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Prevalence of Bacterial Kidney Disease in Natural vs. Hatchery-Reared Adult Chinook Salmon Spawned in a Hatchery and in Nature

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Prevalence of Bacterial Kidney Disease in Natural vs. Hatchery-Reared Adult Chinook Salmon Spawned in a Hatchery and in Nature

Timothy L. Hoffnagle
Northeast Region Fish Research
Oregon Department of Fish and Wildlife
203 Badgley Hall
Eastern Oregon University
La Grande, Oregon

Glenda O'Connor
Northeast Region Fish Health
Oregon Department of Fish and Wildlife
217 Badgley Hall
Eastern Oregon University
La Grande, Oregon

Richard W. Carmichael and Sally Gee
Northeast Region Fish Research
Oregon Department of Fish and Wildlife
203 Badgley Hall
Eastern Oregon University
La Grande, Oregon

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Abstract

Bacterial kidney disease (BKD) is a major health problem of cultured Pacific salmon, *Oncorhynchus* sp. It has been particularly problematic in captive broodstock programs, where the interests of gene conservation and fish health can conflict when spawning females with signs of BKD. Not rearing those fish reduces the genetic diversity of an already depleted population, while rearing those fish may increase the prevalence of BKD in the natural population. We used data collected during spawning at Lookingglass Fish hatchery and on spawning ground surveys to examine the prevalence of BKD, based on enzyme-linked immunosorbent assay optical density (ELISA OD) values, to monitor the prevalence of BKD in natural and hatchery-reared Chinook salmon *O. tshawytscha* from Grande Ronde and Imnaha basin streams in northeast Oregon.

Mean ELISA OD levels differed among all sampled streams from 2004-2008 and was lowest in the Imnaha River salmon (0.0839) and highest in the Minam River (0.1750). Salmon spawned at LFH had a lower mean ELISA OD level (0.086) than those collected from carcasses on spawning ground surveys (0.118). Natural salmon mean ELISA OD level was 0.1058 and 97% were from salmon with ELISA OD level <0.2 and in hatchery salmon, 96% had an ELISA OD level <0.2 and mean ELISA OD level was 0.1138, with no difference between the groups. Over 17 years in the Imnaha River we see no difference in mean ELISA OD levels between natural and hatchery Chinook salmon. There was no difference in mean ELISA OD levels between adult Chinook salmon from wilderness (0.1663) vs. supplemented (0.1184) streams. However, when comparing mean ELISA OD for only natural Chinook salmon carcasses recovered in these streams, we found that mean ELISA OD level was higher in the wilderness streams (0.1676) than in the supplemented streams. Returning adults from the Captive Broodstock F_1 generation had a higher mean ELISA OD level (0.1349) than those of Conventional Hatchery Program offspring (0.0957). Annual mean ELISA OD level decreased over time in the Lostine River stock but did not change for any of the other stocks.

The data for BKD in Chinook salmon from northeast Oregon streams and hatcheries show that this disease is not prevalent and we found no evidence that the release of hatchery salmon is causing an increase in BKD prevalence in the monitored streams. However, we will continue to monitor this disease.

Introduction

Bacterial kidney disease (BKD) is a major health problem of cultured salmonids, particularly Pacific salmon, *Oncorhynchus* sp. (Fryer and Lannan 1993; Roberts and Shepherd 1997). It has been particularly problematic in captive broodstock programs being used to restore threatened Chinook *O. tshawytscha* and sockeye *O. nerka* salmon populations in Idaho and Oregon because the salmon are held much longer in captivity (until maturation) than a conventional hatchery program which releases smolts (Frost et al. 2002; Hoffnagle et al. 2003; Venditti et al. 2003). BKD is caused by *Renibacterium salmoninarum* (Rs), a fastidious, slow-growing and strongly gram-positive diplobacillus that produces a chronic, systemic infection characterized by granulomatous lesions in the kidney and other organs, often resulting in death (Roberts and Shepherd 1997; Winton 2001). Infections can occur at any life stage but clinical signs of disease are uncommon in fish less than six months old. The standard method for diagnosing BKD is the enzyme-linked immunosorbent assay (ELISA) conducted on kidney tissue from mortalities or spawned fish (Thoesen 1994). Particularly problematic is the ability of Rs to be transmitted both horizontally and vertically and the inability of available antibiotics to completely control the disease (Mitchum and Sherman 1981; Evelyn et al. 1986).

Chinook salmon populations in the Grande Ronde and Imnaha basins are a portion of the ESA-listed Snake River ESU (Federal Register 1992, volume 57, number 78). In the 1990s, populations in three Grande Ronde Basin streams, Catherine Creek, Lostine River and upper Grande Ronde River (Grand Ronde River), were severely depleted and in danger of extirpation, so supplementation programs were developed. Chinook salmon supplementation in the Grande Ronde and Imnaha basins is conducted under two programs - a Conventional Hatchery Program (Catherine Creek, Grande Ronde River, Imnaha River, Lookingglass Creek and Lostine River) and Captive Broodstock Program (Catherine Creek, Grande Ronde River and Lostine River). These two complimentary programs were developed to preserve these populations and restore numbers of Chinook salmon to the basins (Hoffnagle et al. 2003). These programs are cooperatively managed by the Oregon Department of Fish and Wildlife, Nez Perce Tribe, Confederated Tribes of the Umatilla Indian Reservation and NOAA Fisheries.

The Grande Ronde Basin Spring Chinook Salmon Captive Broodstock Program is funded by the Bonneville Power Administration and was started in 1995 to increase the numbers of natural spawners in the three program streams to a level at which a Conventional Hatchery Program could take over. The Captive Broodstock Program collects natural parr from each of the program streams and rears them to maturation in captivity. Adults are spawned and their offspring reared to smoltification at Lookingglass Fish Hatchery (LFH) before being released into their parents' natal stream. Captive Broodstock offspring that return as adults are allowed to spawn in nature to reduce the risk of domestication (except for some Catherine Creek adults which are being used to reestablish a Chinook salmon population in Lookingglass Creek, above the hatchery). The final Captive Broodstock Program spawn for the Catherine Creek and Lostine River programs will occur in 2009 because the numbers of salmon spawning in nature has reached the target for discontinuing the program in these two streams. The upper Grande Ronde River was the most impacted population and fish will continue to be reared in captivity in a Safety Net Program to support the Conventional Hatchery Program, when needed.

The Conventional Hatchery Program is funded by the Lower Snake River Compensation Plan (LSRCP; Herrig 1998). This program captures adults as they return to their natal spawning grounds, spawns them at LFH and raises the resulting offspring to smolt before releasing them into their parents' natal stream. Differing from the Captive Broodstock Program, some of the returning Conventional Program offspring are collected for use as hatchery broodstock for their natal stream. Conventional Hatchery programs in the Grande Ronde Basin have been operating continuously since 2000 in the Lostine River (adults were also collected in 1997) and since 2001 in Catherine Creek and the Grande Ronde River. The Imnaha River Supplementation Program is a conventional hatchery program that has been running continuously since 1982 (Carmichael and Wagner 1983; Carmichael and Messmer 1985; Carmichael et al. 1986; 1987; 1988; 1989; 1999; 2004; Messmer et al. 1989; 1990; 1991; 1992; 1993; Hoffnagle et al. 2005; Monzyk et al.2005 a; b; 2006c; 2006d; 2006e; 2007; 2008a; b).

There are additional Grande Ronde Basin Chinook salmon populations that are of concern, as well. The native Chinook salmon population in Lookingglass Creek was extirpated when LFH was completed and salmon were no longer allowed above the hatchery for disease prevention measures. Non-endemic Carson and Rapid River stock Chinook salmon were reared at LFH to replace the native stock but with minimal success and this was discontinued with release of the 1999 brood year 2000 (Monzyk et al. 2005b). Chinook salmon from Catherine Creek (Captive Broodstock Program offspring returning as adults) are now being used to replace the ancestors of the previously stocked non-endemic stocks (Carson and Rapid River), of which, a few still spawn naturally below the hatchery. The Catherine Creek adults are released to spawn in nature far above the hatchery and/or spawned at LFH, from which their offspring will be released as smolts (the first was the 2002 cohort, released in 2004). The Minam and Wenaha rivers are located in wilderness areas of northeast Oregon and southeast Washington. No salmon have been stocked in them, although hatchery strays have been recovered there. Protection of these endemic populations from hatchery strays and their potential effects (e.g., genetic or disease) is a concern of the supplementation programs (Hoffnagle et al. 2003).

Bacterial kidney disease is a concern for both the Captive Broodstock and Conventional Hatchery programs. All females spawned in each program are tested for this disease. It is the most prevalent and problematic fish health concern in the Captive Broodstock Program, where BKD is the greatest cause of mortality, causing as much as 63% of the total mortality for a cohort (Hoffnagle et al. 2003). In the Conventional Hatchery Program and many other hatchery programs, BKD is controlled, at least in part, by preventing vertical and subsequent horizontal transmission of Rs through aggressive culling of eggs based on ELISA optical density (OD) levels of spawned females, an indirect measure of the presence of BKD in that fish (Elliott et al. 1989; 1995; Pascho et al. 1991; Miriam et al. 1997; Gumundsdottir et al. 2000). Since BKD can be vertically transmitted, culling of eggs from females with high ELISA OD levels can be effective in reducing population prevalence of the disease and its subsequent spread via horizontal transmission (Mitchum and Sherman 1981). However, culling may not be feasible for severely depleted populations, especially where BKD is very common and/or gene conservation is a program goal, such as captive broodstock programs.

Many states have a standard ELISA level at which they cull eggs. In northeast Oregon, the standard practice for production hatchery programs is to cull eggs from females with ELISA OD ≥ 0.2. Elsewhere in Oregon, where salmon are more abundant, culling may be implemented at ELISA OD levels ≥ 0.1. However, given the threatened status and extremely low population size of Grande Ronde Basin Chinook salmon populations, culling is counter to the gene conservation objectives of the Captive Broodstock Program, where culling levels have varied annually and inversely with the amount of rearing space available (Hoffnagle et al. 2003). Also the Captive Broodstock Program has found that ELISA has not proven to be a good predictor of the likelihood of vertical transmission of BKD, especially in the moderate range of optical densities (0.2-1.0), resulting in the likelihood that eggs have been unnecessarily culled from these severely depleted populations. The program's Technical Oversight Team has developed a compromise between disease prevention and gene conservation which allows the standard practice of culling offspring from females with ELISA OD levels ≥0.8 while allowing the segregated rearing and release of offspring with higher OD levels whenever rearing space is available. Offspring from females with ELISA OD levels ≥0.2 are reared at a lower density and in isolation from the offspring of females with lower ELISA OD levels, in order to reduce the likelihood of horizontal transmission and an outbreak of BKD in these salmon (Pascho et al. 1991).

The managers of the Captive Broodstock Program recognize the possibility that releasing offspring of females with moderate (or higher) ELISA OD levels may increase the prevalence of BKD in nature in the program streams (Goede 1986; Moffitt et al. 2004). Therefore, we have begun to monitor BKD levels in northeast Oregon Chinook salmon populations. We monitor BKD prevalence in females spawned in the Grande Ronde and Imnaha basin Conventional Hatchery programs by collecting kidney samples during spawning at LFH. Since Captive Broodstock Program offspring are not collected for hatchery broodstock but are allowed to spawn naturally, we have begun collecting kidney samples on spawning ground surveys in the Grande Ronde Basin (O'Connor and Hoffnagle 2007). Collecting kidney samples on spawning ground surveys is also necessary to monitor reference streams - those streams from which no broodstock are collected.

Thoesen (1994) states that "fresh or frozen tissues or blood plasma from infected salmon are used" for ELISA, which is not possible for monitoring salmon on the spawning grounds. However, O'Connor and Hoffnagle (2007) demonstrated that kidney samples collected during spawning ground surveys can be analyzed using ELISA and provide the same results as if that sample had been collected from a freshly killed fish. For samples collected at LFH, there is a different concern. We inject female salmon with erythromycin and oxytetracycline, at collection (May-July) and in early August (the August injection was discontinued in 2008), to prevent or slow the progress and reduce the risk of vertical transmission of BKD. Although this is a standard practice in many hatcheries and culling decisions are based on ELISA OD levels from females that have been injected with antibiotics, the injections may affect antigen titers and, therefore, the ELISA OD result. We are currently examining the use of prophylactic antibiotic injections on antigen titers in Chinook salmon and found that females have both a higher mean ELISA OD level and higher antibiotic levels than males, even though they are injected with the same dose of antibiotic (azithromycin or erythromycin; ODFW unpublished data). Hawkenes and Moffitt (2002) also found sex-related metabolic differences between sexes. We have also found that mean ELISA OD at the time of spawning and survival to spawning did not vary between injected and uninjected males. We will compare mean ELISA OD levels between treated and untreated females during the 2009 Captive Broodstock Program spawn to see if these results hold up for females.

In this report, we use these monitoring data to examine the prevalence of BKD, based on ELISA OD level, in natural and hatchery-reared Chinook salmon from Grande Ronde and Imnaha basin streams. These samples were collected during spawning at LFH and from carcasses recovered on the spawning grounds. We have four objectives. First, we further examine the efficacy of collecting kidney samples for detection of BKD by ELISA from carcasses recovered on Grande Ronde and Imnaha basin spawning ground surveys by comparing ELISA OD levels within populations between samples collected on spawning ground surveys and those collected during spawning at LFH. Second, we compare ELISA OD levels in natural vs. hatchery-reared Chinook salmon, within populations, to compare BKD prevalence between these groups and the potential effect of hatchery supplementation on BKD prevalence in the Grande Ronde and Imnaha river basins. Third, we compare ELISA OD levels in Chinook salmon among all Grande Ronde Basin streams and between streams in which salmonids have been supplemented from hatcheries vs. the two wilderness streams which have had no direct stocking of hatchery salmonids. The wilderness populations can be used as a control for further examining the effect of hatchery supplementation on BKD prevalence. Fourth, we examine changes over time in ELISA OD levels in each Grande Ronde and Imnaha basin stream. In doing so, we present baseline data for future examinations of the potential effect of hatchery stocking on BKD in nature.

Study Area

The Grande Ronde Basin drains portions of the Wallowa and Blue mountains of northeast Oregon and southeast Washington and flows into the Snake River at river kilometer (RK) 270 (Figure 1). Catherine Creek, and the Lostine and Minam rivers drain the northern and western parts of the Wallowa Mountains, while the upper Grande Ronde River (Grand Ronde River), Lookingglass Creek and Wenaha River drain the eastern and southern sides of the Blue Mountains. Catherine Creek joins the Grand Ronde River at RK 191 near La Grande, Oregon, and Lookingglass Creek joins the Grande Ronde River at RK 137. The Lostine and Minam rivers are tributaries of the Wallowa River at RKs 42 and 16, respectively, and the Wallowa River joins the Grand Ronde River at RK 132. The Wenaha River is the furthest downstream, entering the Grande Ronde River at RK 74. The Imnaha River drains the eastern portion of the Wallowa Mountains and flows into the Snake River at RK 317.

Methods

We collected kidney samples from 1992 - 2008 from mature Imnaha River Chinook salmon and from 1997 - 2008 from mature Grande Ronde Basin salmon spawned at Lookingglass Fish Hatchery. We also collected kidney samples from carcasses recovered on spawning ground surveys conducted on Grande Ronde Basin streams from 2004 - 2008 (also in 2001 in the Minam River). We collected samples from the following populations and years: Catherine Creek (2001-2008), upper Grande Ronde River (2001-2008), Lookingglass Creek (2004-2008), Lostine River (1997; 2000-2008), Imnaha River (1992 - 2008), Minam River (2001, 2004-2008) and Wenaha River (2004-2008).

At LFH, we collect kidney samples as a part of standard fish health monitoring and for BKD prevention (by culling eggs from females with high ELISA OD levels). We collected the kidney samples immediately after spawning by excising a 1 cm^3 (~0.5-1 g) sample of mid-kidney

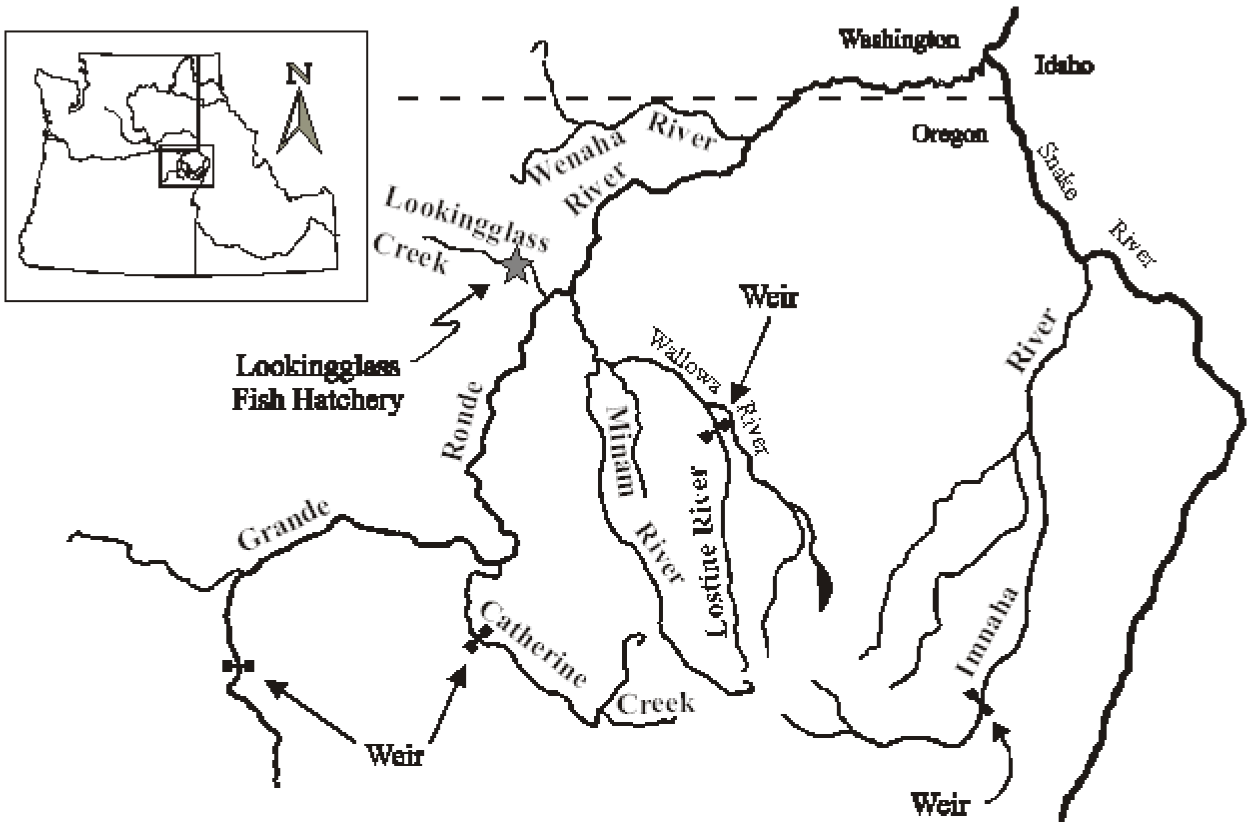

Figure 1. Locations of Lookingglass Fish Hatchery, fish collection weirs and sampled streams in the Grande Ronde and Imnaha river basins, northeast Oregon.

tissue with a clean scalpel or forceps. We placed the sample in a whirl-pac bag and kept it refrigerated or on ice before freezing it later that day. On spawning ground surveys, we collected kidney samples only from carcasses of which the body cavity had not been open to the environment, to prevent contamination. Carcasses from which we collected samples lay in water with temperature <18˚ C and were unlikely to have been dead for longer than three days. Pre-numbered kidney sample collection kits were prepared ahead of time and contained a popsicle stick, a plastic spoon and a whirl-pack. To collect the kidney sample, we opened each salmon and cleared the internal organs and fascia with a knife for access to the kidney. We used the popsicle stick to break into the kidney and loosen the tissue, then collected a sample of approximately 1 cm^3 using the plastic spoon and sealed it in the whirl-pac. Samples were kept as cool as possible (in backpacks) and upon return to town each afternoon, they were frozen until sample processing and analysis. O'Connor and Hoffnagle (2007) simulated the conditions present in a decomposing carcass and after sample collection during a spawning ground survey. They demonstrated that a kidney sample collected in this manner on a spawning ground survey would provided results similar to those, had the sample been collected under the more controlled conditions at a hatchery.

We analyzed kidney samples for BKD using ELISA and used mean ELISA OD values to compare BKD prevalence among treatment groups. Each comparison used a specific subset of the total data set to insure that comparable data were used for all groups being compared. We made four comparisons using ANOVA on ELISA OD values that were transformed by squaring (Sokal and Rohlf 1995). First, we used data from supplemented streams in 2004 - 2008 to compare mean ELISA OD values between sampling sites (hatchery vs. streams) in order to confirm (field test) the results of O'Connor and Hoffnagle (2007) - i.e., that we could validly collect and analyze kidney samples from spawning ground survey carcasses and whether these samples could be compared with samples collected at LFH. Samples for this analysis came from both natural- and hatchery-origin salmon from each stream and are considered to be samples from the same population. Therefore, mean ELISA OD levels between samples collected at the hatchery and on spawning ground surveys should be the same, unless the prophylactic antibiotic treatments given to the hatchery broodstock affected the antigen level measured by ELISA. Second, we used 2004 - 2008 data from supplemented streams (collected both at the hatchery and the spawning grounds) to compare mean ELISA OD values between the two origins of the salmon (hatchery vs. natural). Natural salmon are hatched from eggs deposited in nature and rear in nature, regardless of the origin of their parents. If supplementation is not affecting BKD prevalence, then the mean ELISA OD values for hatchery and natural salmon should be similar. Third, we compared mean ELISA OD values between stream types (supplemented vs. wilderness) using 2004 - 2008 data that was collected on spawning grounds. Again, if supplementation has not affected BKD prevalence, then supplemented and unsupplemented streams should have similar mean ELISA OD values. Fourth, we used 2004 - 2008 data collected on spawning ground surveys to compare mean ELISA OD values among all streams using ANOVA and the least significant difference test (Sokal and Rohlf 1995). If supplementation has affected BKD prevalence, then those streams with the largest supplementation programs should have higher mean ELISA levels. We also used ANOVA (on arcsine-transformed data; Krebs 1989) to test for differences in percent of fish with ELISA OD values in each of three ELISA OD categories: Low <0.2: Moderate 0.2-0.799; High ≥ 0.8. Salmon with ELISA OD level <0.2 are generally considered to be free of BKD. Lastly, we used regression (Sokal and Rohlf 1995) on annual mean ELISA OD levels from samples collected at LFH to determine whether ELISA OD levels have changed over time in all of the sampled streams. These are the baseline data that will be used for monitoring.

Results and Discussion

We collected 1,694 kidney samples from salmon from Grande Ronde Basin streams between 1997 - 2008 (Table 1). Of the total, 1,029 came from hatchery-reared salmon and 665 from natural salmon. We collected 1,090 samples from salmon spawned at LFH and 604 from salmon that spawned in nature and were recovered as carcasses during spawning ground surveys. Individual ELISA OD levels ranged from 0.057 - 2.392 but were generally low, with 97% of the samples being <0.2 OD units (Table 2). Mean ELISA OD levels for each Grande Ronde Basin stream over all years sampled ranged from 0.0929 - 0.1694 (Figure 2).

We collected 2,039 kidney samples from Imnaha River Chinook salmon spawned at LFH from 1992 - 2008 (Table 1). Of those, 1,462 came from hatchery-reared salmon and 577 from natural salmon. 1,921 samples were collected at LFH and 118 from carcasses recovered on spawning ground surveys. Individual ELISA OD levels ranged from 0.053-2.749 and 97% were

Table 1. Number sampled and mean, standard deviation (STD), minimum and maximum ELISA OD levels for hatchery-reared [Captive (CBS) and Conventional (CONV) broodstock programs] and natural adult Chinook salmon from streams in the Grande Ronde and Imnaha river basins and sampled at Lookingglass Fish Hatchery (LFH) or as carcasses on spawning ground surveys (SGS), 1992-2008.

Population,			Sampling		ELISA OD			
run year	Origin	Program	location	N	Mean	STD	Minimum	Maximum
Catherine Creek								
2001	Natural		LFH	19	0.0856	0.0134	0.073	0.128
2002	Natural		LFH	20	0.0954	0.0184	0.082	0.164
2003	Natural		LFH	28	0.0830	0.0086	0.071	0.106
2004	Hatchery	CBS	SGS	16	0.1884	0.1412	0.081	0.510
	Hatchery	CONV	SGS	8	0.1206	0.0234	0.081	0.151
	Natural		LFH	9	0.0948	0.0399	0.063	0.182
	Natural		SGS	9	0.1361	0.0319	0.105	0.212
2005	Hatchery	CONV	LFH	10	0.0745	0.0172	0.062	0.116
	Hatchery	CBS	SGS	12	0.1873	0.1259	0.081	0.414
	Hatchery	CONV	SGS	8	0.5955	0.9003	0.084	2.274
	Natural		LFH	7	0.0761	0.0112	0.063	0.096
	Natural		SGS	11	0.0956	0.0168	0.073	0.128
2006	Hatchery	CONV	LFH	29	0.0754	0.0102	0.064	0.105
	Hatchery	CBS	SGS	5	0.0964	0.0294	0.067	0.144
	Hatchery	CONV	SGS	11	0.1269	0.0540	0.079	0.234
	Natural		LFH	8	0.0821	0.0185	0.064	0.116
	Natural		SGS	10	0.1014	0.0235	0.067	0.139
2007	Hatchery	CONV	LFH	34	0.0796	0.0215	0.061	0.154
	Hatchery	CBS	SGS	8	0.0875	0.0192	0.070	0.123
	Hatchery	CONV	SGS	9	0.0816	0.0083	0.067	0.092
	Natural		LFH	15	0.0749	0.0085	0.065	0.092
	Natural		SGS	4	0.0795	0.0121	0.064	0.093
2008	Hatchery	CONV	LFH	21	0.1009	0.0244	0.065	0.165
	Hatchery		SGS	6	0.1145	0.0226	0.092	0.156
	Natural		LFH	11	0.0976	0.0238	0.065	0.148
	Natural		SGS	10	0.1160	0.0236	0.077	0.152
Grande Ronde River								
2001	Natural		LFH	16	0.0847	0.0128	0.071	0.126
2002	Natural		LFH	25	0.1165	0.0793	0.085	0.492
2003	Natural		LFH	32	0.0898	0.0219	0.071	0.190
2004	Hatchery	CBS	SGS	31	0.1317	0.0701	0.084	0.486
	Hatchery	CONV	SGS	3	0.1403	0.0212	0.121	0.163

Population, run year	Origin	Program	Sampling location	N	ELISA OD Mean	STD	Minimum	Maximum
Grande Ronde River (cont'd)								
	Natural		LFH	7	0.1286	0.0505	0.087	0.213
	Natural		SGS	2	0.1175	0.0530	0.080	0.155
2005	Hatchery	CONV	LFH	38	0.0830	0.0220	0.060	0.166
	Hatchery	CBS	SGS	21	0.1141	0.0361	0.075	0.204
	Hatchery	CONV	SGS	14	0.1363	0.0320	0.087	0.191
	Natural		LFH	2	0.0760	0.0028	0.074	0.078
	Natural		SGS	2	0.1175	0.0106	0.110	0.125
2006	Hatchery	CONV	LFH	72	0.0772	0.0118	0.061	0.113
	Hatchery		SGS	3	0.1350	0.0356	0.101	0.172
	Natural		LFH	12	0.0845	0.0229	0.064	0.136
	Natural		SGS	1	0.1010	.	0.101	0.101
2007	Hatchery	CBS	LFH	2	0.0645	0.0021	0.063	0.066
	Hatchery	CONV	LFH	29	0.0709	0.0099	0.057	0.104
	Hatchery		SGS	15	0.0619	0.0046	0.055	0.071
	Natural		LFH	8	0.0700	0.0139	0.057	0.098
	Natural		SGS	3	0.0653	0.0015	0.064	0.067
2008	Hatchery	CONV	LFH	9	0.0756	0.0156	0.053	0.097
	Hatchery		SGS	1	0.1200	.	0.120	0.120
	Natural		LFH	4	0.0683	0.0120	0.057	0.085
	Natural		SGS	11	0.1523	0.0882	0.078	0.405
Lookingglass Creek								
2004	Hatchery	CBS	LFH	51	0.1362	0.2613	0.058	1.644
	Hatchery	CONV	LFH	3	0.0707	0.0075	0.063	0.078
	Hatchery	CBS	SGS	23	0.1323	0.1602	0.071	0.861
	Hatchery	CONV	SGS	2	0.1050	0.0339	0.081	0.129
	Natural		SGS	2	0.1010	0.0424	0.071	0.131
2005	Hatchery	CBS	SGS	27	0.1093	0.0471	0.076	0.323
	Natural		SGS	3	0.0703	0.0049	0.067	0.076
2006	Hatchery	CBS	SGS	13	0.0852	0.0206	0.064	0.132
	Hatchery	CONV	SGS	11	0.0771	0.0178	0.060	0.125
	Natural		SGS	5	0.0872	0.0188	0.068	0.115
2007	Hatchery	CONV	LFH	25	0.1562	0.4073	0.063	2.108
	Hatchery	CBS	SGS	13	0.1471	0.2257	0.070	0.897
	Hatchery	CONV	SGS	15	0.0815	0.0109	0.068	0.103
	Natural		SGS	3	0.0813	0.0050	0.076	0.086
2008	Hatchery	CONV	LFH	84	0.0997	0.0263	0.056	0.181
	Hatchery		SGS	16	0.1174	0.0169	0.094	0.155

Population, run year	Origin	Program	Sampling location	N	ELISA OD Mean	STD	Minimum	Maximum
Lostine River								
1997	Natural		LFH	4	0.1648	0.0587	0.118	0.245
2000	Natural		LFH	30	0.1367	0.0232	0.118	0.221
2001	Hatchery	CONV	LFH	13	0.0859	0.0146	0.073	0.123
	Natural		LFH	48	0.0781	0.0072	0.070	0.112
2002	Natural		LFH	28	0.0924	0.0203	0.080	0.192
2003	Natural		LFH	21	0.0772	0.0093	0.068	0.100
2004	Hatchery	CONV	LFH	35	0.0834	0.0313	0.057	0.231
	Hatchery	CBS	SGS	7	0.4161	0.5230	0.072	1.220
	Hatchery	CONV	SGS	7	0.1069	0.0360	0.073	0.178
	Natural		LFH	26	0.1224	0.1083	0.061	0.622
	Natural		SGS	6	0.1038	0.0469	0.078	0.199
2005	Hatchery	CONV	LFH	39	0.0716	0.0133	0.058	0.120
	Hatchery	CBS	SGS	10	0.0933	0.0181	0.078	0.139
	Hatchery	CONV	SGS	17	0.0843	0.0139	0.066	0.115
	Natural		LFH	17	0.0814	0.0371	0.059	0.184
	Natural		SGS	8	0.1024	0.0325	0.082	0.182
2006	Hatchery	CONV	LFH	45	0.0937	0.0747	0.058	0.542
	Hatchery	CBS	SGS	11	0.0945	0.0218	0.074	0.147
	Hatchery	CONV	SGS	8	0.0871	0.0115	0.070	0.104
	Natural		LFH	12	0.0949	0.0264	0.068	0.144
	Natural		SGS	6	0.0793	0.0081	0.073	0.094
2007	Hatchery	CONV	LFH	49	0.0678	0.0107	0.056	0.103
	Hatchery	CBS	SGS	7	0.1091	0.0398	0.076	0.175
	Hatchery	CONV	SGS	8	0.0848	0.0102	0.070	0.101
	Natural		LFH	23	0.0659	0.0071	0.057	0.084
	Natural		SGS	5	0.0960	0.0141	0.082	0.117
2008	Hatchery	CONV	LFH	48	0.0762	0.0120	0.055	0.102
	Hatchery		SGS	21	0.1277	0.0739	0.069	0.400
	Natural		LFH	22	0.0789	0.0179	0.061	0.131
	Natural		SGS	9	0.0942	0.0215	0.067	0.124
Minam River								
2001	Hatchery	CONV	SGS	1	0.1790	.	0.179	0.179
	Natural		SGS	6	0.1048	0.0147	0.085	0.129
2004	Natural		SGS	12	0.1145	0.0343	0.073	0.190
2005	Natural		SGS	17	0.1032	0.0453	0.071	0.271
2006	Hatchery	CONV	SGS	1	0.0850	.	0.085	0.085
	Natural		SGS	13	0.0991	0.0242	0.076	0.168
2007	Natural		SGS	7	0.4097	0.8741	0.069	2.392

Population, run year	Origin	Program	Sampling location	N	ELISA OD Mean	STD	Minimum	Maximum
Minam River (cont'd)								
2008	Hatchery		SGS	3	0.1877	0.1092	0.113	0.313
	Natural		SGS	14	0.2709	0.4179	0.097	1.715
Wenaha River								
2004	Hatchery	CONV	SGS	1	0.0640	.	0.064	0.064
	Natural		SGS	13	0.1216	0.0726	0.070	0.340
2005	Natural		SGS	6	0.0947	0.0161	0.076	0.124
2006	Natural		SGS	6	0.1387	0.0627	0.072	0.232
2007	Natural		SGS	6	0.2927	0.5239	0.070	1.362
2008	Natural		SGS	1	0.1070	.	0.107	0.107
Imnaha River								
1992	Hatchery	CONV	LFH	115	0.0973	0.0140	0.083	0.158
	Natural		LFH	84	0.1154	0.1838	0.082	1.775
1993	Hatchery	CONV	LFH	208	0.1457	0.2739	0.064	2.622
	Natural		LFH	90	0.1081	0.0629	0.063	0.462
1994	Hatchery	CONV	LFH	22	0.0805	0.0132	0.066	0.110
	Natural		LFH	15	0.0911	0.0160	0.074	0.125
1995	Hatchery	CONV	LFH	20	0.1987	0.1602	0.107	0.747
	Natural		LFH	6	0.3597	0.4060	0.134	1.182
1996	Hatchery	CONV	LFH	7	0.1524	0.0370	0.104	0.200
	Natural		LFH	17	0.1459	0.0285	0.110	0.210
1997	Hatchery	CONV	LFH	49	0.3140	0.5117	0.115	2.661
	Natural		LFH	6	0.1800	0.0212	0.159	0.214
1998	Hatchery	CONV	LFH	34	0.1135	0.0136	0.098	0.164
	Natural		LFH	27	0.1116	0.0091	0.098	0.143
1999	Hatchery	CONV	LFH	31	0.3211	0.5354	0.115	2.749
	Natural		LFH	6	0.1363	0.0278	0.117	0.192
2000	Hatchery	CONV	LFH	59	0.1302	0.0253	0.114	0.308
	Natural		LFH	8	0.1248	0.0079	0.117	0.140
2001	Hatchery	CONV	LFH	99	0.1033	0.1180	0.071	1.123
	Natural		LFH	69	0.0860	0.0165	0.069	0.157
2002	Hatchery	CONV	LFH	132	0.1351	0.3212	0.080	2.739
	Natural		LFH	22	0.0930	0.0146	0.078	0.143
2003	Hatchery	CONV	LFH	123	0.1274	0.3029	0.064	2.647
	Natural		LFH	27	0.1983	0.4413	0.066	2.188
2004	Hatchery	CONV	LFH	112	0.0720	0.0135	0.056	0.124
	Natural		LFH	47	0.0812	0.0315	0.056	0.265
2005	Hatchery	CONV	LFH	88	0.0812	0.0473	0.056	0.496

Population, run year	Origin	Program	Sampling location	N	ELISA OD Mean	STD	Minimum	Maximum
Imnaha River (cont'd)								
	Natural		LFH	29	0.1565	0.3134	0.062	1.675
2006	Hatchery	CONV	LFH	74	0.0734	0.0107	0.056	0.108
	Hatchery	CONV	SGS	28	0.0941	0.0222	0.062	0.171
	Natural	CONV	LFH	24	0.0740	0.0228	0.058	0.170
	Natural		SGS	19	0.1012	0.0289	0.071	0.188
2007	Hatchery	CONV	LFH	106	0.0849	0.1169	0.056	1.189
	Hatchery	CONV	SGS	28	0.0785	0.0083	0.064	0.094
	Natural		LFH	30	0.0739	0.0117	0.060	0.110
	Natural		SGS	16	0.0799	0.0137	0.065	0.123
2008	Hatchery	CONV	LFH	106	0.0824	0.0257	0.053	0.213
	Hatchery	CONV	SGS	21	0.1048	0.0346	0.070	0.200
	Natural		LFH	29	0.0810	0.0170	0.056	0.143
	Natural		SGS	6	0.1197	0.0283	0.076	0.156

Table 2. Number and percent of natural and hatchery-reared [Captive (CBS) and Conventional (CONV) broodstock programs] adult Chinook salmon from streams in the Grande Ronde and Imnaha basins sampled for BKD with ELISA OD levels in each category, 1992-2008.

			ELISA category						
			Low (<0.2)		Moderate (0.2 - <0.8)		High (≥0.8)		
Population, run year	Origin	Program	N	%	N	%	N	%	Total
Catherine Creek									
2001	Natural		19	100	0	0	0	0	19
2002	Natural		20	100	0	0	0	0	20
2003	Natural		28	100	0	0	0	0	28
2004	Hatchery	CBS	13	81	3	19	0	0	16
2004	Hatchery	CONV	8	100	0	0	0	0	8
2004	Natural		17	94	1	6	0	0	18
2005	Hatchery	CBS	8	67	4	33	0	0	12
2005	Hatchery	CONV	15	83	1	6	2	11	18
2005	Natural		18	100	0	0	0	0	18
2006	Hatchery	CBS	5	100	0	0	0	0	5
2006	Hatchery	CONV	38	95	2	5	0	0	40
2006	Natural		18	100	0	0	0	0	18
2007	Hatchery	CBS	8	100	0	0	0	0	8
2007	Hatchery	CONV	43	100	0	0	0	0	43

Population, run year	Origin	Program	ELISA category: Low (<0.2) N	%	Moderate (0.2 - <0.8) N	%	High (≥0.8) N	%	Total
Catherine Creek (cont'd)									
2007	Natural		19	100	0	0	0	0	19
2008	Hatchery	CONV	27	100	0	0	0	0	27
2008	Natural		21	100	0	0	0	0	21
Grande Ronde River									
2001	Natural		16	100	0	0	0	0	16
2002	Natural		24	96	1	4	0	0	25
2003	Natural		32	100	0	0	0	0	32
2004	Hatchery	CBS	30	97	1	3	0	0	31
2004	Hatchery	CONV	3	100	0	0	0	0	3
2004	Natural		8	89	1	11	0	0	9
2005	Hatchery	CBS	20	95	1	5	0	0	21
2005	Hatchery	CONV	52	100	0	0	0	0	52
2005	Natural		4	100	0	0	0	0	4
2006	Hatchery	CONV	75	100	0	0	0	0	75
2006	Natural		13	100	0	0	0	0	13
2007	Hatchery	CBS	2	100	0	0	0	0	2
2007	Hatchery	CONV	44	100	0	0	0	0	44
2007	Natural		11	100	0	0	0	0	11
2008	Hatchery	CONV	10	100	0	0	0	0	10
2008	Natural		14	93	1	7	0	0	15
Lookingglass Creek									
2004	Hatchery	CBS	71	96	0	0	3	4	74
2004	Hatchery	CONV	5	100	0	0	0	0	5
2004	Natural		2	100	0	0	0	0	2
2005	Hatchery	CBS	26	96	1	4	0	0	27
2005	Natural		3	100	0	0	0	0	3
2006	Hatchery	CBS	13	100	0	0	0	0	13
2006	Hatchery	CONV	11	100	0	0	0	0	11
2006	Natural		5	100	0	0	0	0	5
2007	Hatchery	CBS	12	92	0	0	1	8	13
2007	Hatchery	CONV	39	98	0	0	1	3	40
2007	Natural		3	100	0	0	0	0	3
2008	Hatchery	CONV	100	100	0	0	0	0	100
Lostine River									
1997	Natural		3	75	1	25	0	0	4
2000	Natural		29	97	1	3	0	0	30

Population, run year	Origin	Program	ELISA category: Low (<0.2) N	%	Moderate (0.2 - <0.8) N	%	High (≥0.8) N	%	Total
Lostine River (cont'd)									
2001	Hatchery	CONV	13	100	0	0	0	0	13
2001	Natural		48	100	0	0	0	0	48
2002	Natural		28	100	0	0	0	0	28
2003	Natural		21	100	0	0	0	0	21
2004	Hatchery	CBS	4	57	1	14	2	29	7
2004	Hatchery	CONV	41	98	1	2	0	0	42
2004	Natural		31	97	1	3	0	0	32
2005	Hatchery	CBS	10	100	0	0	0	0	10
2005	Hatchery	CONV	56	100	0	0	0	0	56
2005	Natural		25	100	0	0	0	0	25
2006	Hatchery	CBS	11	100	0	0	0	0	11
2006	Hatchery	CONV	52	98	1	2	0	0	53
2006	Natural		18	100	0	0	0	0	18
2007	Hatchery	CBS	7	100	0	0	0	0	7
2007	Hatchery	CONV	57	100	0	0	0	0	57
2007	Natural		28	100	0	0	0	0	28
2008	Hatchery	CONV	67	97	2	3	0	0	69
2008	Natural		31	100	0	0	0	0	31
Minam River									
2001	Hatchery	CONV	1	100	0	0	0	0	1
2001	Natural		6	100	0	0	0	0	6
2004	Natural		12	100	0	0	0	0	12
2005	Natural		16	94	1	6	0	0	17
2006	Hatchery	CONV	1	100	0	0	0	0	1
2006	Natural		13	100	0	0	0	0	13
2007	Natural		6	86	0	0	1	14	7
2008	Hatchery	CONV	2	67	1	33	0	0	3
2008	Natural		10	71	3	21	1	7	14
Wenaha River									
2004	Hatchery	CONV	1	100	0	0	0	0	1
2004	Natural		12	92	1	8	0	0	13
2005	Natural		6	100	0	0	0	0	6
2006	Natural		5	83	1	17	0	0	6
2007	Natural		5	83	0	0	1	17	6
2008	Natural		1	100	0	0	0	0	1
Grande Ronde Basin Total			1,650	97	32	2	12	1	1,694

Population, run year	Origin	Program	ELISA category: Low (<0.2) N	%	Moderate (0.2 - <0.8) N	%	High (≥0.8) N	%	Total
Imnaha River									
1992	Hatchery	CONV	115	100	0	0	0	0	115
1992	Natural		83	99	0	0	1	1	84
1993	Hatchery	CONV	192	92	12	6	4	2	208
1993	Natural		85	94	5	6	0	0	90
1994	Hatchery	CONV	22	100	0	0	0	0	22
1994	Natural		15	100	0	0	0	0	15
1995	Hatchery	CONV	16	80	4	20	0	0	20
1995	Natural		3	50	2	33	1	17	6
1996	Hatchery	CONV	6	86	1	14	0	0	7
1996	Natural		16	94	1	6	0	0	17
1997	Hatchery	CONV	28	57	18	37	3	6	49
1997	Natural		5	83	1	17	0	0	6
1998	Hatchery	CONV	34	100	0	0	0	0	34
1998	Natural		27	100	0	0	0	0	27
1999	Hatchery	CONV	25	81	3	10	3	10	31
1999	Natural		6	100	0	0	0	0	6
2000	Hatchery	CONV	58	98	1	2	0	0	59
2000	Natural		8	100	0	0	0	0	8
2001	Hatchery	CONV	97	98	1	1	1	1	99
2001	Natural		69	100	0	0	0	0	69
2002	Hatchery	CONV	128	97	2	2	2	2	132
2002	Natural		22	100	0	0	0	0	22
2003	Hatchery	CONV	120	98	0	0	3	2	123
2003	Natural		25	93	0	0	2	7	27
2004	Hatchery	CONV	112	100	0	0	0	0	112
2004	Natural		46	98	1	2	0	0	47
2005	Hatchery	CONV	87	99	1	1	0	0	88
2005	Natural		27	93	1	3	1	3	29
2006	Hatchery	CONV	102	100	0	0	0	0	102
2006	Natural		43	100	0	0	0	0	43
2007	Hatchery	CONV	131	98	2	1	1	1	134
2007	Natural		46	100	0	0	0	0	46
2008	Hatchery	CONV	124	98	3	2	0	0	127
2008	Natural		35	100	0	0	0	0	35
Imnaha Basin Total			1,958	96	59	3	22	1	2,039

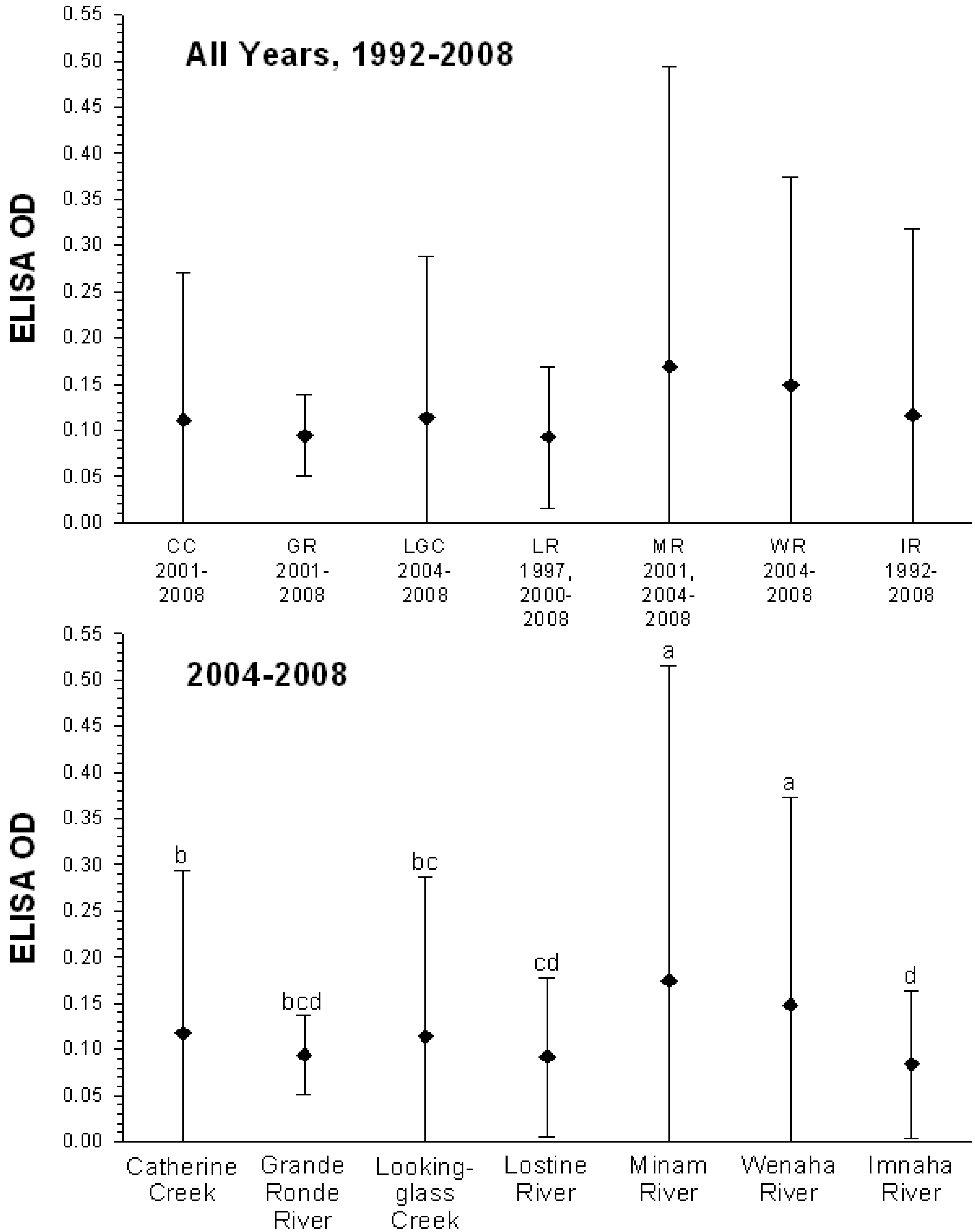

Figure 2. Mean (±1 SD) ELISA OD levels for adult Chinook salmon from streams in the Grande Ronde and Imnaha river basins for all available years sampled (1992-2008, top) and years for which data are available for all streams (2004-2008, bottom). Streams with the same letter (bottom graph) had means that were not statistically different (α=0.05).

from salmon with ELISA OD level <0.2 (Table 2). Mean ELISA OD level for Imnaha River salmon from 1992-2008 was 0.1165 (Figure 2).

Mean ELISA OD levels differed among all sampled streams from 2004-2008 ($P<0.0001$; Figure 2). Mean ELISA OD level was lowest in the Imnaha River salmon (0.0839) and highest in the Minam River (0.1750). Imnaha River mean ELISA OD level was lower than that of the Minam River, Wenaha River, Lookingglass Creek and Catherine Creek, but did not differ from that of the Lostine River or Grande Ronde River. Mean ELISA OD in the two wilderness streams (Minam and Wenaha rivers) were the highest, with both streams having a higher mean ELISA OD level than all of the supplemented streams The proportion of fish in each of the categories did/did not vary among the sampled streams ($P\geq0.1810$; Figure 3). 90-99% of the fish sampled from each stream had mean ELISA OD levels <0.2 (Low category) and only 0.5-4% had mean ELISA OD levels ≥0.8 (High category).

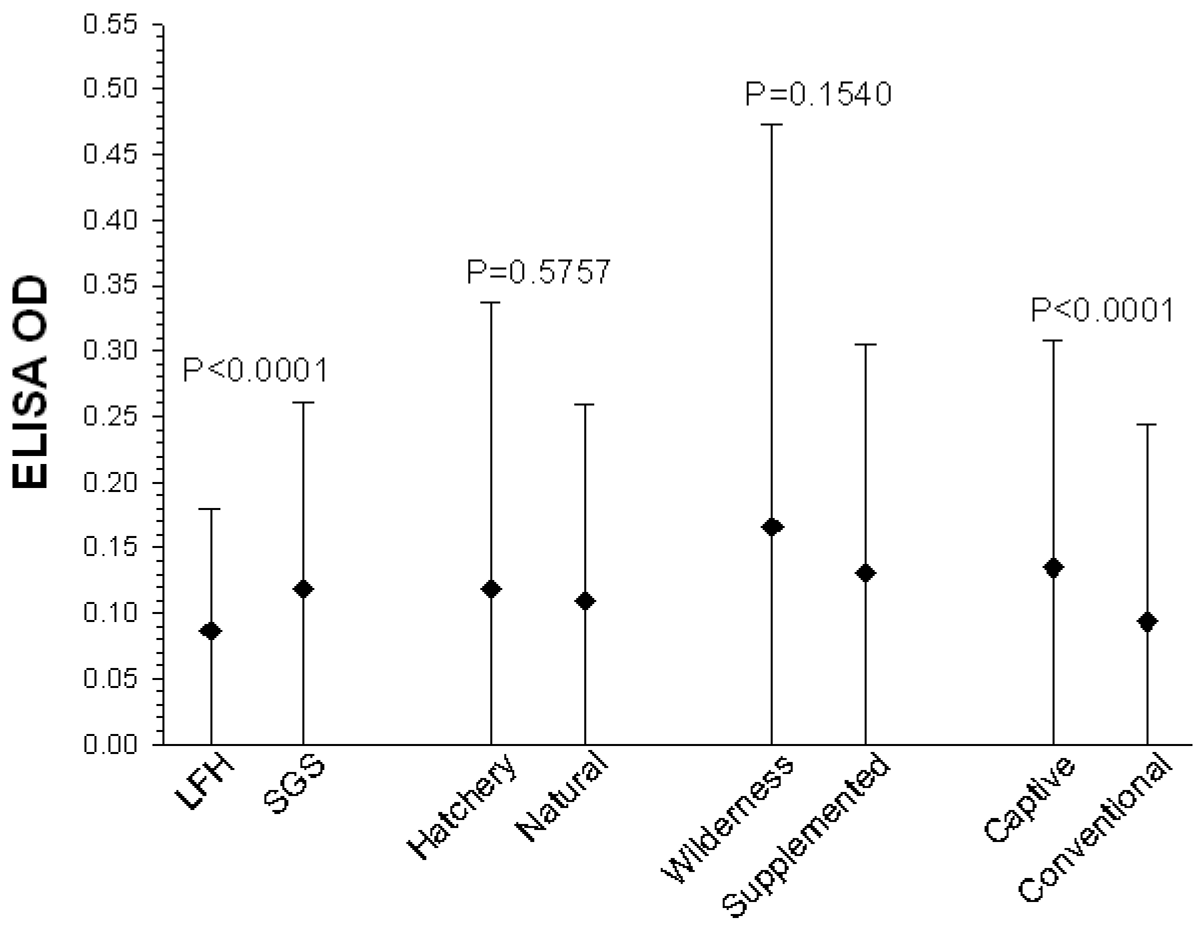

Figure 3. Mean (±1 SD) ELISA OD levels for adult Chinook salmon for various paired comparisons: fish sampled at Lookingglass Fish Hatchery (LFH) vs. those sampled on spawning ground surveys (SGS), hatchery origin vs. natural origin, wilderness (unsupplemented) vs. supplemented streams, and progeny of the Captive Broodstock Program vs. those from the Conventional Hatchery Program.

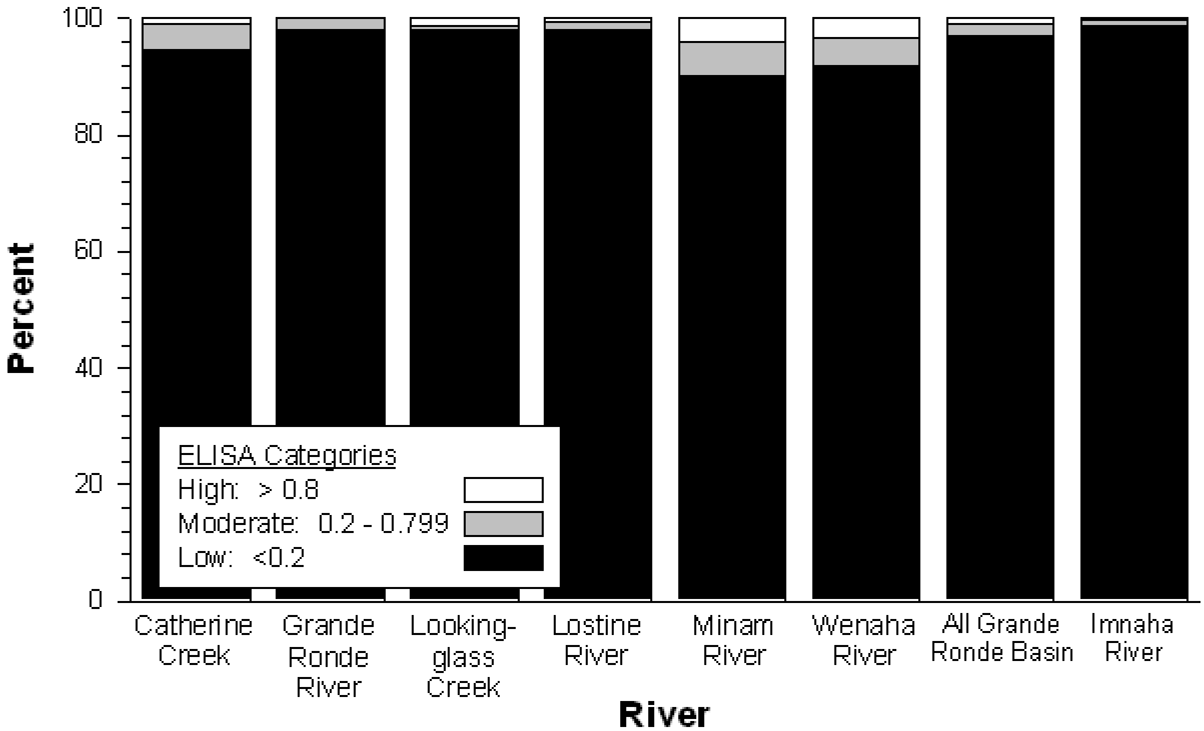

Figure 4. Percent of adult Chinook salmon from streams in the Grande Ronde and Imnaha river basins with ELISA OD levels in each ELISA category.

Stream vs. Hatchery Samples

Kidney samples from salmon spawned at LFH had a lower (P<0.0001) mean ELISA OD level (0.086; range: 0.053-2.108) than those collected from carcasses on spawning ground surveys (0.118; range: 0.055-2.274) (Figure 4). For hatchery-reared salmon, the samples collected at LFH had a mean ELISA OD value of 0.0867 while those collected on the spawning grounds had a mean ELISA OD of 0.1236 (P<0.0001). There was also a difference in mean ELISA OD for natural salmon between sampling locations (P<0.0001), with those sampled at LFH having a mean ELISA OD level of 0.0843 and 0.1025 for those on the spawning grounds. In individual streams, mean ELISA OD level was higher for samples collected at LFH than for samples collected from spawning ground carcasses in Catherine Creek, the Grande Ronde River, Lostine River and Imnaha River (P<0.0001) but not for Lookingglass Creek (P=0.9442).

From these results, we draw two conclusions. First, our results confirm the results of O'Connor and Hoffnagle (2007) that valid kidney samples can be collected from intact carcasses collected on spawning ground surveys. The range of ELISA OD values from kidney samples collected during spawning ground surveys (0.055-2.274) was similar to the range of values from samples collected at LFH (0.053-2.108), which may be biased by drug treatment (see below). This confirms that we can monitor BKD in streams on which there are no traps and can compare BKD prevalence in unsupplemented vs. supplemented populations. It is important that we are able to monitor BKD in both nature and the hatchery for the Grande Ronde Basin Chinook salmon population, due to its threatened status and our practice of sometimes rearing and releasing offspring from females with higher ELISA OD levels than are usually reared at production hatcheries. Therefore, we will need to continue to collect kidney samples from intact carcasses on spawning ground surveys in order to monitor BKD in wilderness streams and to compare those streams with supplemented streams.

Second, we conclude that ELISA OD levels in kidney samples collected from carcasses recovered during spawning ground surveys are not the same as those collected in a hatchery. This is most likely due to the erythromycin/oxytetracycline injections given to combat BKD in adults collected for broodstock. Antibiotic injections are given at capture (May-June) and again in early August (the August injection was discontinued in 2008), approximately two weeks before spawning begins at LFH. It seems likely that the erythromycin treatment and its expected effect on Rs causes a reduction in the amount of Rs antigen, thus a reduction in ELISA OD levels. This is good from a fish health perspective but it also means that we need to take care when comparing ELISA OD levels using samples collected at both the hatchery and on spawning ground surveys.

Natural vs. Hatchery Salmon

There was no difference (P=0.5757) in overall mean ELISA OD levels between hatchery-reared and natural Chinook salmon adults, nor within any of the sampled streams ($P \geq 0.0955$; Figures 3 and 5). Natural salmon mean ELISA OD level was 0.1058 (range: 0.056-2.188) and 97% were from salmon with ELISA OD level <0.2 (Table 2). For hatchery salmon, 96% had an ELISA OD level <0.2 and mean ELISA OD level was 0.1138 (0.053-2.749). For individual years, mean ELISA OD levels were higher in hatchery salmon in 1999 (Imnaha River data, only)

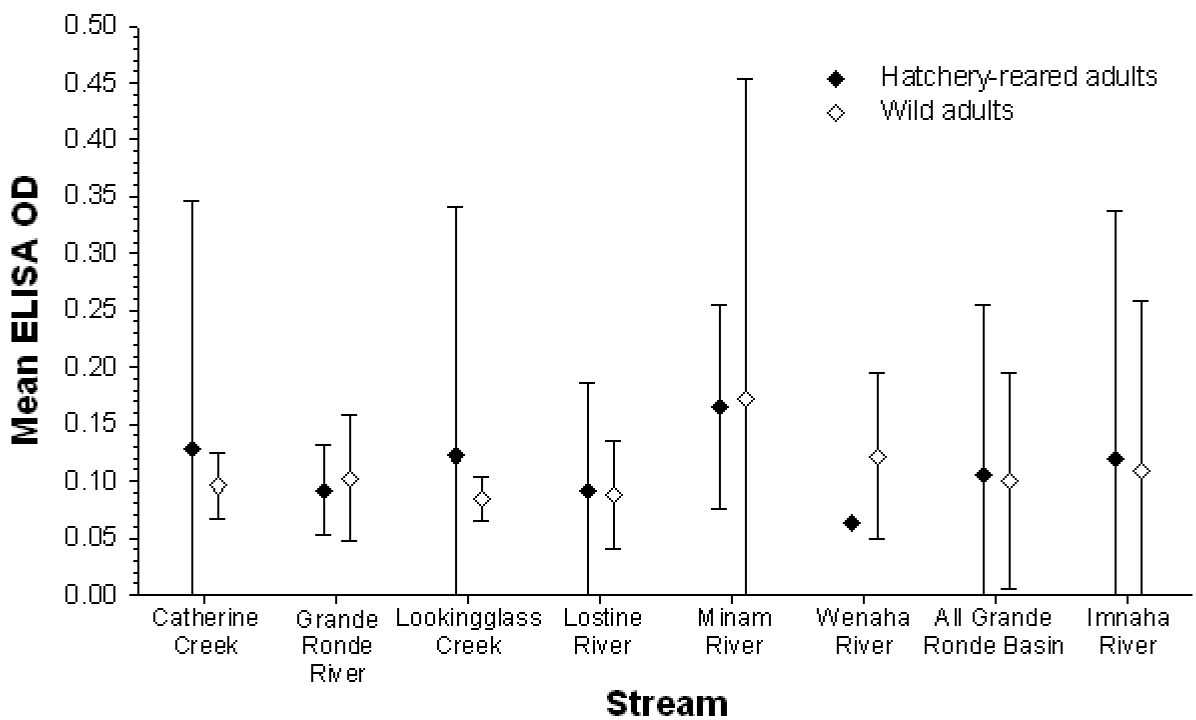

Figure 5. Mean (±1 SD) ELISA OD levels for hatchery-reared and natural adult Chinook salmon returning to streams in the Grande Ronde Basin and the Imnaha River, 1992-2008. There were no differences in mean ELISA OD levels between hatchery and natural adults (α=0.05).

and 2001 but were higher in the natural salmon in 1994 (Imnaha River data, only), 2006 and 2008 (all streams; $P \leq 0.0372$). Mean ELISA OD level was higher in hatchery salmon than in natural salmon from Catherine and Lookingglass creeks ($P \leq 0.0490$). Although the differences were not statistically significant ($P \geq 0.1669$), it should be noted that mean ELISA OD levels were higher for hatchery-reared adults than for natural adults overall and in the other supplemented streams (also in the Minam and Wenaha rivers, where few hatchery adults were recovered), except the Grande Ronde River. As of now, these differences are probably not biologically significant since the means were near or below 0.1 OD units, well below the culling level of 0.2 OD units, except the Minam River, where the natural salmon mean was 0.1730 (n=33) and stray hatchery salmon had a mean of 0.1654 (n=5).

We have 17 years of monitoring data for the Imnaha River and see little effect of hatchery supplementation on ELISA OD levels. There was no overall significant difference in mean ELISA OD levels between natural and hatchery Chinook salmon (P=0.3333) and only three years in which there was a difference. In 1994 and 2004 the natural salmon had a higher mean ELISA OD level than the hatchery salmon. However, in both of these years, the mean ELISA OD level of both origins was <0.1, so this result is unlikely to be biologically significant. In 1999, the mean ELISA OD level of the hatchery salmon was higher (0.321) due to six hatchery salmon with ELISA OD ranging from 0.21 to 2.749. Our data also show no trend in changes in mean ELISA OD levels over time for the Imnaha River (see Trends Over Time, below) and mean ELISA OD levels were < 0.2 for all years, except for two years for each group, when three or fewer females had extremely high ELISA OD levels. Although we do not have data from the inception of the Imnaha River Supplementation Program, the low and steady mean ELISA OD values for each group indicates that supplementation has probably not affected BKD prevalence or intensity in Imnaha River Chinook salmon.

Rhodes et al. (2006) found no difference in prevalence of Rs in hatchery and natural juvenile Chinook salmon sampled in Puget Sound. The differences that we found between adult hatchery and natural salmon were few, small and unlikely to be biologically significant. However, there is a concern that these differences could become larger, particularly due to the release of potentially infected hatchery salmon, such as offspring of Captive Broodstock females that had elevated ELISA OD levels. It is possible that the presence of hatchery-reared salmon with elevated BKD titers could cause an increase in the prevalence and affect of BKD in the general population. Annual monitoring will allow us to take management actions, if necessary.

<u>Wilderness vs. Supplemented Streams</u>

There was no difference in mean ELISA OD levels between adult Chinook salmon from wilderness (0.1663) vs. supplemented (0.1184) streams (P=0.0700; Figure 3). However, when comparing mean ELISA OD for only natural Chinook salmon carcasses recovered in these streams, we found that mean ELISA OD level was higher in the wilderness streams (0.1676) than in the supplemented streams (0.1025; P=0.0205). There was no difference (P=0.0659) in the mean proportion of salmon with ELISA OD levels <0.2 (Low category) between wilderness (91%) and supplemented (98%) streams when all salmon were included (Figure 4). Again, when comparing only natural salmon, the supplemented streams have a higher percentage of salmon in the Low category (99%) than the wilderness streams (91%; P=0.0360).

The difference between mean ELISA OD levels in the wilderness and supplemented streams is unlikely to be biologically significant, since both means were below the culling cutoff of 0.2 and the proportions of fish in the Low category were high in both groups. However, the Minam and Wenaha rivers had the lowest proportions of adults with ELISA ODs in the Low category (90.1% and 91.9%, respectively), whereas the supplemented streams each had at least 94.6% of the salmon with ELISA ODs in the Low category (all, except Catherine Creek exceeded 97%). These results may be due to the culling that occurs in the Conventional Hatchery Program.

Captive Broodstock vs. Conventional Hatchery Programs

Returning adults from the Captive Broodstock F_1 generation had a higher ($P<0.0001$) mean ELISA OD level (0.1349; range: 0.058-1.644) than those of the Conventional Hatchery Program offspring (0.0957; 0.056-2.274) (Figure 3). In individual streams, the returning Captive Broodstock adults had a higher mean ELISA OD level in the Grande Ronde and Lostine rivers ($P\leq0.0228$) but not in Catherine Creek (P=0.1780). The Captive Broodstock Program offspring had a lower percentage of fish in the Low ELISA category (93%) than the Conventional Hatchery Program (97%) but the difference was not significant (P=0.3573).

The Captive Broodstock Program has released offspring of females with ELISA OD levels >1.0, particularly into the upper Grande Ronde River. Conversely, both natural and Conventional Hatchery Program females returning to Grande Ronde Basin streams tend to have low ELISA OD levels and those >0.2 are culled. Therefore, smolts released from the Conventional Hatchery Program are always from females with ELISA OD levels <0.2. All smolts released from each program are differentially marked so we can associate each returning adult with the raceway in which it was reared and the disease history of that raceway and the females that produced those fish. We also have data on ELISA levels of females that contributed to the offspring in each of the raceways and will examine these data for relationships between their disease history and survival rates and disease prevalence of returning adults.

Trends Over Time

Annual mean ELISA OD level decreased over time in the Lostine River stock (P=0.0281) but did not change for any of the other stocks ($P\geq0.1369$) (Figures 6-12). The change in the Lostine River mean ELISA OD levels was due to a decrease in the ELISA OD of natural salmon (P=0.0159) from 1997-2008 - the mean ELISA OD level of the hatchery salmon did not change from 2001-2008 (P=0.7044). Prevalence of BKD (as indicated by the percentage of fish in each ELISA category) varied only for Grande Ronde River hatchery salmon, where the percentage of fish in the Low category increased over time and those in the Moderate category decreased (P=0.0497).

We found no evidence that the release of hatchery salmon is causing an increase in BKD prevalence in the monitored streams. The only change that we saw was a decrease in mean ELISA OD level in natural salmon and at the levels that we measured, it was probably biologically meaningless. Even in the Grande Ronde River, where we have released smolts that were offspring of females with very high ELISA OD levels and from raceways in which there were BKD outbreaks, we saw no change in mean ELISA and a decrease in BKD prevalence. It seems likely that any sick salmon that we may have released were either unable to survive in nature, leaving only the healthy fish to survive to maturation, or they were able to fight off the infection and return to spawn.

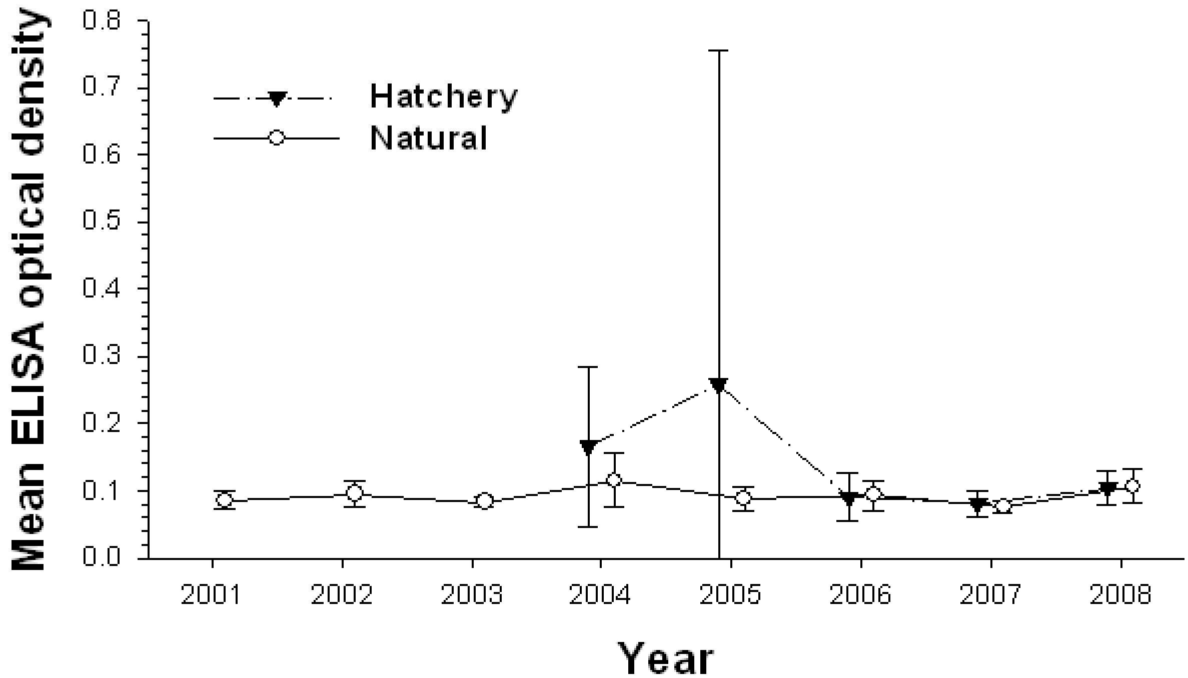

Figure 6. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from Catherine Creek, 2001-2008.

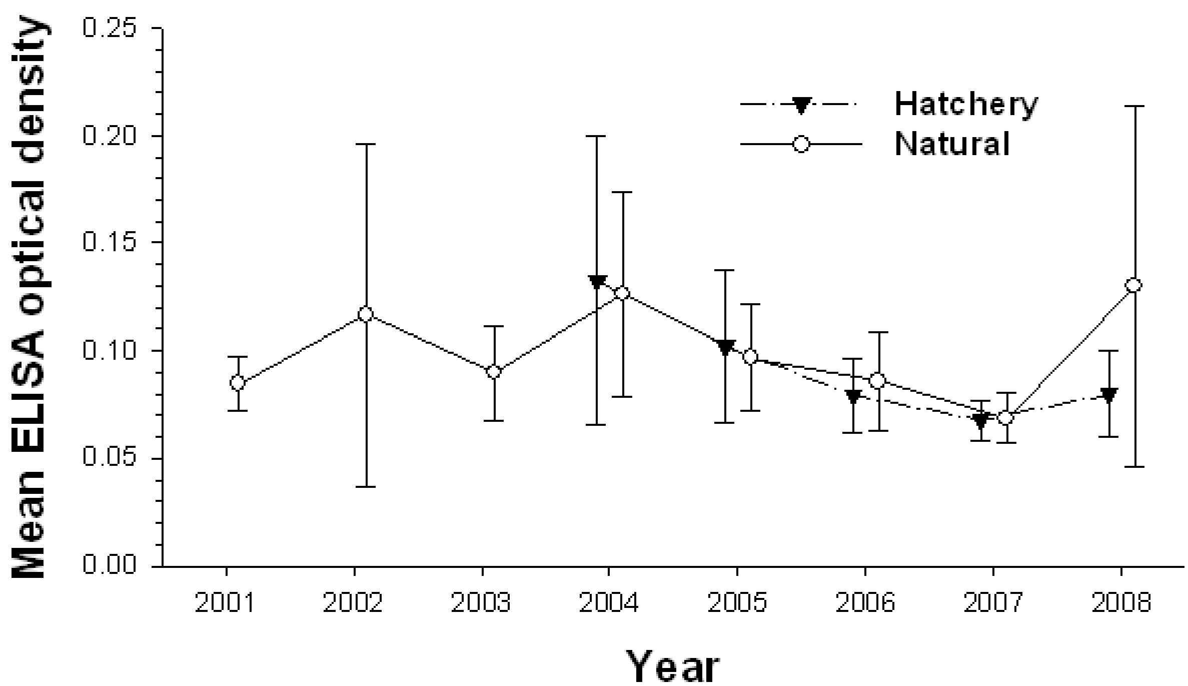

Figure 7. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from the Grande Ronde River, 2001-2008.

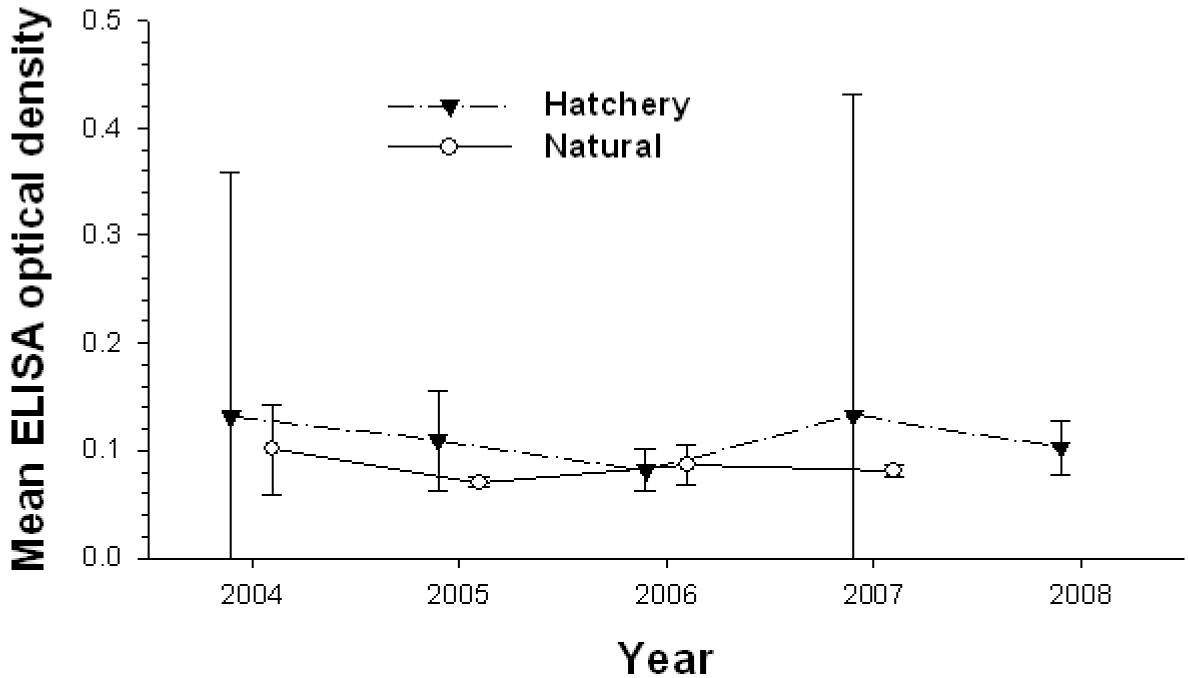

Figure 8. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from Lookingglass Creek, 2004-2008.

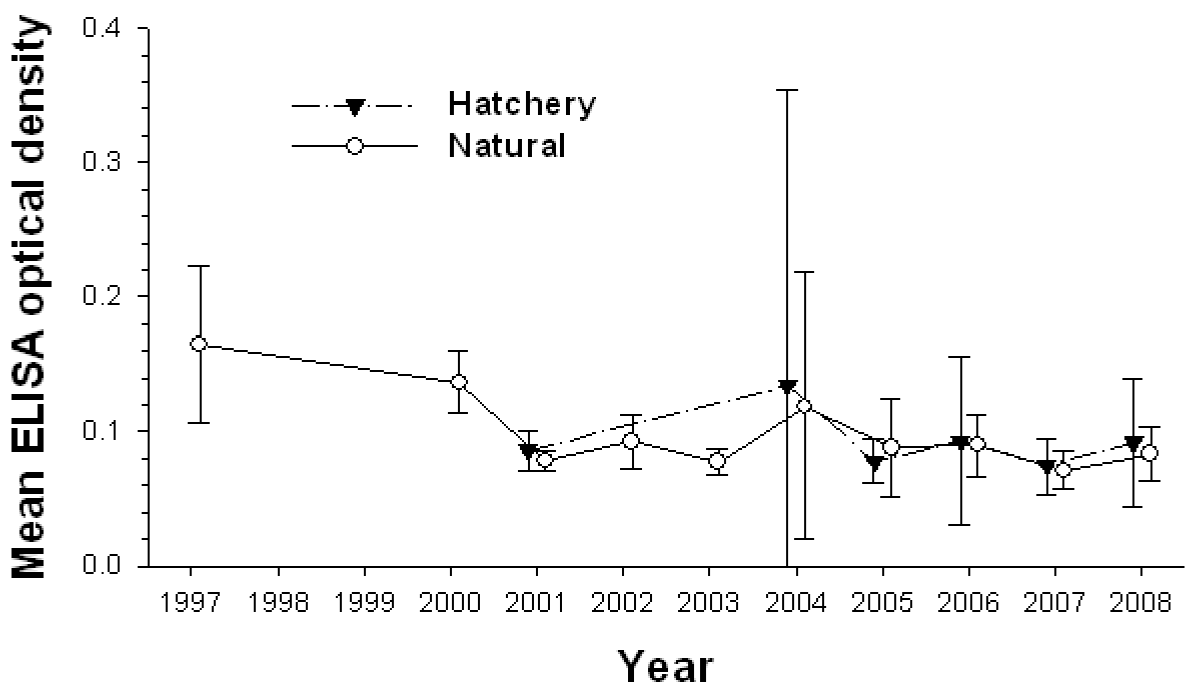

Figure 9. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from the Lostine River, 1997-2008.

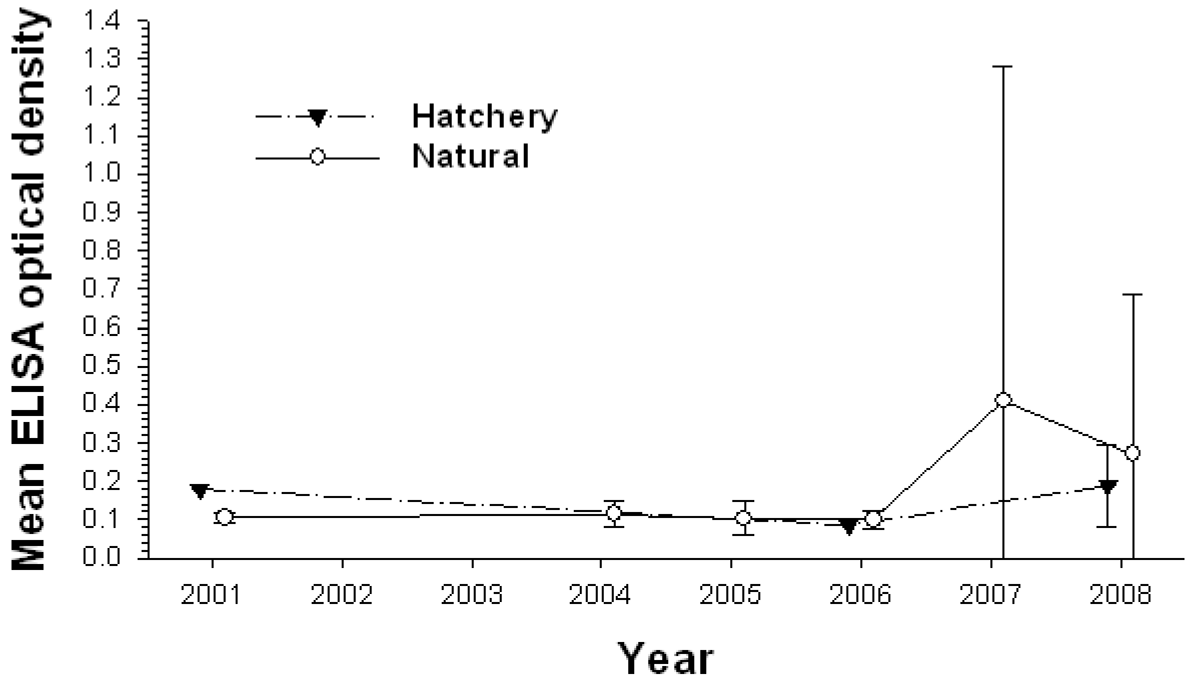

Figure 10. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from the Minam River, 2001-2008.

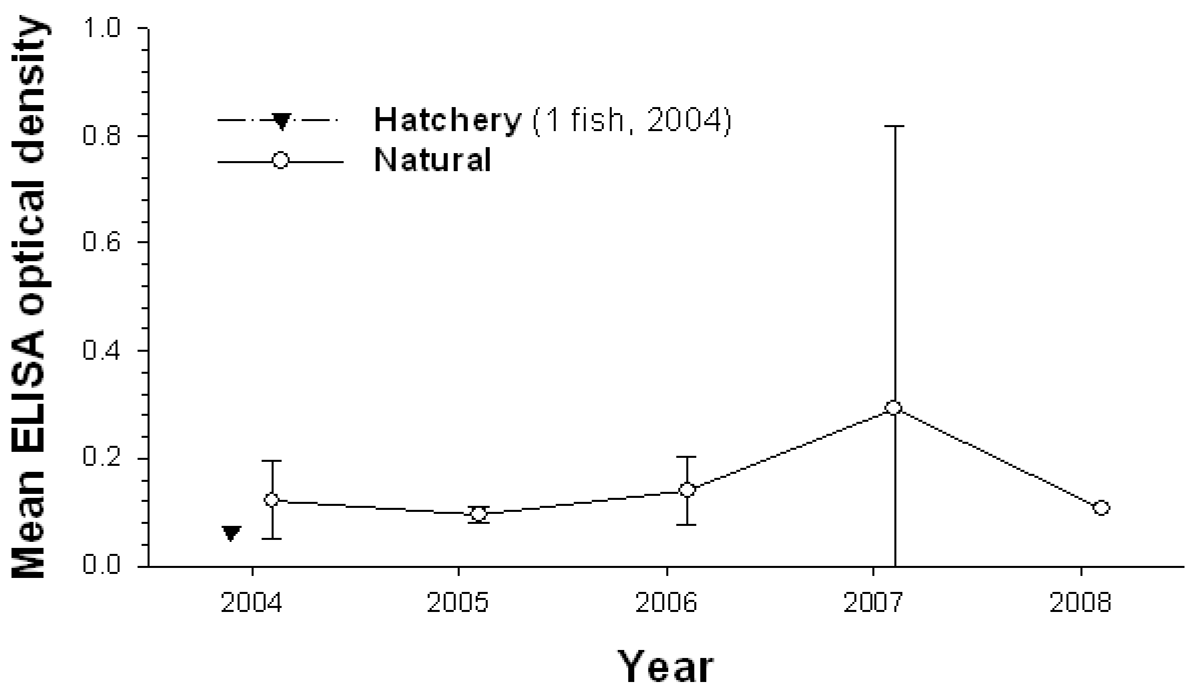

Figure 11. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from the Wenaha River, 2004-2008.

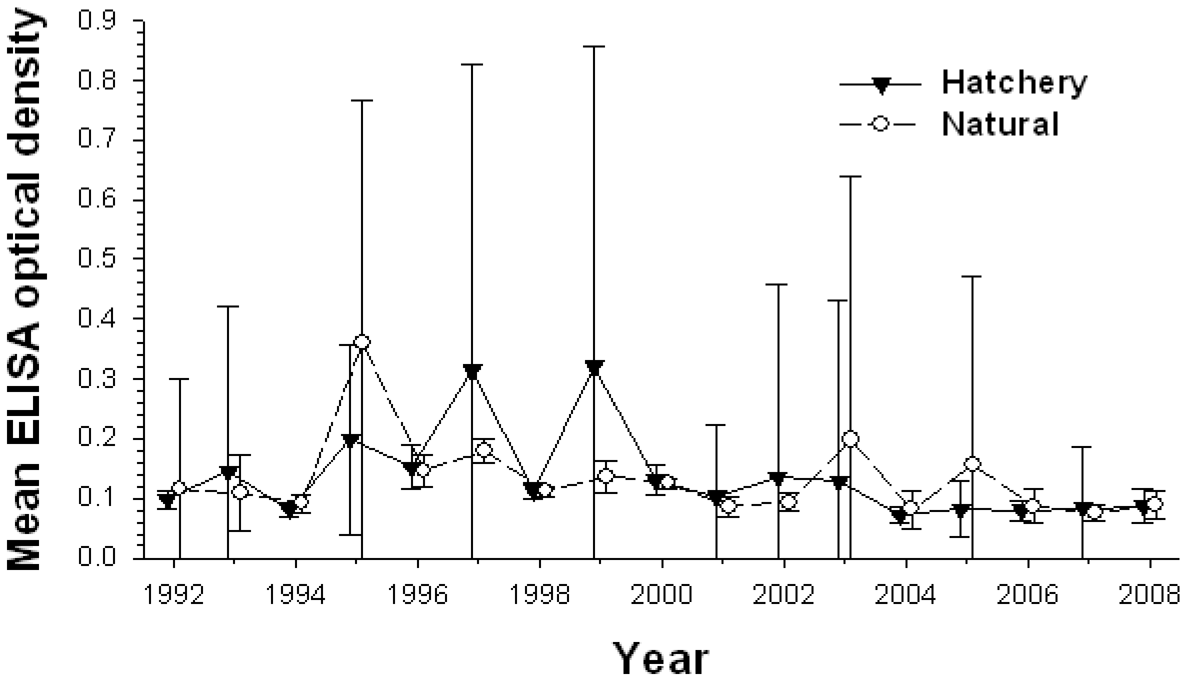

Figure 12. Mean (±1 SD) ELISA OD levels for hatchery and natural adult Chinook salmon from the Imnaha River, 1992-2008.

Conclusion

The data for BKD in Chinook salmon from northeast Oregon streams and hatcheries show that this disease is not prevalent. The two trends in changes in BKD over time are toward reduced prevalence of this disease. Those results showing no difference in ELISA OD levels between hatchery-reared and natural salmon and no difference between supplemented and wilderness streams indicate that supplementation has not affected BKD prevalence in Grande Ronde and Imnaha basin streams, as of 2008. However, this does not mean that this could not happen with increased supplementation and, particularly, when offspring of females with ELISA OD levels > 0.2 are released. The fact that each of these programs was initiated using endemic salmon and in streams where BKD is naturally low is key.

We recognize that rearing and releasing offspring of high ELISA OD females carries risk and that, on average, survival to maturation of offspring of those females is lower than that of offspring of females with low ELISA OD levels (see Pascho et al. 1993). Release of higher risk offspring is only done as part of the gene conservation mission of the Captive Broodstock Program (Hoffnagle et al. 2003). No adult Chinook salmon (hatchery or natural) returned to the upper Grande Ronde River in 1995 and 1999, evidence of the threatened status of these stocks and the need to conserve as much genetic diversity as possible. Given the threatened status and low number of returning adults, the Captive Broodstock Program's Technical Oversight Team felt that, when hatchery space was available, rearing and releasing smolts from females with elevated ELISA OD levels (usually <0.8 but occasionally higher) would provide a net benefit to the populations of these streams if any of the salmon survived to maturation. However, as numbers have increased (largely due to the Captive Broodstock Program) and the Conventional Hatchery Program took over, releases of offspring from females with elevated ELISA OD values has become a rare occurrence and will cease altogether. Broodstock collected from nature as adults and used for the Conventional Hatchery Program rarely have ELISA OD levels >0.2 (and those are culled).

It appears that BKD is a disease that at least some salmon can survive. Indeed, Pascho et al. (1991) reported that the proportion of Chinook salmon parr in the medium BKD category (ELISA OD 0.1-0.349) changed from 0% in April to 58% in July, back down to 2% in November and up to 4% in the following March. Those in the high ELISA category had a similar change: 3% in April, 8% in July, 2% in November and 16% in March. Indeed, in July, 100% of the 360 parr sampled had ELISA OD levels greater than the positive-negative cutoff. In March, as the salmon went through smoltification, 74% had positive ELISA OD values but the percentage with ELISA OD levels below the positive-negative cutoff increased from 4% in November to 26%. During that same period, cumulative mortality in low and high BKD groups of Chinook salmon parr was approximately 5.1% and 16.8%, respectively. Cumulative mortality was less than the maximum total percentage of salmon in the medium and high ELISA categories (70% in July) and the percentage of salmon with ELISA OD levels below the negative-positive cutoff increased from April through March. Pascho's results indicate that many of the salmon that had been infected by Rs (as indicated by an ELISA OD level greater than the positive-negative cutoff) were able to survive the infection and recover. Similarly, Elliott et al. (1997a) reported that 86-100% of the Chinook salmon migrating past Columbia and Snake river dams between 1988-1991 tested positive (ELISA OD level greater than the positive-negative cutoff) annually for Rs antigens, indicating exposure to this pathogen. However, in those same samples, only 4-17% tested positive by the fluorescent antibody technique (FAT), which tests for the presence of actual Rs cells and indicates an active infection. Again, this indicates that a large percentage of the smolts, some of which had ELISA OD levels > 0.2, had been exposed to this pathogen (as indicated by a high ELISA) and were able to fight off the infection (as indicated by a negative FAT).

There is also evidence that some salmon are resistant to BKD. Suzumoto et al. (1977) reported differential resistance to BKD in coho salmon *O. kisutch* with three different genotypes for transferrin, iron-binding plasma protein. The least resistant transferrin genotype was three times as likely to die from BKD than the most resistant genotype. Withler and Evelyn (1990) found differential resistance to BKD in two strains of coho salmon. The more resistant strain had nearly twice the survival rate as the less resistant strain and the mean time to death was 20% longer (68.5 days after challenge). Hard et al. (2006) found high heritability for resistance to Rs in Chinook salmon but that selective culling (based on ELISA) for BKD control in hatcheries does not affect the resistance of the hatchery progeny. However, it is highly unlikely that culling at most production hatcheries, such as the one studied by Hard et al., approaches the levels sometimes experienced in the Grande Ronde Basin Captive Broodstock Program (>50%). It seems highly likely that there is a genetic effect on populations exposed to these high levels of culling and low population numbers.

Since the Captive Broodstock Program has been rearing offspring from females with ELISA OD values >0.2, we have found that ELISA is a poor predictor of the risk of vertical transmission of Rs, especially in the middle ranges of optical densities (approximately 0.2-1.0).

This means that we have probably culled eggs even from females with ELISA OD levels >0.8 but for which no vertical transmission had taken place and may have also reared some eggs from females with low ELISA OD levels that were infected with BKD from their maternal parent. Pascho (1991) provided evidence for "a direct relationship between the presence and level of *R. salmoninarum* in a female spring Chinook salmon parent and the effect of infection on her progeny." However, they also noted that "no reports have been published on the relation between *R. salmoninarum* infection levels in kidneys of adult salmonids and the probability of vertical transmission." Pascho et al. (1991) advocated segregated rearing for the offspring of females in different ELISA categories (but not necessarily culling).

These data indicate that a better method of estimating the risk of vertical transmission than kidney ELISA OD level is needed so that we don't unnecessarily reduce the genetic variability in these threatened populations. Pascho et al.(1991) demonstrated a strong relationship between Rs concentrations in ovarian fluid (as measured by FAT) and the success of vertical transmission. We plan to test other methods and tissues to find a better predictor. Arbitrarily culling offspring from females with ELISA OD levels greater than a specific threshold can reduce the incidence of BKD in a cohort but it may also remove salmon from the population that may be resistant to BKD, despite their high ELISA OD level at the time of spawning. This would make the population more susceptible to BKD outbreaks and subject to higher mortality when they occur.

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