BONNEVILLE POWER ADMINISTRATION

Grande Ronde Basin Chinook Salmon Captive Brood and Conventional Supplementation Programs

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Fish Research Project
Oregon

Project Title**:** Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Program

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INTRODUCTION

BPA Fish and Wildlife Program Project Number 1998-01-001 provides funding for the Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Program. This report satisfies the requirement that an annual report be submitted for FY 2003.

The Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Project is designed to rapidly increase numbers of salmon in stocks that are in imminent danger of extirpation. Parr are captured in Catherine Creek, upper Grande Ronde River and Lostine River and reared to adulthood in captivity. Upon maturation, these fish are spawned (within stocks) and their progeny reared to smoltification before being released into the natal stream of their parents. This program is co-managed by ODFW, National Marine Fisheries Service, Nez Perce Tribe and Confederated Tribes of the Umatilla Indian Reservation.

This report covers activities conducted and provides data analyses for the Grande Ronde Spring Chinook Salmon Captive broodstock Program from 1 January – 31 December 2003. Since the fiscal year ends in the middle of the spawning period, an annual report based on calendar year is more logical. This document is the FY 2003 annual report. Detailed information on historic and present population status, project background, goals and objectives, significance to regional programs and relationships to other programs, methods and previous results are available in the 1995-2002 Project Status Report (Hoffnagle et al 2003).

DESCRIPTION OF STUDY AREA & FACILITIES

The Grande Ronde River drainage basin covers approximately 10,700 km^2 of northeast Oregon. The headwaters are in the Blue and Wallowa mountains and it enters the Snake River at RK 272, upstream from Asotin, WA. Catherine Creek and Lostine River are two of the larger tributaries to the Grande Ronde. Catherine Creek flows directly into the Grande Ronde River at RK 224 downstream from La Grande, OR. The Lostine River flows into the Wallowa River near the town of Wallowa, and the Wallowa River enters the Grande Ronde at RK 132. Salmon in the Grande Ronde basin must migrate 1,120 km between the ocean and spawning and rearing habitat in the upper basin. This takes them through eight dams and associated reservoirs on the Columbia and Snake rivers.

Prior to 2003 parr were collected and taken to Lookingglass Fish Hatchery for pre-smolt rearing. Parr collected in 2003 (2002 cohort) were transported to Wallowa Fish Hatchery (WFH) near Enterprise, Oregon. Post-smolt rearing takes place at Bonneville Fish Hatchery (BOH; freshwater) on the Columbia River near Cascade Locks, Oregon, and at Manchester Research Station (MRS; saltwater) on Puget Sound near Port Orchard, Washington. Detailed information on the study area and facilities is available in the 1995-2002 Project Status Report (Hoffnagle et al 2003).

METHODS

Pre-smolt phase

We attempt to collect 500 parr from each of the study streams during August using passive seining techniques. We collected the parr from throughout the drainage to incorporate as

much genetic variability as possible into the captive broodstock. At the end of each day's collections fish were transported to Wallowa Fish Hatchery (WFH) where they were weighed, measured and separated into either accelerated or natural growth regime groups. At WFH they are being reared in twelve 1.2 m diameter circular tanks to smolts. Accelerated growth regime groups are being reared on well water at constant 13^{o}C. The natural growth regime groups are being reared on spring water that varies seasonally from 4.6^{o}C to 13^{o}C. While at Wallowa fish are inventoried, injected with antibiotics for bacterial kidney disease (BKD) prevention, given individual PIT-tags and inoculated for vibrio.

Post-smolt phase

As fish reached the smolt phase they were transferred to either Bonneville Fish Hatchery (BOH) for rearing in freshwater, or Manchester Research Station (MRS) for rearing in saltwater. During this phase fish are reared in separate tanks for each population and cohort. If numbers are low, five and six year old fish from the same stock may be combined. Fish are sampled periodically for growth and treated for disease as needed.

All fish are sampled for maturity each spring and all maturing fish are transported to and/or kept at BOH where spawning takes place between late August and mid October. Sex ratio of maturing fish is calculated and that information is used to determine number and type of spawning matrices for each population and treatment during spawning. Our objectives are to mimic the natural sex ratio in the spawning population, maximize the number of family groups and minimize potential for sibling crosses. Whenever there are too few live males available for a spawning matrix cryopreserved semen is used. Spawning protocols change when cryopreserved semen is used since mean fertilization rate is much lower (~35%) than for fresh sperm (~85%).

Fertilized eggs are transported to Oxbow Fish Hatchery (OFH) for incubation to the eyed stage. Once eyed, eggs are transported to Irrigon Fish Hatchery for hatching. After hatching fry are transported to Lookingglass Fish Hatchery for rearing until smoltification when they are acclimated and released in their parents' natal stream.

Throughout the program, hatchery and fish health personnel regularly monitor fish health and condition and disease treatments are carried out as appropriate. The most common and survival-threatening infection has proven to be BKD. All mortalities are inspected by fish health specialists to determine cause of death.

RESULTS

1998 – 2001 Cohorts

Activities Conducted

During 2003, 1,516 parr from the 2002 cohort were collected from Catherine Creek, upper Grande Ronde River and Lostine River. During the same year 1,526 fish were removed from the captive population: 671 fish were spawned, 153 additional mature fish were killed but not spawned (due to either gross BKD or tumors in the 2000 GR fish) and 702 fish died from various other causes (Table 1). Pre-smolt rearing was conducted at WFH, followed by rearing to adulthood at either BOH or MRS. Mortalities in 2003 were sent to either the Clackamas, La Grande or Manchester fish health laboratories for examination and determination of cause of death. A detailed account of the Fish Health Lab findings is provided in the Fish Health

Monitoring and Disease section of this report. Here, we account for all fish remaining or removed from each cohort of the captive population from 1 January - 31 December 2003. No fish from the 1994-1997 cohorts were alive in 2003.

1998 Cohort

A total of 500, 500 and 498 parr were collected in 1999 from Catherine Creek and upper Grande Ronde and Lostine rivers, respectively. As of 31 December 2003, there were no fish remaining alive (Table 1). During 2003, the final 68 fish from the 1998 cohort were removed from the captive population: 21 survived to gamete collection, 12 died due to BKD and 35 died and remain to be examined by pathology.

Table 1. Number of fish collected, mortalities, surviving to gamete collection and remaining for each cohort and stock (CC = Catherine Creek, GR = upper Grand Ronde River, LR = Lostine River) of the Grande Ronde Basin chinook salmon captive broodstock, as of 31 December 2003.

		Mortalities							
Cohort/ stock	Number collected	BKD	Other diseases	Opera-tional	Other	Un-known	Undeter-mined[1]	Survived to gamete production	Number remaining
1998									
CC	500	7					18	9	0
GR	500	2					7	2	0
LR	498	3					10	10	0
1999									
CC	503	1					55	192	17
LR	500	1				1	83	162	13
2000									
CC	503			1			49	148	255
GR	502	1		5			134	140	182
LR	503			1			114	92	178
2001									
CC	500			2			30	20	445
GR	461						13	13	430
LR	500		3	1	4		74	32	375
2002									
CC	513	2	10	14	8	1	0	0	479
GR	503	2		8	2		0	0	488
LR	500		6	4	6	4	2	0	477

[1]Many of the 'Undetermined' mortalities will be changed at a later date as complete pathology data become available.

1999 Cohort

A total of 503 and 500 parr were collected in 2000 from Catherine Creek and Lostine River, respectively. No upper Grande Ronde River fish were captured in 2000. As of 31 December 2003, there were 30 fish remaining alive (Table 1). During 2003, 496 fish were removed from the captive population: 354 survived to gamete collection, 2 died due to BKD and 1 died due to unknown causes. One hundred thirty-nine mortalities remain to be examined by pathology.

2000 Cohort

A total of 503, 502 and 503 parr were collected in 2001 from Catherine Creek and upper Grande Ronde and Lostine rivers, respectively. As of 31 December 2003, there were 615 fish remaining alive (Table 1). During 2003, 686 fish were removed from the captive population: 380 survived to gamete collection, 1 died due to BKD and 7 due to operational causes. Two hundred ninety-eight are undetermined and remain to be examined by pathology.

2001 Cohort

A total of 500, 461 and 500 spring chinook salmon parr were collected from each of Catherine Creek and Grande Ronde and Lostine rivers in August 2002. Of the original 1,461 fish collected 1,250 fish were still alive as of 31 December 2003. Of the 193 fish removed from the population, 65 produced gametes, 3 fish died from BKD or other diseases, 3 from operational causes and 4 from other/unknown causes. An additional 118 fish remain to be examined by pathology.

Growth

Fork length and weight were measured on captive fish during inventories, maturity sorting, samplings and transfers. After the first maturity sort (late March/early April for 1998-2000 cohorts and mid-July for 2001 cohort), only those fish classified as immature were examined and measured. Here we provide information on size of the 1998-2001 cohorts, by stock and treatment, during April, July (immature fish) and September/October (spawning fish) (Tables 2-5).

Growth of the captive broodstock fish is somewhat different than expected when this program was designed (Tables 2-5). Pre-smolt growth was expected to result in simulated natural fish attaining a mean fork length of 120 mm by the time of smoltification. This is the only group that has attained its target size. The accelerated growth treatment group was expected to reach 186 mm at smoltification. Although the 2001 cohort accelerated growth fish were significantly larger than the natural growth fish, mean length did not approach the target at the April inventory (Table 5).

Post-smolt growth in both freshwater and saltwater has also been different than expected (Tables 2-5). In nearly all stocks and cohorts, the saltwater fish grew more slowly than those in freshwater. The anticipated growth rate would have the natural growth fish reaching 120 mm fork length at the time of smoltification, 220 mm in August of their second year, 480 mm in August of their third year, 730 mm in August of their fourth year and 870 mm in August of their fifth year. The accelerated growth fish were expected to be 186 mm at smoltification and 278, 538, 788 and 928 mm in August of their second, third, fourth and fifth years, respectively. At all ages, the captive broodstock were smaller than expected. It is noteworthy that, although the accelerated pre-smolt growth fish were larger at smoltification, size of the natural and

accelerated fish was similar within one year after smoltification. This may be due to one or a combination of compensatory growth on the part of the natural fish or removal of the larger accelerated fish due to maturation.

Maturity, Spawning and Incubation

Fish from the 1998-2000 cohorts were first examined for signs of maturity on 9-10 April 2003 at MRS and 16-18 April at BOH. The 2001 cohort fish were first examined for signs of maturity on 21-24 July 2003 at BOH and MRS. Additional maturity sorts were conducted for each cohort and stock based on guidelines in the 2003 Captive Broodstock Annual Operating Plan (AOP). Maturity data include all fish that were assumed to be maturing, whether they survived to gamete collection, died prior to gamete collection or were later determined to be immature and returned to immature tanks. Maturing fish were transferred form MRS to BOH and were examined for ripeness at BOH approximately weekly from 30 August - 11 October 2002.

Gametes were collected from the 1998-2001 cohorts in 2003 (Table 6). Males in excess of those needed to spawn had their semen collected and cryo-preserved – forty-nine semen samples were collected for cryo-preservation in 2003. Approximately one-half of these samples are being stored at BOH and the other half at the regional repository at the University of Idaho, Moscow. All spawning was done using spawning matrices developed following guidelines identified in the 2003 Captive Broodstock AOP. One hundred thirty-six spawning matrices were used in 2003.

Table 2. Mean fork length (FL) and weight (W) in April (all fish), July (immature fish) and September/October (spawning fish) of 1998 cohort chinook salmon collected from Catherine Creek, Grande Ronde River and Lostine River and reared in either freshwater (FW; Bonneville Fish Hatchery) or saltwater (SW; Manchester Marine Laboratory), 2003.

							September/October (mature)					
	April (all fish)			July (immature)			Male			Female		
Stock, growth regime	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)
Catherine Creek												
FW	13	528	1974	0	-	-	0	-	-	6	539	1860.1
SW	13	500	1728.3	0			1	574	1918.4	3	527	1734.5
Grande Ronde River												
FW	6	577	2646.6	3	593	2909.2	0	-	-	1	577	2083.7
SW	3	589	2457	2	589	2163	2	586	1987.2	1	556	1970.5
Lostine River												
FW	9	585	2510	0	-	-	0	-	-	4	581	2153.3
SW	11	536	2222.8	0	-	-	0	-	-	7	552	1967

Table 3. Mean fork length (FL) and weight (W) in April (all fish), July (immature fish) and September/October (time of spawning of mature fish) of 1999 cohort chinook salmon collected from Catherine Creek, Grande Ronde River and Lostine River and reared under either a freshwater accelerated (FA) or freshwater natural (FN) growth regime at Bonneville Fish Hatchery or saltwater natural (SN) growth regime at Manchester Marine Laboratory, 2003.

							September/October (mature)					
	April (all fish)			July (immature)			Male			Female		
Stock, growth regime	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)
Catherine Creek												
FA	81	518	2041.3	1	557	2529.3	7	512	1415.7	66	525	1637.4
FN	93	521	2088.6	1	469	1238.5	18	518	1463.5	63	540	1709.7
SN	72	493	1904.4	6	489	1594.9	4	510	1275.7	27	536	1919.6
Grande Ronde River												
FA	0	-	-	0	-	-	0	-	-	0	-	-
FN	0	-	-	0	-	-	0	-	-	0	-	-
SN	0	-	-	0	-	-	0	-	-	0	-	-
Lostine River												
FA	78	527	2128.2	2	494	1767.5	5	472	1015.4	54	543	1704.7
FN	84	555	2450.8	4	526	1920.2	9	562	1767	54	563	1952.3
SN	92	487	1897.8	0	-	-	7	475	1096.7	58	518	1684.7

1998 Cohort

Forty-four fish from the 1998 cohort were determined to be maturing during maturity sorts: 21 were spawned (1 male and 20 females) and no males had semen cryo-preserved (Table 6). A total of 21,454 eggs were collected and 16,030 of those eggs (74.7%) survived to the eyed stage (Table 7).

1999 Cohort

A total of 459 fish from the 1999 cohort were determined to be maturing during maturity sorts: 357 were spawned (57 males and 300 females) and no males had semen cryo-preserved (Table 6). A total of 439,909 eggs were collected and 358,341 of those eggs (81.5%) survived to the eyed stage (Table 7).

2000 Cohort

Five hundred and sixteen 2000 cohort fish were determined to be maturing during maturity sorts: 381 males and one female were spawned. Of the males, 32 had semen cryo-preserved (Table 6).

Table 4. Mean fork length (FL) and weight (W) in April (all fish), July (immature fish) and September/October (time of spawning of mature fish) of 2000 cohort chinook salmon collected from Catherine Creek, Grande Ronde River and Lostine River and reared under either a freshwater accelerated (FA), freshwater natural (FN) or saltwater natural (SN) growth regime at Bonneville Fish Hatchery (freshwater) or Manchester Marine Laboratory (saltwater), 2003.

							September/October (mature)					
	April (all fish)			July (immature)			Male			Female		
Stock, growth regime	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)
Catherine Creek												
FA	110	353	645.6	0	-	-	45	380	607.4	0	-	-
FN	119	362	704.9	0	-	-	42	400	694	0	-	-
SA	101	344	629.7	0	-	-	41	375	566	0	-	-
SN	118	350	638.2	0	-	-	38	373	540.2	1	372	809.5
Grande Ronde River												
FA	112	366	725.8	0	-	-	17	395	690.2	0	-	-
FN	118	368	754.6	0	-	-	12	393	699.8	0	-	-
SA	103	367	752.3	0	-	-	19	384	617.6	4	401	987.9
SN	119	362	742.1	0	-	-	13	391	670.9	0	-	-
Lostine River												
FA	91	366	726.8	2	381	717	27	394	633.4	1	402	774.9
FN	100	375	891.6	3	412	1004.6	33	403	684.7	0	-	-
SA	94	358	698.4	4	381	840.4	24	382	592.2	1	390	822.3
SN	91	364	725.5	2	335	488.8	27	528	623.6	1	370	635.4

2001 Cohort

Seventy-nine fish were sorted as mature. Of these 64 males (precocial) were spawned, including 16 that had semen cryo-preserved (Table 6).

2002 Cohort

Activities Conducted

A total of 513, 503, and 500 spring chinook salmon parr were collected from each of Catherine Creek and Grande Ronde and Lostine rivers, respectively, in August 2003. During each day of collection, all collected fish were transferred to WFH, anesthetized with 40-50 ppm MS-222 and measured for length and weight (Table 7). These fish were allowed to recover and placed in troughs according to stock (CC, GR or LR). Approximately two months following collection, all fish were again weighed and measured and given an injection of azithromycin. At this time they were randomly separated into treatment groups (accelerated or natural growth) and placed in separate circular tanks (two tanks for each sock and treatment). A month later (November) fish were again weighed, measured, and 134.2 kHz PIT tags were implanted and

Table 5. Mean fork length (FL) and weight (W) in April (all fish), July (immature fish) and September/October (time of spawning of mature fish) of 2001 cohort chinook salmon collected from Catherine Creek, Grande Ronde River and Lostine River and reared under either a freshwater accelerated (FA) or freshwater natural (FN) growth regime at Bonneville Fish Hatchery, or saltwater accelerated (SA) or saltwater natural (SN) growth regime at Manchester Marine Laboratory, 2003.

							September/October (mature)					
	April (all fish)			July (immature)			Male			Female		
Stock, growth regime	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)	N	FL (mm)	W (g)
Catherine Creek												
FA	124	147	38	127	194	94.7	5	216	106.8	0	-	-
FN	126	122	21.1	131	180	78.3	6	186	73	0	-	-
SA	125	152	42.4	121	194	102.5	8	208	97.2	0	-	-
SN	125	121	21.3	124	186	93	5	183	61.5	0	-	-
Grande Ronde River												
FA	112	147	39.5	118	191	90.6	8	216	126	0	-	-
FN	116	119	20.7	119	180	76	0	-	-	0	-	-
SA	113	147	39.3	113	194	104.5	6	209	115.5	0	-	-
SN	115	120	21.1	115	188	109.2	1	197	95.9	0	-	-
Lostine River												
FA	120	138	32.9	126	177	71.9	14	207	87.2	0	-	-
FN	121	118	20.7	131	163	56.4	9	188	64.8	0	-	-
SA	122	140	34	116	187	93.1	11	200	86.8	0	-	-
SN	122	119	20.7	120	187	95.7	5	199	91	0	-	-

tissue samples (fins clips) taken for genetic analysis. Prior to tagging, fish were collected from each tank and anesthetized with 40-50 ppm MS-222. Fish were tagged using needles sterilized in 70% ethanol for a minimum of ten minutes.

Of the original 1,516 fish collected 1,441 fish were still alive at WFH as of 31 December 2003 (Tables 1 and 2). Of the 69 fish removed from the population, 4 fish died from BKD, 16 from other diseases, 26 from operational causes, 21 from other/unknown causes and 2 remain to be examined by pathology. Mortality has been higher than average and, in part, is due to a substantial number of the collected parr being smaller than normal and not converting to artificial food. An additional six fish are unaccounted for and are assumed to have jumped out or were lost early in the operational stages prior to installation of the new circular rearing tanks.

Table 6. Number of chinook salmon from each cohort, stock and treatment maturing, number surviving to gamete collection, percent of maturing fish that survived to gamete collection and number of males from which semen samples were collected for cryopreservation in 2003.

Cohort	Stock	Treatment	Number sorted as mature	Number surviving to gamete maturation		Percent survival to spawn	Number of males cryoed
				Male	Female		
1998	CC	FW	11	0	6	54.5	
		SW	8	1	2	37.5	
	GR	FW	4	0	1	25.0	
		SW	3	0	1	33.3	
	LR	FW	9	0	3	33.3	
		SW	9	0	7	77.8	
1999	CC	FA	78	10	63	93.6	
		FN	88	26	63	101.2	
		SN	53	4	26	56.6	
	LR	FA	75	3	50	70.7	
		FN	83	8	47	65.4	
		SN	82	6	51	69.5	
2000	CC	FA	46	45	0	97.8	
		FN	44	39	0	88.6	1
		SA	47	33	0	70.2	1
		SN	49	33	0	68.8	
	GR	FA	41	31	0	75.6	7
		FN	46	39	0	84.4	7
		SA	59	37	0	62.7	6
		SN	43	32	0	74.4	5
	LR	FA	33	23	1	72.7	
		FN	40	30	0	75.0	
		SA	34	18	0	52.9	2
		SN	34	21	0	61.8	3
2001	CC	FA	5	4	0	80.0	
		FN	7	5	0	71.4	
		SA	8	7	0	87.5	1
		SN	5	4	0	80.0	2
	GR	FA	7	5	0	71.4	5
		FN	0	0	0	0.0	
		SA	6	6	0	100.0	2
		SN	1	1	0	100.0	
	LR	FA	15	12	0	80.0	
		FN	8	7	0	87.5	1
		SA	12	10	0	83.3	5
		SN	5	3	0	60.0	0
Total			1098	503	321	75.0	48

Table 7. Take and disposition of 2002 cohort spring chinook salmon collected and transported to Wallowa Fish Hatchery (WFH) for captive broodstock in 2003.

	Stock		
Activity	Catherine Creek	Grande Ronde River	Lostine River
Collection dates	4-6 AUG	18-20 AUG	11-13 AUG
Number collected	513	503	500
Number transported	513	503	500
Number ponded	513	503	500
Number inoculated and separated	492	499	484
Number PIT-tagged / genetic samples taken	485	490	475
Number at WFH on 31 December 2003	479	485	477

Growth

The 2002 cohort is the third cohort in which we were able to achieve our planned accelerated and natural growth regimes and is demonstrating a separation in size of fish reared under the accelerated and natural growth treatments (Table 8). However, it appears unlikely that the accelerated group will meet the target length, which may be unattainable under the environmental constraints at WFH. This will be monitored and evaluated as the fish reach smoltification in May.

Table 8. Mean fork length (FL) and weight (W) in August (time of collection), October (inoculation) and November (PIT-Tagging) of 2002 cohort chinook salmon collected from Catherine Creek, Grande Ronde River and Lostine River and reared at Wallowa Fish Hatchery, 2003.

Stock,	Capture (August)		Inoculation and Separation (October)		Tagging (November)	
growth regime	FL (mm)	W (g)	FL (mm)	W (g)	FL (mm)	W (g)
Catherine Creek	62.6	3				
Accelerated			78.2	5.9	94	10.7
Natural			79.4	6.4	90	9.4
Grande Ronde River	64.9	3.2				
Accelerated			77.7	5.5	91	10
Natural			78.5	5.6	88	8.2
Lostine River	67.6	4				
Accelerated			80.6	6.4	93	10.4
Natural			81.3	6.5	89	8.9

F_1 Generation Production

2001 F_1 cohort

The F_1 2001 cohort was spawned at Bonneville Fish Hatchery where we collected a total of 929,210 eggs from all three stocks in 2001 (Table 9). These eggs were transported to Oxbow Fish Hatchery and incubated, where 817,808 (88.0) reached the eyed stage. A total of 111,402 eggs (12.0%) died prior to eye-up and 171,579 (21.0%) were culled at the eyed stage from females with high ($\geq$0.8) BKD ELISA values. Eyed eggs were then transferred to Irrigon Fish Hatchery in late 2002 where 601,451 (93.1%) of the eggs hatched in 2002. The resulting fry were transferred to Lookingglass Fish Hatchery in April 2002 where 516,075 parr were coded-wire tagged. A total of 464,616 smolts (50.0% of the green eggs collected) were released in March/April 2003.

2002 F_1 cohort

The F_1 2002 cohort was spawned at Bonneville Fish Hatchery where we collected a total of 602,124 eggs from all three stocks in 2002 (Table 10). These eggs were transported to Oxbow Fish Hatchery and incubated, where 532,270 (88.4%) reached the eyed stage. A total of 69,854 eggs (11.6%) died prior to eye-up and 82,379 (15.5%) were culled at the eyed stage from females with high ($\geq$0.8) BKD ELISA values. Eyed eggs were then transferred to Irrigon Fish Hatchery in late 2002 where 438,304 (97.4%) of the eggs hatched in 2003. The resulting fry were transferred to Lookingglass Fish Hatchery in April 2003 where 361,924 parr wee coded-wire tagged. A total of 359,498 remained, alive as of 31 December 2003.

2003 F_1 cohort

The F_1 2003 cohort was spawned at Bonneville Fish Hatchery where we collected a total of 466,677 eggs from all three stocks in 2003 (Tables 11 and 12). These eggs were transported to Oxbow Fish Hatchery and incubated, where 379,250 (81.3%) reached the eyed stage. A total of 87,347 eggs (18.7%) died prior to eye-up and 68,379 (23.4%) were culled at the eyed stage from females with high ($\geq$0.8) BKD ELISA values. Eyed eggs were then transferred to Irrigon Fish Hatchery for hatching.

Fish Health and Mortalities

The fish health section of this report covers some of the significant findings and results for the eighth consecutive year of captive broodstock reporting. Included in this report are the initial fish health findings for the 2002 cohort, the ninth cohort to be collected and the first to undergo presmolt rearing at Wallowa Hatchery. In the first annual report (Hoffnagle et al 2003) we stated that the predominant fish health problem in this program was bacterial kidney disease. This has been the most consistent pathogen problem since the collection of the first cohort (1994). In 2003 alone, BKD caused 318 mortalities at MRS and BOH, combined. A new approach to BKD management was tried with the 2002 cohort fish, collected in 2003. These were injected with azithromycin at 20 mg/kg in October of 2003 at WFH. This was possible through a veterinary prescription and a letter of approval from the Oregon Department of Environmental Quality. Azithromycin injections were also given to the Lostine River 2000

Table 9. Number of eggs collected, mean fecundity, number of eyed eggs, fertilization rate (percent reaching the eyed stage), percent of eyed eggs culled for BKD prevention, number of fry, hatching rate (percent of green eggs hatching), number of parr coded wire tagged, number of smolts released and egg-to-smolt survival rate for 2001 F_1 cohort captive broodstock chinook salmon from Catherine Creek, upper Grande Ronde River and Lostine River, 2001-2003.

Variable	Catherine Creek	Grande Ronde River	Lostine River
Eggs collected	227,926	434,668	266,616
Mean fecundity	1,838	2,184	2,035
Eyed eggs	200,226	375,345	242,237
Fertilization rate	87.8	86.4	90.9
Number of eggs culled for BKD	43,520	77,914	50,145
Percent culled for BKD	21.7	20.8	20.7
Fry	139,970	283,967	177,514
Hatching rate	89.3	95.5	92.4
Parr CWT-tagged	124,115	244,538	147,422
Smolts released	92,105	242,913	129,598
Egg-to-smolt survival rate	40.4*	55.9*	48.6*

* Does not account for eggs culled for BKD prevention.

Table 10. Number of eggs collected, mean fecundity, number of eyed eggs, fertilization rate (percent reaching the eyed stage), percent of eyed eggs culled for BKD prevention, number of fry and hatching rate (percent of green eggs hatching) for 2002 F_1 cohort captive broodstock chinook salmon from Catherine Creek, upper Grande Ronde River and Lostine River, 2001-2003.

Variable	Catherine Creek	Grande Ronde River	Lostine River
Eggs collected	232,843	104,873	264,408
Mean fecundity	1,819	1,872	2,319
Eyed eggs	205,528	98,077	228,665
Fertilization rate	88.3	93.5	86.5
Number of eggs culled for BKD	35,990	0	46,389
Percent culled for BKD	17.5	0	20.3
Fry	161,966	95,079	181,259
Hatching rate	95.5	96.9	99.4
Parr CWT-tagged	147,782	79,598	134,544
Parr alive as of 31 December 2003	146,091	79,374	134,033

Table 11. Total and mean number of green and eyed eggs collected from female spring chinook salmon of each cohort stock and treatment and mean percent survival of eggs to the eyed stage, 2003.

Stock/ Cohort	Treatment	Number of females	Green eggs		Eyed eggs		Percent survival
			Total	Mean	Total	Mean	
Catherine Creek							
1998	FX	6	7,915	1,319	4,712	785	0.595
	SX	2	1,587	794	1,517	759	0.956
1999	FA	63	96,470	1,531	76,946	1,221	0.798
	FN	61	104,488	1,713	77,622	1,272	0.743
	FX	2	3,094	1,547	1,618	809	0.523
	SN	26	51,045	1,963	40,750	1,567	0.798
Unknown	Unknown	2	4,387	2,194	4,194	2,097	0.956
Grande Ronde River							
	FX	1	0	0	0	0	-
	SX	1	1,548	1,548	1,133	1,133	0.732
Lostine River							
1998	FX	3	1,915	638	1,799	600	0.939
	SX	7	8,499	1,214	6,869	981	0.808
1999	FA	49	61,310	1,251	53,061	1,083	0.865
	FN	44	68,654	1,560	59,317	1,348	0.864
	FX	1	1,389	1,389	1,383	1,383	0.996
	SN	49	53,459	1,091	47,644	972	0.891
2000	FA	1	817	817	685	685	0.838
Unknown	Unknown	1	0	0	0	0	-
Total		317	466,577	1,210	379,250	982	0.813

Table 12. Number of eggs collected, mean fecundity, number of eyed eggs, fertilization rate (percent reaching the eyed stage) and percent of eyed eggs culled for BKD prevention for 2003 F_1 cohort captive broodstock chinook salmon from Catherine Creek, upper Grande Ronde River and Lostine River, 2001-2003.

Variable	Catherine Creek	Grande Ronde River	Lostine River
Eggs collected	268,986	1,548	196,143
Mean fecundity	1,681	1,548	1,508
Eyed eggs	207,359	1,133	170,758
Fertilization rate	77.1	73.2	87.1
Number of eggs culled for BKD	26,335	0	59,595
Percent culled for BKD	12.7	0	34.9

cohort at MRS and BOH due to increased BKD loss. This approach was taken in addition to the more standard erythromycin injections and fish pill treatments given in previous years.

An unusual problem of unknown cause was encountered when the Grande Ronde 2000 cohort experienced a high prevalence of oral carcinomas at both MRS and BOH. Help was sought from various professionals and the Registry of Tumors in Lower Animals (RTLA). A Grande Ronde 2000 fish with oral carcinoma was sacrificed in April 2003 and the RTLA ultimately diagnosed this condition as squamous cell carcinoma (SCC). A second opinion from the School of Veterinary Medicine, University of Wisconsin was pending as of 31 December 2003. This same condition was also found in some hatchery and naturally-reared adult chinook salmon returning to the Grande Ronde and Imnaha rivers in 2003 but has not been observed in any other stocks or cohorts within the captive broodstock program.

Bonneville Fish Hatchery

The leading cause of mortality at BOH was BKD, where 50% of all mortalities were caused by this disease (Table 13). As previously mentioned, there was an unusual occurrence of oral carcinomas in the Grande Ronde River 2000 cohort. Thirty-eight of 71 (53.5%) Grande Ronde 2000 mortalities were categorized as gross tumor lip fish. Although no Grande Ronde 2000 mortalities were directly attributed to this malady, their presence and prevalence were alarmingly high. All samples collected for culturable viruses were negative including assays of tissues from fish with visible tumors. Eight of 20 (40%) LR99, 11/28 (39.3%) LR00 and 5/15 (33.3%) LR01 fish were positive for the presence of *Myxobolus cerebralis*; two LR98 fish were negative. Seventeen CC99, 20 CC00, 12 CC01 and 23 GR00 fish tested negative for *M. cerebralis*. Results from 46 fish sampled for *M. cerebralis* in 2003 are pending.

Manchester Research Station

The leading cause of mortality at MRS was also BKD. One hundred and two of 183 (55.7%) mortalities for all stocks and cohorts were attributed to BKD (Table 14). The unknown cause of death category (N=56) comprised 30.6% of all mortalities. Oral carcinoma Grande Ronde 2000 fish were first noticed at Manchester in December 2002 and this condition was diagnosed in a sacrificed fish in April 2003. Six of 67 (8.9%) Grande Ronde 2000 mortalities at MRS were attributed to squamous cell carcinoma and all occurred in December 2003. All samples collected for culturable viruses were negative.

Adult Spawners at Bonneville Fish Hatchery

All samples collected from spawners for culturable viruses were negative including a large number of samples from the killed-not-spawned Grande Ronde 2000 cohort fish with tumors. The Lostine River stock had a very high proportion of fish with ELISA values ≥ 0.800 OD units (Figure 1). Sixty-eight of 155 (43.9%) spawned and killed-not-spawned Lostine River fish had ELISA values ≥ 0.800 OD units. The Catherine Creek stock had 19/163 (11.7%) and the Grande Ronde stock had 1/2 (50%) with ELISA values ≥ 0.800 OD units.

Lookingglass Fish Hatchery

There were 12 captive brood 2001 cohort mortalities at Lookingglass Hatchery in 2003 (Table 15). Two of these were Catherine Creek handling mortalities and the remainder were Lostine River fish: three were fungus mortalities, two were precocial males (fungus was the secondary cause of death), one mortality had systemic flavobacteriosis and the remaining six

Table 13. Causes of mortality in captive broodstock spring chinook salmon at Bonneville Hatchery, 1 January – 31 December 2003.

Stock / Cohort	Bacterial Kidney Disease		Senesence[a]	*Aeromonas/ Pseudomonas* Septicemia	Fungus	Handling/ Operational	Wasting[b]	Killed	Failure to osmoregulate	Unknown	Total
	Typical kidney pathology	Enlarged hindgut syndrome									
Catherine Creek											
1998	13	0	0	2	0	0	0	0	2	2	19
1999	18	0	3	1	0	0	0	0	8	4	34
2000	4	4	19	0	0	2	1	0	7	4	41
2001	6	0	3	0	0	6	0	0	0	13	28
Unknown	1	0	0	0	0	0	0	0	0	0	1
Total	42	4	25	3	0	8	1	0	17	23	123
Grande Ronde River											
1998	9	0	0	0	0	0	0	0	0	0	9
1999	0	0	0	0	0	0	0	0	0	0	0
2000	23	4	18	2	0	2	0	4	6	12	71
2001	0	0	1	0	0	5	1	0	0	4	11
Total	32	4	19	2	0	7	1	4	6	16	91
Lostine River											
1998	10	0	0	1	0	0	0	0	2	0	13
1999	63	0	5	3	0	0	0	1	4	5	81
2000	58	0	18	1	0	0	0	0	3	2	82
2001	0	2	6	4	1	7	3	0	0	18	41
Unknown	1	0	0	0	0	0	0	0	0	0	1
Total	132	2	29	9	1	7	3	1	9	25	218
Grand Total	206	10	73	14	1	22	5	5	32	64	432
Percentage of Total Mortalities	47.7	2.3	16.9	3.2	0.2	5.1	1.2	1.2	7.4	14.8	
All BKD	50.0										

[a] Fish which were sexually mature but died before being spawned, assumed to be due to the general physical decline associated with sexually maturity in *Oncorhynchus* spp.
[b] Immature fish showing signs of starvation (ample amounts of food were presented to all fish)

Table 14. Causes of mortality in captive broodstock spring chinook salmon at Manchester Marine Laboratory, 1 January – 31 December 2003.

Stock / Cohort	BKD	Unknown	Bloat	Transfer	Handling	Mature	Vibriosis	Wasting	SCC[d]	Totals
Catherine Creek										
1998	4	1	0	0	0	0	0	0	0	5
1999	16	5	0	0	0	0	0	1	0	22
2000	4	5[a]	0	0	0	0	1	1	0	11
2001	0	2	0	0	0	0	0	0	0	2
Total	24	13	0	0	0	0	1	2	0	40
Grande Ronde River										
2000	43	18	0	0	0	0	0	0	7[b]	68
2001	0	0	0	0	1	1	0	0	0	2
Total	43	18	0	0	1	1	0	0	7	70
Lostine River										
1999	3	2	0	0	0	0	0	0	0	5
2000	31[c]	4	0	0	0	0	0	0	0	35
2001	1	19	1	3	5	1	2	1	0	33
Total	35	25	1	3	5	1	2	1	0	73
Grand total	102	56	1	3	6	2	3	3	7	183
Percentage of total mortalities	55.7	30.6	0.6	1.6	3.3	1.1	1.6	1.7	3.8	

[a]Includes one killed fish due to exophthalmia.
[b]Includes one sacrificed for tumor diagnosis on 4/16/03.
[c]Includes one killed fish due to exophthalmia.
[d]Diagnosed in 2003 as squamous cell carcinoma by the Registry of Tumors in Lower Animals (RTLA).

mortalities were categorized as dropouts, handling or unknown specific causes of death. There were no 2001 cohort BKD mortalities at Lookingglass Hatchery in 2003. One of nine (11.1%) Lostine River fish was positive for *M. cerebralis*. Two Catherine Creek fish tested negative for *M. cerebralis*.

Wallowa Fish Hatchery

There were 69 captive brood 2002 cohort mortalities at Wallowa Hatchery in 2003: 35 from Catherine Creek, 22 from the Lostine River and 12 from the Grande Ronde River stock (Table 16). Handling accounted for the greatest number of mortalities (26) followed by dropout

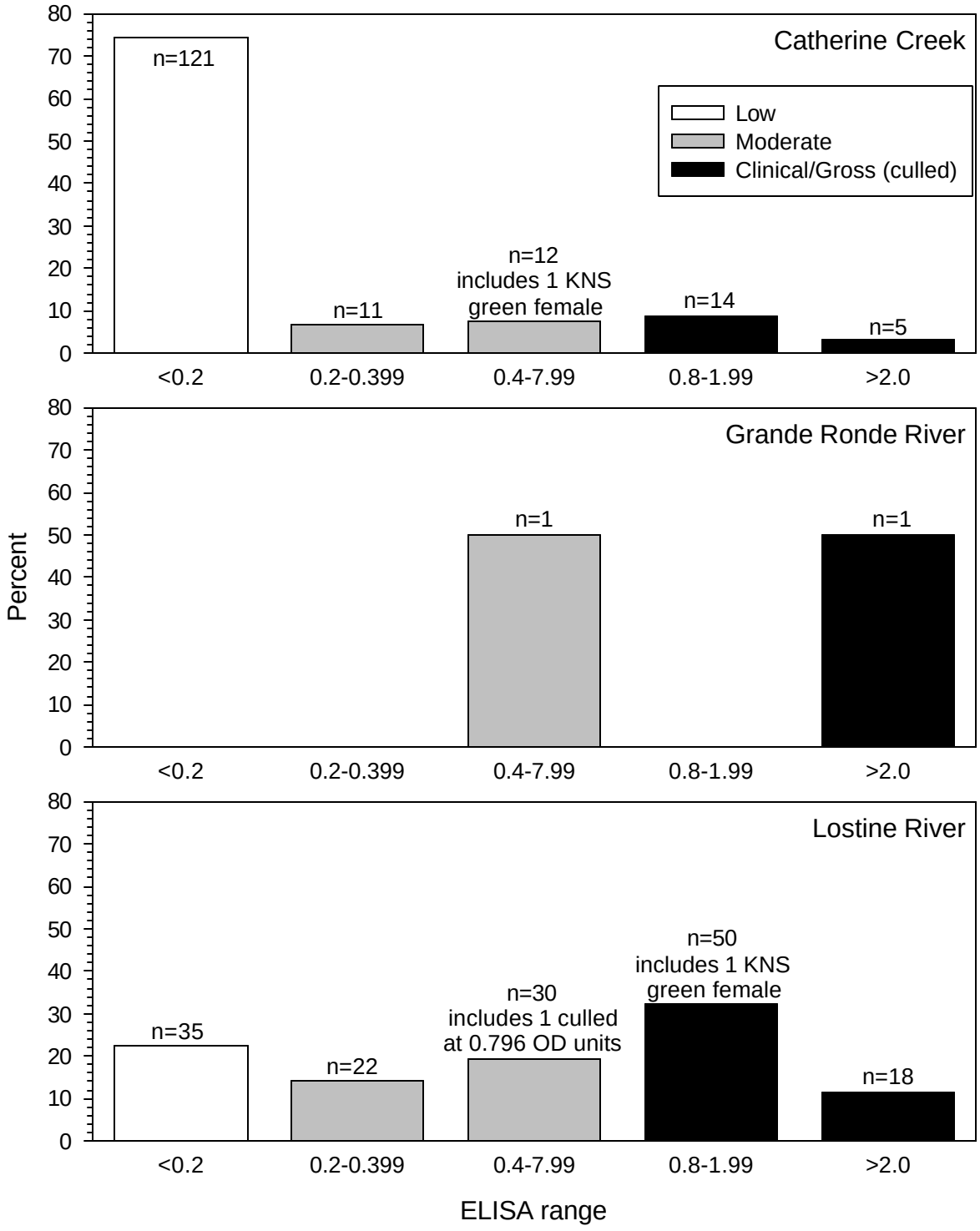

Figure 1. Percent of maturing female Catherine Creek, Grande Ronde River and Lostine River chinook salmon in each ELISA category, 2003.

Table 15. Causes of mortality among 2001 cohort captive broodstock spring chinook salmon at Lookingglass Fish Hatchery, 1 January – 31 May 2003.

	BKD	Handling	Dropout	Fungus	Unknown	Precocial[a]	Flavo-bacteriosis	Totals
Catherine Creek	0	2	0	0	0	0	0	2
Lostine River	0	1	2	3	1	2	1	10
Grande Ronde River	0	0	0	0	0	0	0	0
Totals	0	3	2	3	1	2	1	12

[a]Fungus was the secondary cause of death on these two precocial mortalities.

Table 16. Causes of mortality among 2002 cohort captive broodstock spring chinook salmon at Wallowa Fish Hatchery, 1 August – 31 December 2003.

	BKD	Handling	Dropout	Fungus	Unknown	Jumpout	CWD[a]	Totals
Catherine Creek	2	13	8	10	1	1	0	35
Lostine River	0	5	7	5	4	0	1	22
Grande Ronde River	2	8	2	0	0	0	0	12
Totals	4	26	17	15	5	1	1	69

[a]Bacterial coldwater disease.

(17) and fungus (15). One of the Lostine River mortalities died due to bacterial cold water disease. Bacterial kidney disease caused two Catherine Creek and two Grande Ronde River mortalities. The 2002 cohort was given an injection of azithromycin (20 mg/kg) for BKD management control on 9-10 October. Tissues were collected for antibiotic level analysis (results pending) but there were no adverse reactions to the azithromycin injections reported by the hatchery crew. On 3-5 September, one hour formalin treatments were implemented for fungus control in the Lostine River and Catherine Creek stocks at 167 ppm for three consecutive days. One Lostine River fish tested positive for the presence of *M. cerebralis* while 31 Catherine Creek and 12 Grande Ronde fish were negative.

DISCUSSION AND MANAGEMENT RECOMMENDATIONS

Fish Culture Practices

Inability to Achieve Temperature Separation and Treatment Objectives

Problems with the chiller at LFH appear to be resolved and we have succeeded in producing true accelerated and natural pre-smolt growth groups since the 1999 cohort. The accelerated group has still not achieved the size expected when this program was designed. This growth target may not have been realistic but having a significant difference in size between the accelerated and natural growth regimes will allow us to compare those groups.

We moved the parr-rearing phase of the Captive Broodstock Program to Wallowa Fish Hatchery with collection of the 2002 cohort in August 2003. There, we are using spring water, the temperature of which fluctuates seasonally (4.6°C to 13°C), for the natural growth group. Well water (constant 13°C) is being used for the accelerated growth group.

Maturity Sorts and Spawning

We are continuing to use ultrasound and test the use of near infrared spectroscopy (NIR) to determine maturity and sex of fish early in the maturation process. We are now able to determine maturity and sex (of maturing fish, only) in early April. This allows us to transfer maturing fish from saltwater to freshwater at a more natural time, which should improve fecundity and egg quality, particularly of saltwater-reared fish. The visual / manual maturity sorts at BOH and MRS were eliminated because they were found to be unnecessary, which has allowed us to further decrease fish handling. Conducting only one maturity sort in April reduces stress on the fish that is caused by each sort.

Ultrasound Issues

We incorrectly classified 24 immature females as maturing males during maturity sorting in 2003. These fish had been reared in saltwater and were transferred to BOH in April where they died. We have identified the problem and will avoid it in the future. We were seeing small gray masses that we assumed were testes but they were tiny egg masses in females that wouldn't have matured until 2004. We will be able to identify these correctly in the future.

Fish Health and Disease

We continue to have difficulty with prevention and resolution of BKD outbreaks. Erythromycin and azithromycin were used to treat this disease in the form of injections, pills and treated feed. We are examining this issue, as well as investigating other possible treatments and prophylactic measures to deal with this disease.

To further reduce the incidence of BKD in the Captive Broodstock Program, Oregon's Technical Oversight Team (TOT) continues to follow two standard practices regarding culling of eggs to reduce vertical transmission of BKD into the F_1 generation. The first provides culling, prior to fertilization, of eggs (with agreement of the fish culture, health and research personnel present at spawning) from females showing gross external and internal symptoms of BKD (e.g., lesions, bleeding and an obviously infected and swollen kidney). The second practice allows culling of eyed eggs from females with high BKD ELISA values (generally $\geq$0.8 but the cull level may vary), depending on the distribution of ELISA values, number of eggs collected and management considerations. We anticipate that these practices will reduce vertical transmission of BKD, thus improving the health of these stocks. However, the primary goal of this captive broodstock program is to conserve the genetic diversity of these stocks. Culling of entire egg lots is counterproductive to this effort. Therefore, further work is needed to define the threat of vertical BKD transmission to captive broodstock progeny and to find new prophylactic measures and treatments for this disease.

We are evaluating injection of an alternative drug, azithromycin, as a treatment for BKD (injected into females, for water hardening eggs and/or at first feeding of offspring). Azithromycin is able to enter cells to fight the bacteria and remains in the fish for a longer period of time. This should reduce the amount of drug treatment given to the fish, both by injection and feeding, thus improving feeding of the fish and reducing the stress of handling. A small group of