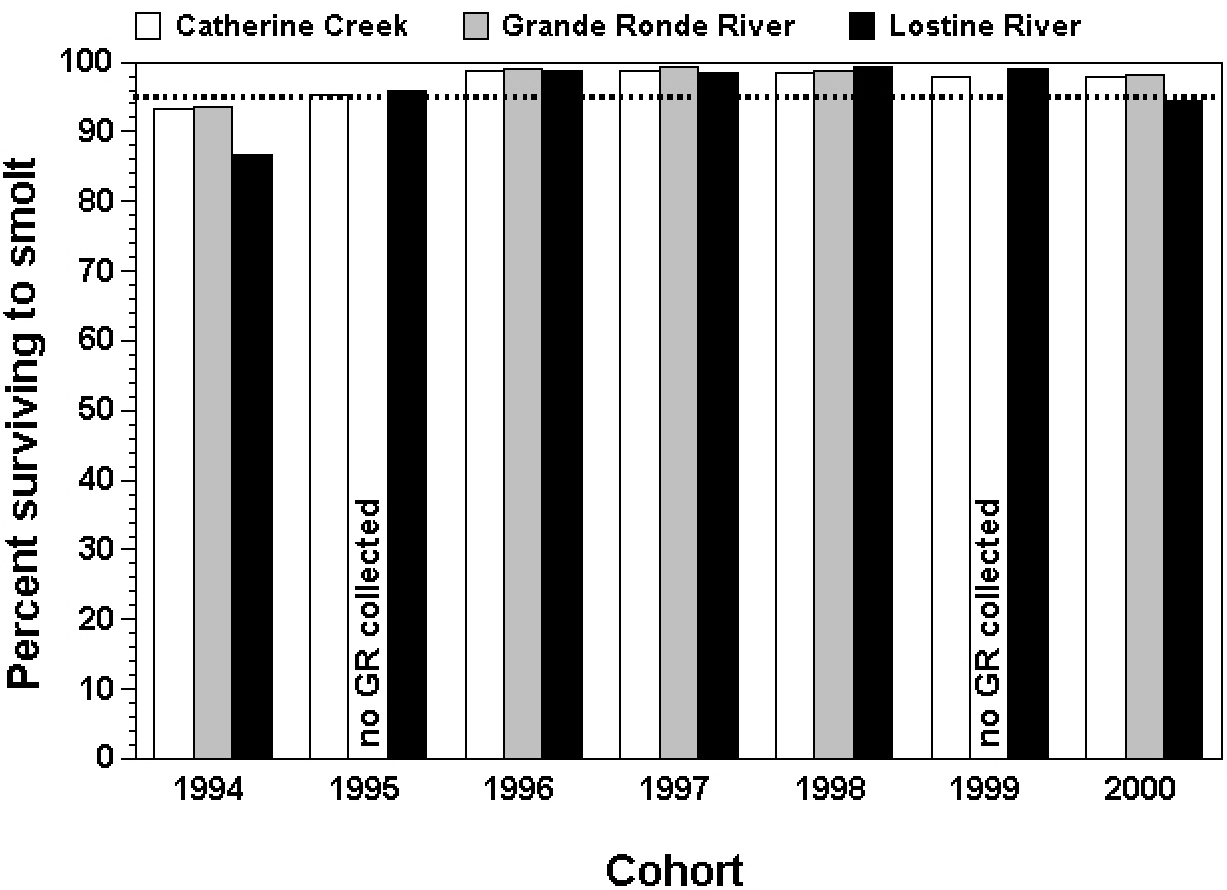


Figure 61. Parr-to-smolt survival for 1994-2000 cohorts of captive broodstock spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River parr collected for the Captive Broodstock Program, 1995-2001. Dashed line indicates anticipated value when the captive broodstock program was initiated.

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

Smolt-to-adult survival varied greatly but has been improving (Figure 62). The expected rate is 55% and we have exceeded this for 8 of 14 cohorts/treatments. Mean survival was 61.5% and ranged from 26.4% (1994 Grande Ronde River) to 83.3% (1997 Grande Ronde River). For treatment groups, we exceeded the expected for 11 of 13 cohorts/treatment groups. Mean smolt-to-adult survival rate was 62.6% and ranged from 36.7% (1995 Freshwater Accelerated) to 75.9% (1997 Freshwater Natural).

<u>Disposition</u>

We have assumed 50% survival from parr to spawn and we exceeded this goal for each of

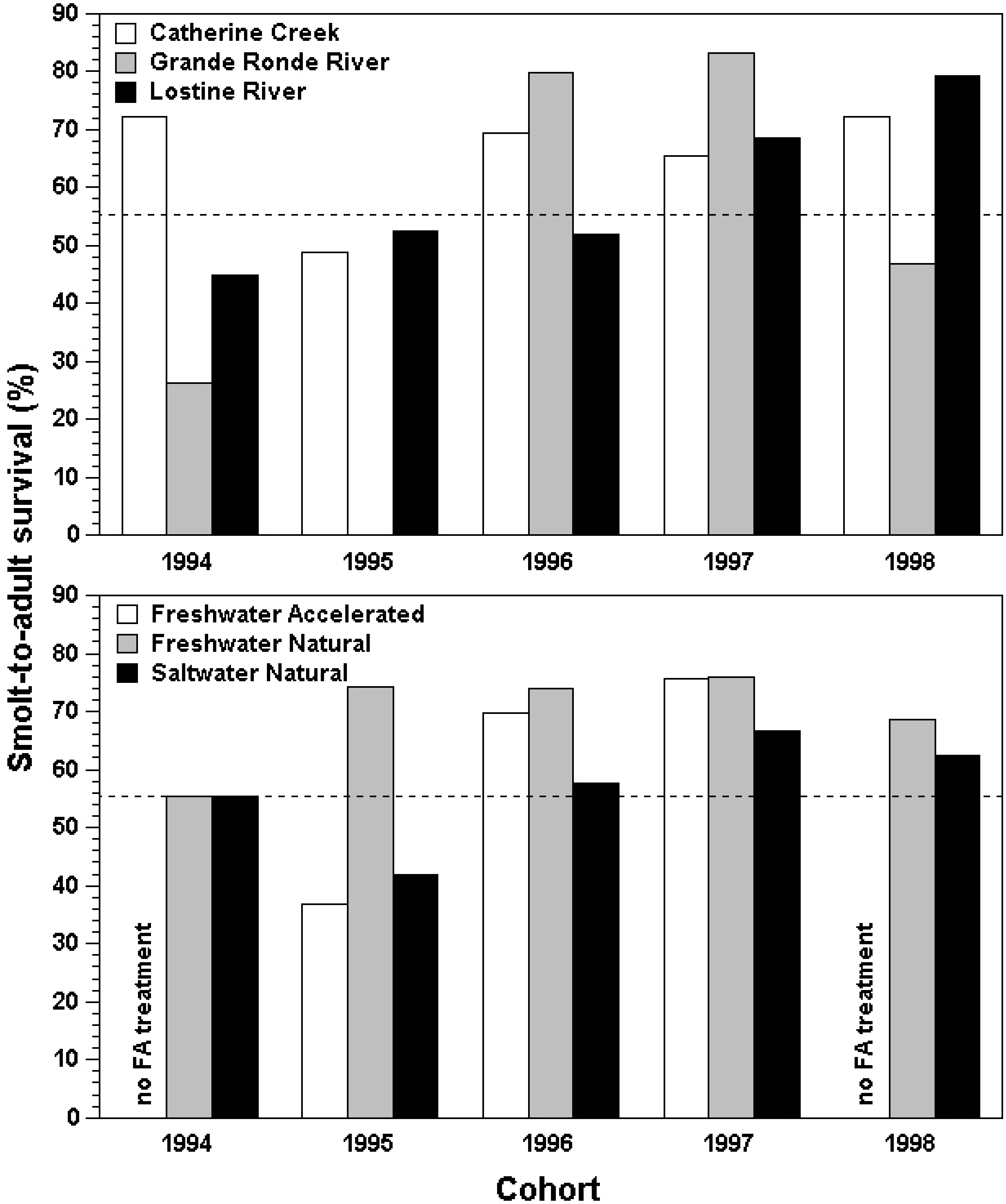


Figure 62. Smolt-to-adult survival for 1994-1998 cohort captive broodstock spring chinook salmon from Catherine Creek, Grande Ronde River and lostine River (top) reared under Freshwater Accelerated, Freshwater Natural and Saltwater Natural treatments (bottom). Dashed lines indicate anticipated value when the captive broodstock program was initiated.

the first four cohorts (Figure 63). Survival to spawn for the 1995-1997 cohorts 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 prespawn 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%.

Age of Maturity

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

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 later than expected (Figure 64). Very few age 3 females were spawned, only 0.4% of those surviving to maturity. 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. The Catherine Creek females tended to mature later than the Grande Ronde River and Lostine River females. For treatments, the Freshwater Accelerated females matured the earliest, followed by the Saltwater Natural and then the Freshwater Natural females. Age of maturity of females varied with treatment group ($P<0.0001$). Females from the Freshwater Accelerated group matured at an earlier mean age (4.1 years) than those of the Freshwater Natural (4.3 years) and Saltwater Natural treatment groups (4.2 years). Females in the Saltwater Natural group also matured significantly earlier 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 substantially earlier than expected (Figure 65). 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. The Catherine Creek and Lostine River males tended to mature earlier than the Grande Ronde River males. Within treatment groups, the Freshwater Natural and Freshwater Accelerated males tended to mature earlier than the Saltwater Natural males. Mean age of maturity of males varied with treatment group ($P<0.0001$). Males from the Freshwater Accelerated and Freshwater Natural groups matured at an earlier mean age (3.0 years) than those of the Saltwater Natural treatment group (3.2 years).

Size of Spawners

Size of 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 66). Mature females of age 6 were also similar in length to those of ages 4 and 5 while age 3 females were significantly smaller than older females. 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 Natural group were smaller

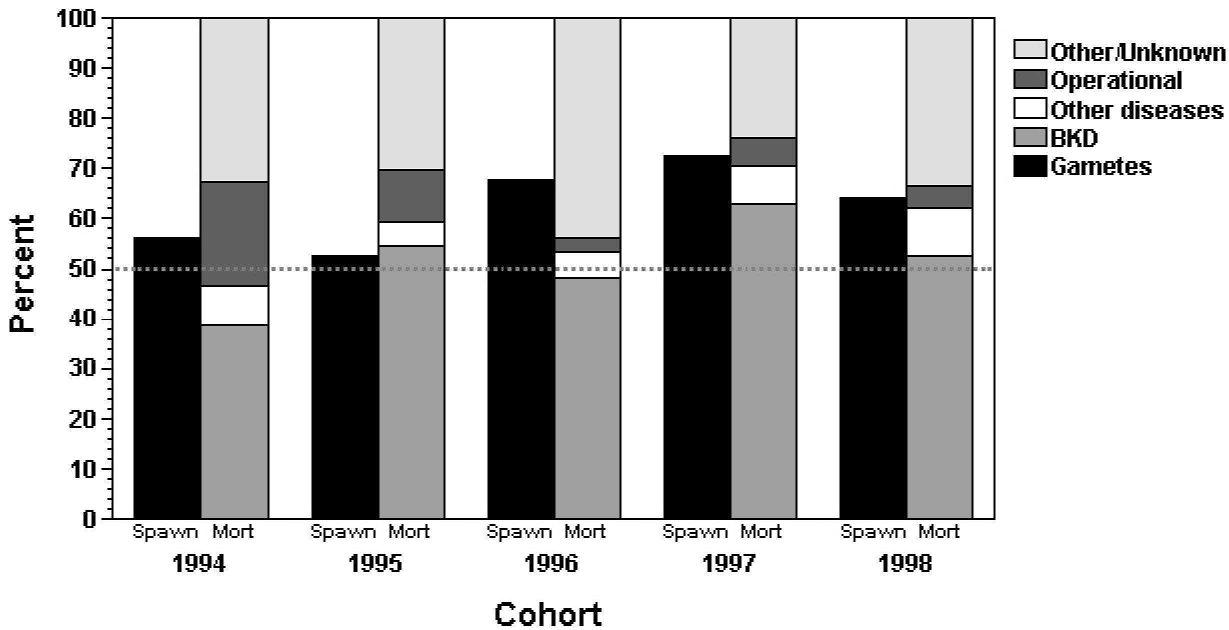


Figure 63. Disposition ("gametes" are fish that survived to reproduce) of 1994-1997 cohort captive broodstock spring chinook salmon. Dashed line indicates the anticipated percentage of fish to mature when the captive broodstock program was initiated.

(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). Age 4 males were larger than those of all other ages (Figure 66). Males of ages 3 and 5 were similar size but larger than ages 2 and 6 and age 6 was larger than age 2. 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 fecundity did not reach expected levels, except for the age 3 females (Figure 67). Mean fecundity for age 3 fish was higher than expected, although very few females matured at this age. Fecundity for older fish was much lower than expected. 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 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 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.

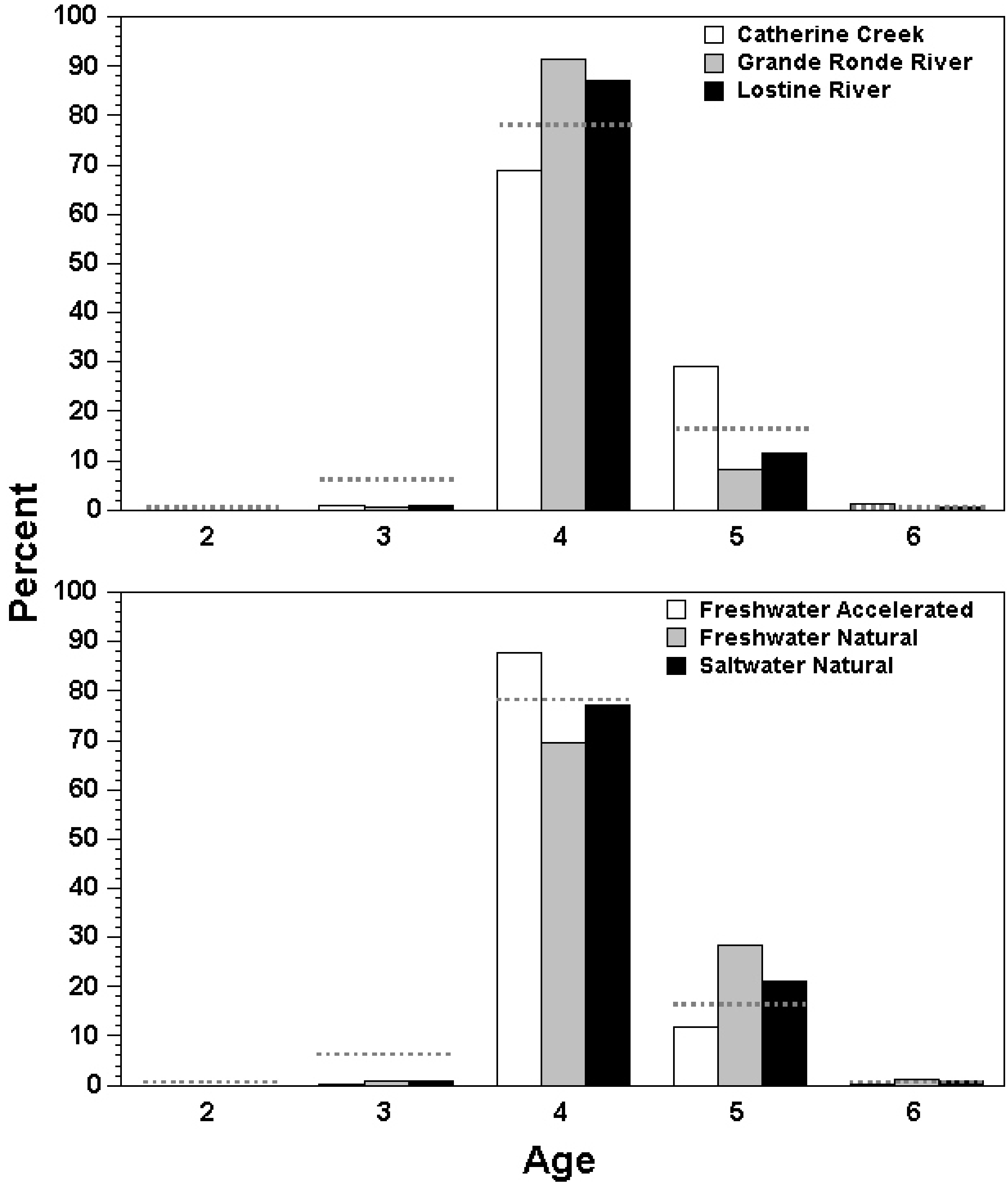


Figure 64. Percent of captive broodstock female spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River (top) and reared under Freshwater Accelerated, Freshwater Natural and Saltwater Natural rearing regimes (bottom) that matured at ages 2-6 (1994-1998 cohorts). Dashed lines show anticipated values for each age when the captive broodstock program was initiated.

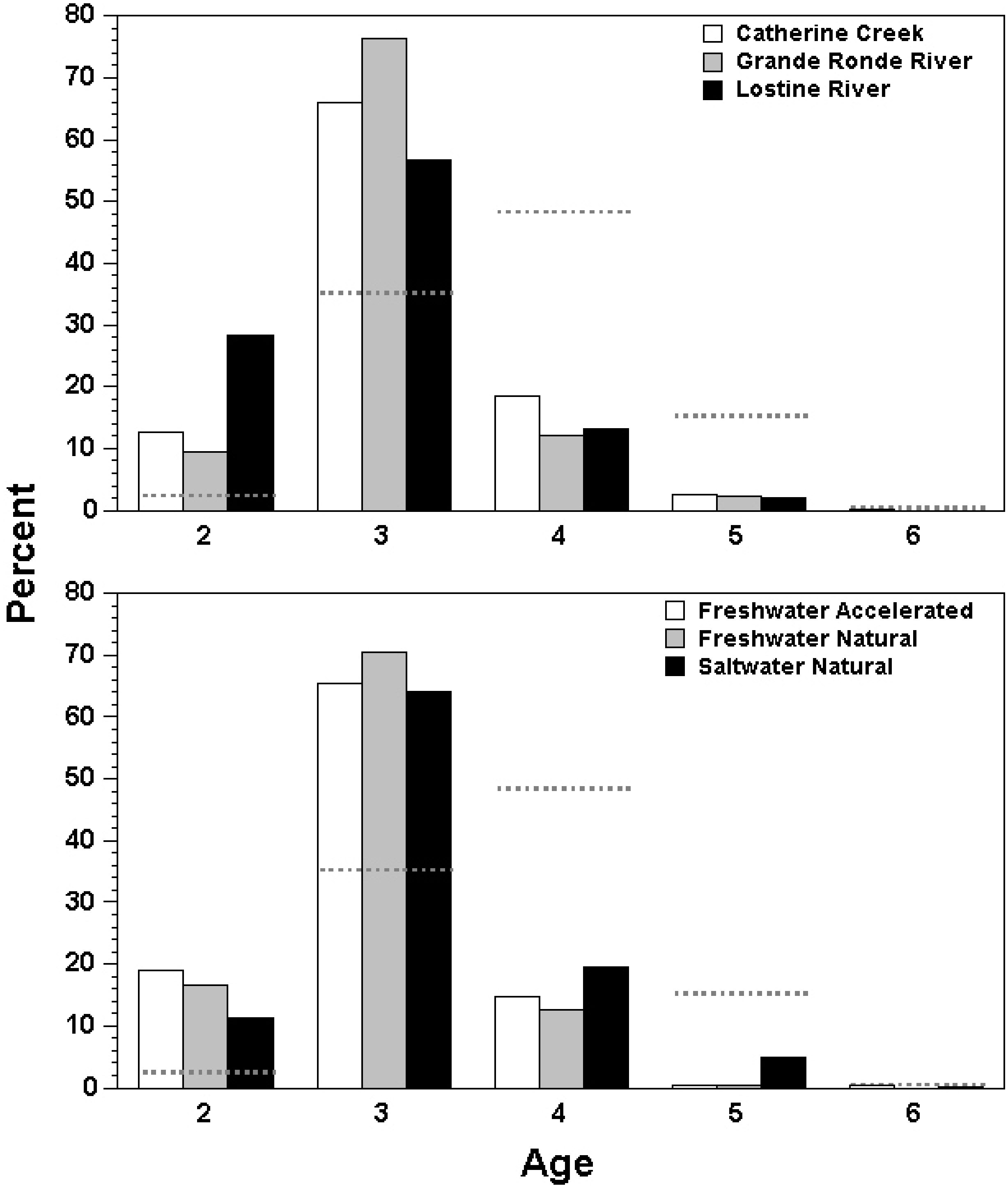


Figure 65. Percent of captive broodstock male spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River (top) and reared under Freshwater Accelerated, Freshwater Natural and Saltwater Natural rearing regimes (bottom) that matured at ages 2-6 (1994-1998 cohorts). Dashed lines show anticipated values for each age when the captive broodstock program was initiated.

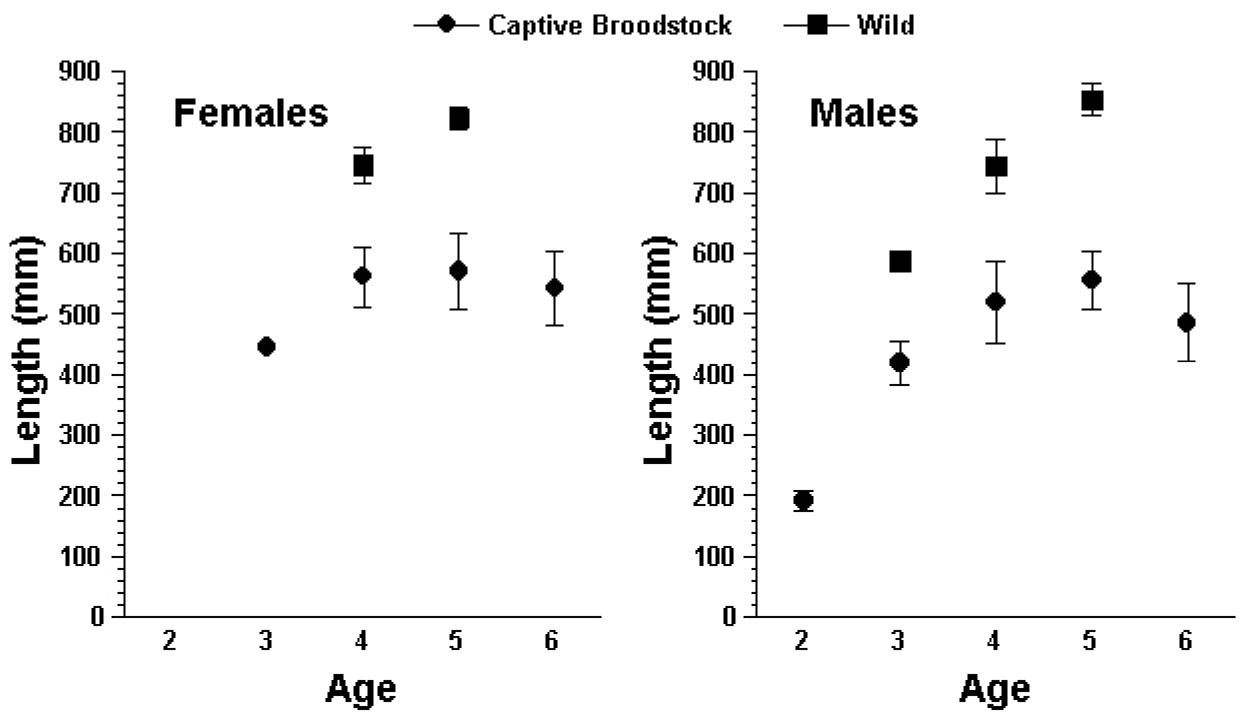


Figure 66. Mean (±1 SD) length for mature wild and captive broodstock female and male spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River at ages 2 - 6.

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.

<u>Egg Weight</u>

Mean weight of eggs varied between treatment groups and age 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 significantly 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 5-year old 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 1396 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).

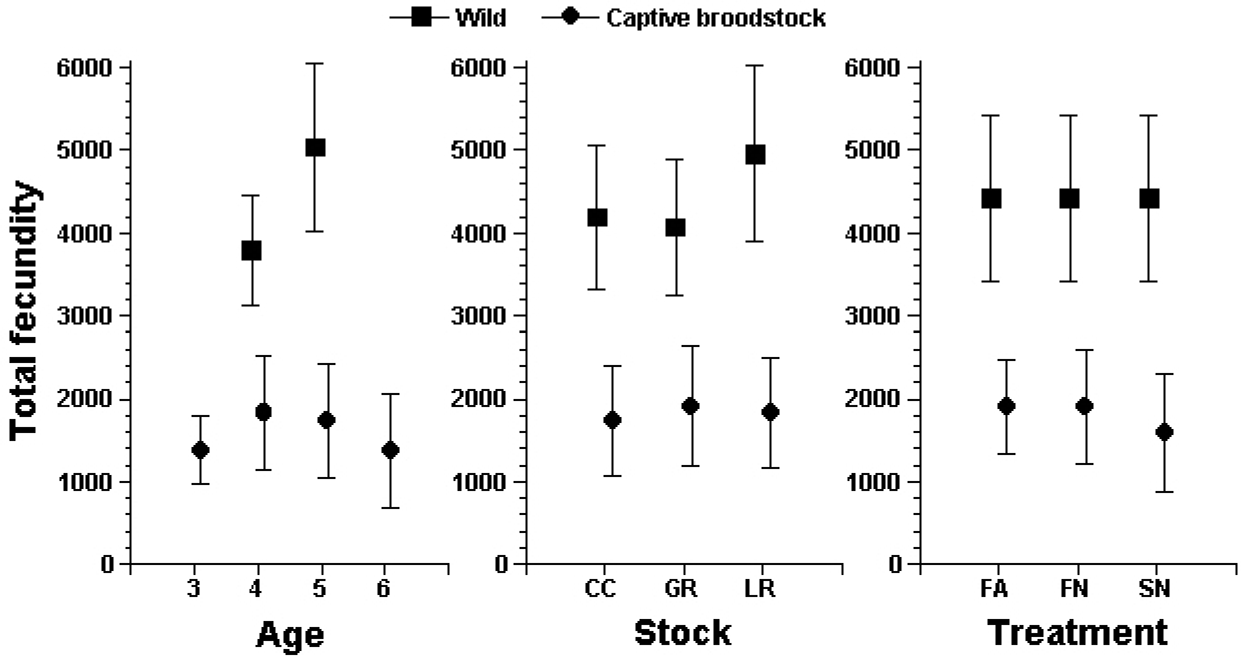


Figure 67. Mean (±1 SD) total fecundity for wild and captive broodstock spring chinook salmon for each age, stock and treatment (treatment values for wild salmon is for all stocks, combined).

Fertility

We assumed 75% embryo viability (percent of total eggs that reach the eyed stage). Mean fertility rate has been 78.4%. 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 2002 Grande Ronde River fish.

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

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 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.

The relationship of spermatocrit vs. fertility was also tested. 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 significantly (P<0.0001) smaller than wild chinook salmon at age of maturity (Figure 66). 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 size, wild females have greater fecundity than captive broodstock females (Figure 67). Captive broodstock salmon spawn an average of four weeks earlier 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 68).

F_1 Survival

Eyed egg-to-smolt survival

We assumed a 80% viable embryo-to-smolt survival. Mean egg-to-smolt survival has been 73.7% for the 1998, 1999 and 2000 cohorts of the three stocks (Figure 69). We have exceeded 80% survival for the 1998 and 2001 cohorts of Catherine Creek and 1998 cohort Lostine River salmon.

Post-smolt Survival

To date, only two years of captive broodstock F_1'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 rate of 0.1% smolt-to-adult survival. 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.

F_1 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: 7% of the Catherine Creek and 6.8% of the Lostine River 1998 cohort returns (and this percentage should 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 in order to maintain the genetic diversity and identity of the endemic Catherine Creek, Lostine River and Grande Ronde River stocks. The primary goal of this program is to conserve of the genetic resources of endemic stocks in each of the project streams. Over the eight years that this project has been underway, we have modified some practices to improve our ability to reach our gene conservation and production goals. The following is a discussion of some of the successes and failures and subsequent modifications that have or will be made to the program.

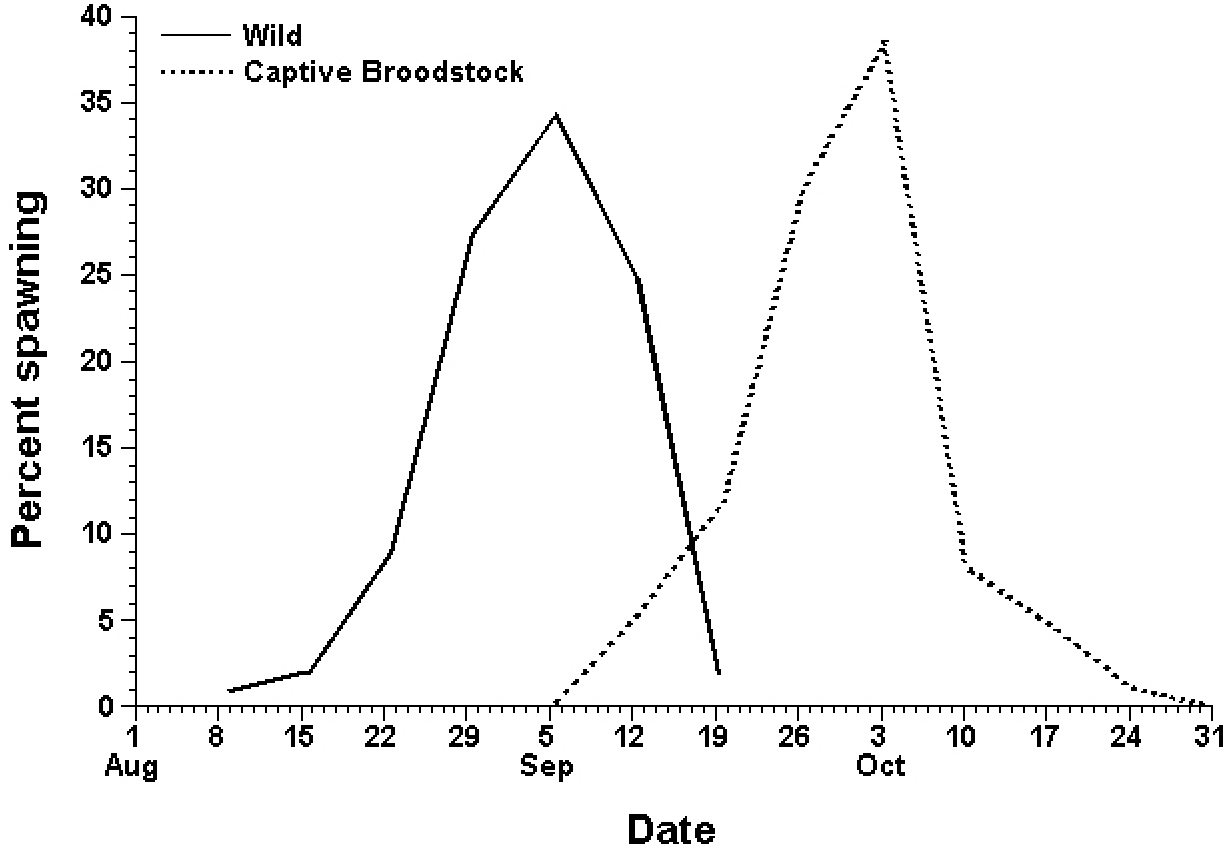


Figure 68. Percent of wild (collected for conventional broodstock spawning at Lookingglass Fish hatchery) and captive broodstock spring chinook salmon spawning at weekly intervals.

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 their 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. 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 erythromycin treatments, with scheduled handling, whenever possible. 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 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 2002 spawning season and will continue to do so in 2003. Initial results are promising for both methods.

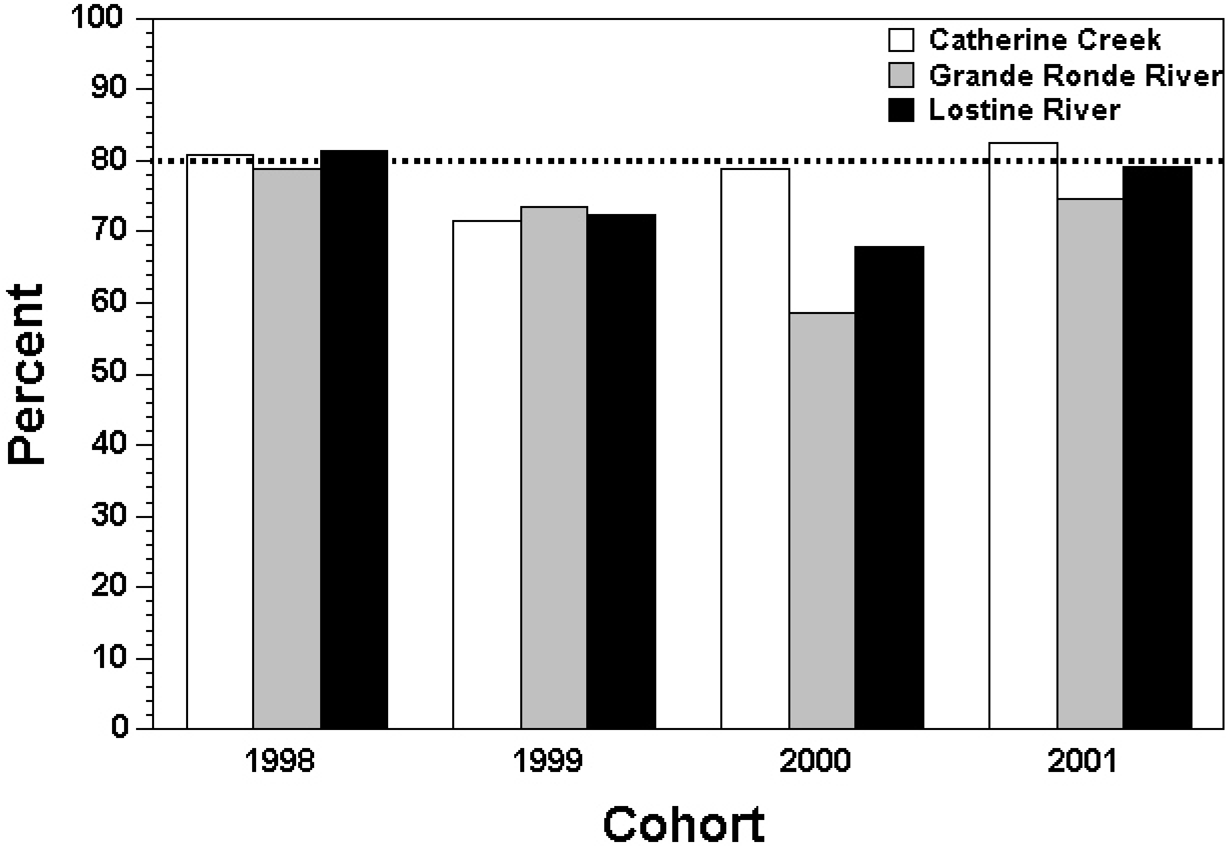


Figure 69. Eyed egg-to-smolt survival for 1998 - 2000 cohort captive broodstock F_1 spring chinook salmon from Catherine Creek, Grande Ronde River and Lostine River stocks. Dashed line indicates the anticipated value when the captive broodstock program was initiated.

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.

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 natural fish being at a mean size at smoltification that is similar to that of wild smolts in this system and the accelerated growth fish are significantly larger. 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).

Table 16. Number of captive broodstock progeny returning as adults for each stock and cohort, as of 20 September 2002. Note: 2002 return year (1998 cohort age 4 and 1999 cohort age 3) numbers are based on captures at weir - actual return is higher and will be adjusted based on results of coded wire tags recovered from spawning ground surveys.

	1998 cohort					1999 cohort
Stock	Age 3 males (1998 cohort +	Age 4 males (1998 cohort)	Age 4 females (1998 cohort)	Total	Return rate	Age 3 males
Catherine Creek	10	62	70	142	0.37%	29
Grande Ronde River	0	0	3	3	0.20%	0
Lostine River	19	121	138	278	0.79%	25

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

We have also modified the experimental design so that the fish are now reared at equal densities at both BOH and MML. The addition of a Saltwater Accelerated group in the 2000 cohort means that 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. The addition of this group would have made spawning too complex, however, so we have simplified our spawning activities to spawn fish within post-smolt (freshwater or saltwater) rearing groups.

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 *Renibacterium salmoninarum*, the causative agent of BKD. Preliminary data were promising and suggested that mortality, for the first 20 months after vaccination, due to BKD of vaccinated fish was lower than that experienced by previous cohorts of unvaccinated fish. However, severe mortalities in late 2001 in both the saltwater- and freshwater-reared 1998 cohort of Grande Ronde River salmon (and lesser, but still high, levels of mortality in other stocks) indicated the vaccination may have only delayed the onset of BKD. In addition, preliminary data on Renogen in chinook salmon (Dr. Mark Strom, NMFS, personal communication) showed it to be ineffective. Therefore, we decided to not vaccinate the 2001 cohort and will wait for a better vaccine to be developed (Dr. Strom has promising results with another vaccine).

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 stock 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-smoltification growth has been substantially lower than expected, spawning fish are much smaller and females have fewer eggs than comparably-aged wild fish, especially for those 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 correct it, if possible. The cause of this is likely 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.

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, PIT tags (134 KHz) 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.

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

In 1999, we measured spermatocrit of males with the thought that variation in this parameter might relate to fertilization rate. As reported above, although there was significant variation in spermatocrit among treatment groups, spermatocrit did not significantly relate to fertilization rate and this activity was discontinued.

Rate of fertility was measured 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 total eggs collected 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 70). 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, then using Method 2 is just as accurate to measure fertilization rate for our purposes and much easier to determine. Therefore, we have discontinued measuring fertilization based on the number of eggs showing cell division and future analyses will determine fertilization rate by the percentage of eggs

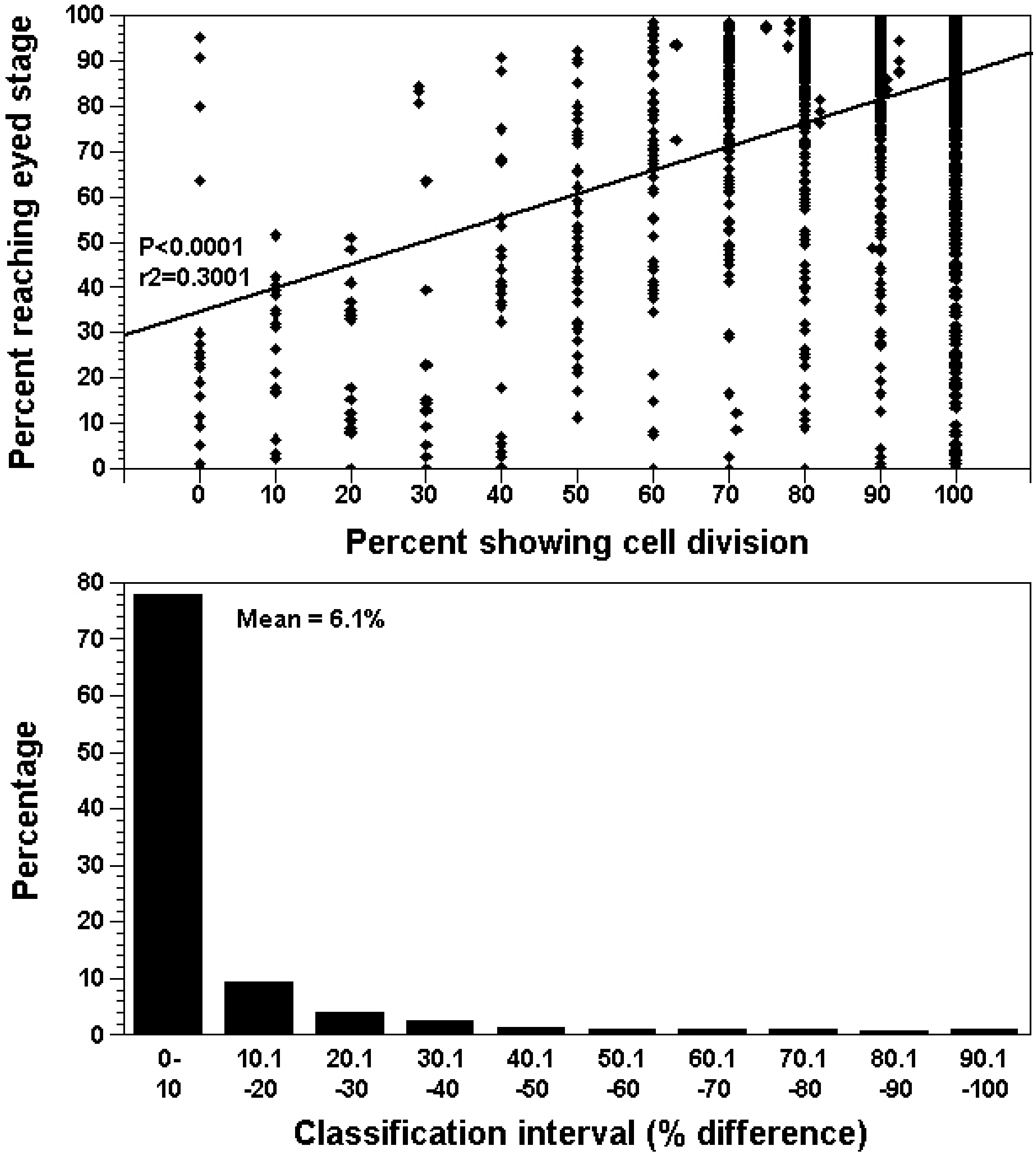


Figure 70. Relationship between percentage of eggs showing cell division and percentage of eggs reaching the eyed stage (top) and distribution of differences in fertilization estimates between the two methods (bottom).

reaching the eyed stage.

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 - MS Access[1]). 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 ready for use 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 also still have some things to learn The comanagers of the stocks 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 of have targeted for release as smolts. The Grande Ronde Hatchery Management Plan will be implemented in 2003.

If sufficient hatchery space is available, they can be raised to smoltification and released into their home stream or one of its tributaries that presently has few or no chinook salmon. If hatchery space is unavailable to raise them to smoltification, then they can be released as fry or parr into their home stream or tributary. We had more F_1 2001 cohort juveniles than we could rear, particularly under treatment and BKD segregations. We have specific outlet streams (tributaries of the program streams or other nearby streams) into which we can release excess fish. In 2002, we released the excess fish as 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 (Figure 67). This means that the captive brood fish may not spawn with wild fish, which would be the ideal result of releasing them. Additionally, any deposited eggs will 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. So, we need to learn the reasons for this late spawning in captive brood fish and correct it before any fish will be released to spawn in the wild. We may also be able to partially spawn them to obtain eggs for the program and then release them to complete their spawning in the wild. We hope to conduct experiments to determine the utility of this option.

If excess eggs are collected, they can be planted in their home streams and allowed to develop and hatch in the wild. However, there is still the problem of late spawning. This 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.

Lastly, if we are able to consistently produce more smolts than targeted for this program,

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

a reduction in the number of parr collected will be warranted. This contingency was expected at some time in the future and 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 experiments. Also, if sufficient numbers of sdults return to these streams, the captive broodstock program may be reduced or terminated for a given stream (in deference to a conventional broodstock program). In that event, we may begin a captive broodstock program in another stream.

As previously stated, bacterial kidney disease is the largest source of mortality in captive broodstock fish and we often spawn females with relatively high infections of *R. salmoninarum*. For the 2000 - 2002 F_1 cohorts, we have culled eggs from these females 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 F_1 generation (by curtailing vertical transmission of this disease) and reduce the amount of mortality that it causes in these fish in the hatchery (from both vertical and horizontal transmission) and wild fish (horizontal transmission) upon their release. It may also reduce the amount of BKD in this system 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 predicatable. The risks of vertical and horizontal transmission are poorly understood in hatcheries and appear to be even less understood in the wild. Vertical transmission is likely a function of the severity and location of an infection in the female. 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 her eggs, which is counter to the primary goal of this project - genetic conservation. 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 possibility or severity of a BKD outbreak? 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? 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 the Snake and Columbia river dams. Answering 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 stocks. 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 F_1 fish, we will rear an entire raceway of high BKD fish from the Grand Ronde River at low density. This will give us more information concerning our ability to rear these fish - the conflicting risks of culling to reduce BKD or rearing potentially sick fish to maintain genetic diversity.

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