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[Article]

Management of Bacterial Kidney Disease in Chinook Salmon Hatcheries Based on Broodstock Testing by Enzyme-Linked Immunosorbent Assay: A Multiyear Study

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Abstract.—From the mid-1980s through the early 1990s, outbreaks of bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* continued in Chinook salmon *Oncorhynchus tshawytscha* in Idaho Department of Fish and Game (IDFG) hatcheries despite the use of three control methods: (1) injection of returning adult fish with erythromycin to reduce prespawning BKD mortality and limit vertical transmission of *R. salmoninarum*, (2) topical disinfection of green eggs with iodophor, and (3) prophylactic treatments of juvenile fish with erythromycin-medicated feed. In addition, programs to manage BKD through measurement of *R. salmoninarum* antigen levels in kidney tissues from spawning female Chinook salmon by an enzyme-linked immunosorbent assay (ELISA) were tested over 13–15 brood years at three IDFG hatcheries. The ELISA results were used for either (1) segregated rearing of progeny from females with high ELISA optical density (OD) values (usually $\geq$0.25), which are indicative of high *R. salmoninarum* antigen levels, or (2) culling of eggs from females with high ELISA OD values. The ELISA-based culling program had the most profound positive effects on the study populations. Mortality of juvenile fish during rearing was significantly lower at each hatchery for brood years derived from culling compared with brood years for which culling was not practiced. The prevalence of *R. salmoninarum* in juvenile fish, as evidenced by detection of the bacterium in kidney smears by the direct fluorescent antibody test, also decreased significantly at each hatchery. In addition, the proportions of returning adult females with kidney ELISA OD values of 0.25 or more decreased 56–85% for fish reared in brood years during which culling was practiced, whereas the proportions of ELISA-negative adults increased 55–58%. This management strategy may allow IDFG Chinook salmon hatcheries to reduce or eliminate prophylactic erythromycin-medicated feed treatments. We recommend using ELISA-based management of BKD in Chinook salmon hatcheries where it is a concern.

Renibacterium salmoninarum, the etiological agent of bacterial kidney disease (BKD), causes significant morbidity and mortality in cultured Chinook salmon *Oncorhynchus tshawytscha* and has been implicated in the decline of adult salmon returns in the Pacific Northwest (Raymond 1988). This gram-positive diplobacillus can be transmitted horizontally (Bell et al. 1984; Murray et al. 1992) and vertically (Evelyn et al. 1986a, 1986b). Chronic losses of fish due to BKD occur during hatchery rearing (Fryer and Sanders 1981), during emigration to the ocean (Park 1985; Banner et al. 1986), and during ocean residence (Banner et al. 1986). Bacterial kidney disease is also a cause of prespawning mortality in returning adults (Groman and Klontz 1983; Evelyn 1988; Elliott et al. 1989; Brown et al. 1990). Infection by *R. salmoninarum* causes a host response characterized by a granulomatous inflammation that may result in abscesses in the kidney, spleen, and liver (Bruno 1986) and progress to a septicemia. Diagnosis and treatment have been difficult because of the dual modes of transmission, the insidious nature of the disease, the intracellular infection by the bacterium, and the protracted manifestation of disease signs. Gross signs of BKD could be confused with proliferative kidney disease and possibly the gross manifestations of *Ichthyophonus hoferi*, but diagnostic tools such as Gram stain, immunological and molecular tests, and histopathology will exclude these other diseases.

Attempts to control BKD with various disease management strategies have provided some benefit,

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but have not entirely removed the bacterium from hatchery populations or alleviated concerns of BKD outbreaks within Chinook salmon hatcheries. Antibiotic injections of returning adults have reduced prespawning mortality due to BKD (Lee and Evelyn 1994; Haukenes and Moffitt 1999), but vertical transmission has not been completely eliminated, and some adult fish have been lost to erythromycin toxicity (Moffitt and Kiryu 1999). Iodophor disinfection of eggs successfully inactivates surface bacteria but does not eliminate intraovum infections (Evelyn et al. 1984, 1986a, 1986b). Prophylactic antibiotic-medicated feed treatments of hatchery juvenile salmon are not entirely protective (Fryer and Sanders 1981; Groman and Klontz 1983; Austin 1985; Elliott et al. 1989), and a potential for development of reduced antibiotic susceptibility exists in *R. salmoninarum* (Bell et al. 1988; Rhodes et al. 2008). Thus, use of nonantibiotic methods for BKD control or prevention is preferable. Unfortunately, vaccination programs have not provided the anticipated protection in Pacific salmon (Evenden et al. 1993). Vaccination of Atlantic salmon *Salmo salar* with *Arthrobacter davidaneli* (commercial vaccine Renogen) has provided adequate protection from BKD (Griffiths et al. 1998; Salonius et al. 2005). The protection provided by *A. davidaneli* is thought to be a function of the close relationship between *Arthrobacter* and *Renibacterium* (Wiens et al. 2008), but similar protection has not been observed in Pacific salmon (Rhodes et al. 2004; Alcorn et al. 2005).

The application of enzyme-linked immunosorbent assay (ELISA) to cull brood Chinook salmon has provided another nonantibiotic management strategy for control of BKD. This strategy uses ELISA optical density (OD) values from kidney tissue testing of each brood female to evaluate risk of vertical transmission. Early research demonstrated an increased prevalence of *R. salmoninarum* and higher incidences of BKD in progeny of adult Chinook salmon females with elevated ELISA OD values (Pascho et al. 1991, 1993; Elliott et al. 1995). Conversely, male Chinook salmon have not been implicated in vertical transmission of *R. salmoninarum* (Evelyn et al. 1986a, 1986b). Segregated rearing of Chinook salmon families, based on ELISA OD values of the female parent, demonstrated that progeny from females with higher ELISA OD values (above 0.25) had higher prevalence of BKD after seawater entry (Elliott et al. 1995), were more susceptible to predation (Mesa et al. 1998), had higher mortality when exposed to gas super saturation (Weiland et al. 1999), and were more likely to horizontally transmit *R. salmoninarum* during coded-wire-tagging (Elliott and Pascho 2001). Higher survival rates of Chinook salmon progeny from low *R. salmoninarum* ELISA OD parentage (Elliott et al. 1989; Pascho et al. 1991, 1993) and lower *R. salmoninarum* prevalence in populations subjected to culling of high RS ELISA OD broodstock (Meyers et al. 2003) further illustrated the potential effectiveness of broodstock segregation and culling as strategies for managing vertical transmission of BKD in this species. Certain other studies indicated variable success in BKD management programs involving broodstock screening, and culling or segregation of egg lots from highly *R. salmoninarum*-infected females, but the management procedures were inconsistently applied (Maule et al. 1996; Vanderkooi and Maule 1999).

Since 1993, the Idaho Department of Fish and Game (IDFG) has implemented an ELISA-based culling and segregation rearing strategy to limit vertical and horizontal transmission of *R. salmoninarum* at all Chinook salmon rearing facilities. The research reported here tested the hypothesis that BKD mortality of juvenile Chinook salmon at IDFG hatcheries could be reduced by use of ELISA-based management to remove from general production the eggs from females at high risk for vertical transmission of *R. salmoninarum*. Other benefits, such as lower prevalence and intensity of *R. salmoninarum* infection in returning adults, were not considered at commencement of the program, but were observed as the program evolved. In this study we report the results of a multiyear ELISA-based program for controlling BKD in Chinook salmon cultured at three IDFG hatcheries Sawtooth Hatchery, Rapid River Hatchery, and McCall Hatchery.

Overview of Hatcheries

The effects of the IDFG ELISA-based BKD program were examined at McCall Hatchery (1987–2005), Rapid River Hatchery (1986–2005), and Sawtooth Hatchery (1985–2005; Figure 1). In addition, Clearwater and Oxbow hatcheries were part of the operations of the Rapid River Hatchery Chinook salmon program. Oxbow and Rapid River hatcheries are funded through Idaho Power Company; the Clearwater, McCall, and Sawtooth hatcheries are funded through the Lower Snake River Compensation Plan of the U.S. Fish and Wildlife Service.

McCall Hatchery is located in McCall, Idaho (UTM 11T569296mE, 4975572mN), and Valley County. The hatchery is located on the banks of the North Fork of the Payette River and is approximately 0.16 river kilometers (rkm) downstream of its water source, Payette Lake. Adult Chinook salmon were trapped at the South Fork trap on the South Fork of the Salmon River (UTM 11T602760mE, 4946775mN) approximately 42 km east of Cascade, Idaho, and 1,158 rkm from the Pacific Ocean. Returning adult Chinook

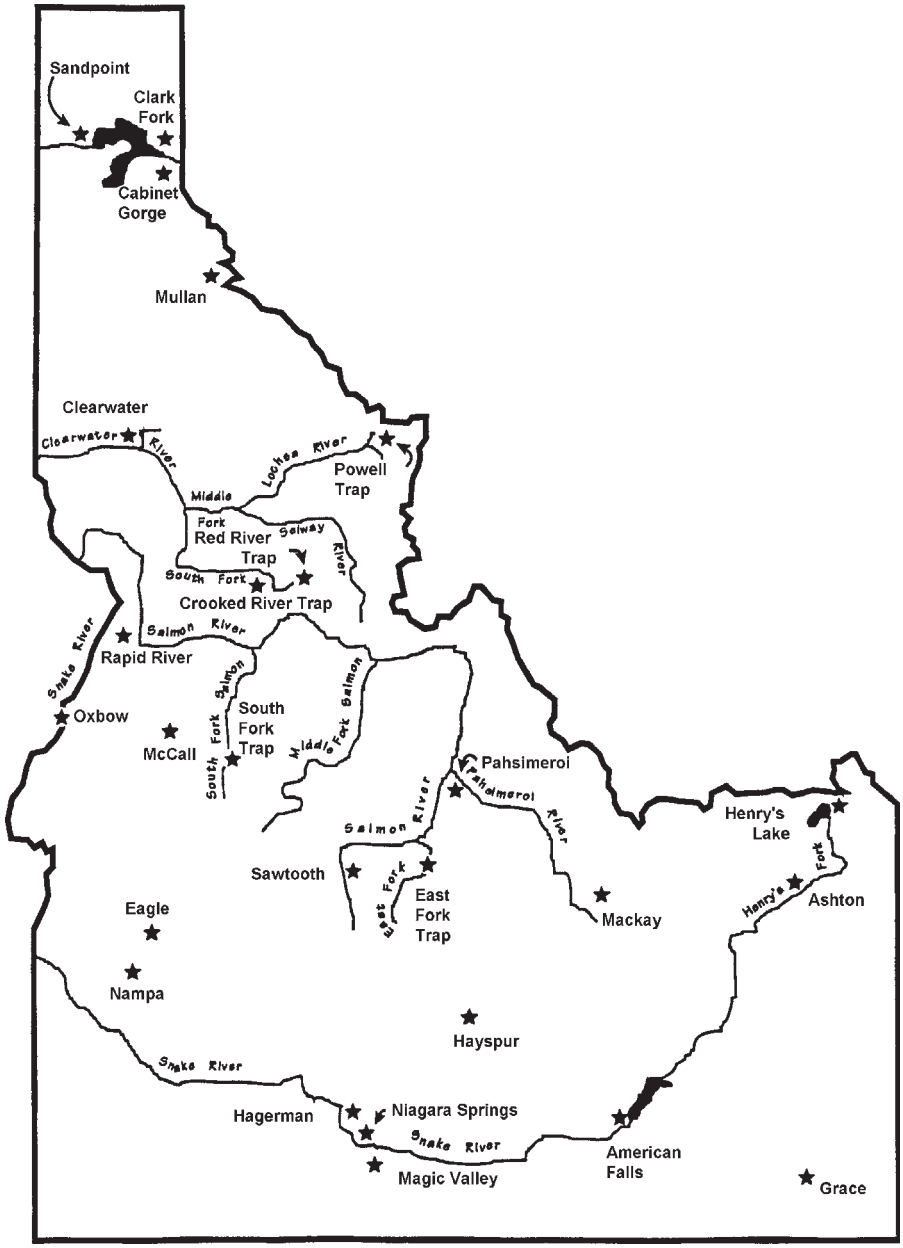

FIGURE 1.—Locations of Idaho Department of Fish and Game hatcheries (stars) and associated watersheds.

salmon were held at the South Fork trap until they were artificially spawned and the green eggs were then transported to McCall Hatchery on the day of spawning. Adult Chinook salmon were not released into the water source of McCall Hatchery to volitionally spawn.

Rapid River Hatchery (UTM 11T547292mE, 5022383mN) is located 11 km southwest of Riggins (Idaho County), Idaho, and is approximately 960 rkm from the Pacific Ocean. Rapid River is the water source for the hatchery and is a tributary to the Little Salmon River. Adult Chinook salmon were released above the hatchery's weir into the hatchery's water source to volitionally spawn. Returning adult Chinook salmon were trapped 4 rkm downstream of the main hatchery, transported to the adult holding ponds at the main

facility, and held until artificially spawned. Additionally, Chinook salmon were trapped below Hells Canyon Dam (UTM 11T523404mE, 5010114mN) on the Snake River, approximately 915 rkm from the Pacific Ocean, and were transported to Oxbow Hatchery (UTM 11T511485E, 4979901N) and held briefly in adult holding raceways before being transported to Rapid River Hatchery. Once at Rapid River Hatchery, these fish were placed into the adult holding ponds with the fish that were trapped at Rapid River Hatchery.

Sawtooth Hatchery (UTM 11T669426mE, 4890344mN) is located 8.05 km south of Stanley (Custer County), Idaho, and is approximately 1,529 rkm from the Pacific Ocean. The Salmon River is the water source for outside raceways of this hatchery. Adult Chinook salmon are trapped at this hatchery and were held until artificially spawned. A portion of the fish trapped at this hatchery were released above the weir into the hatchery's water source to volitionally spawn.

Clearwater Hatchery (UTM 11T551498mE, 5151905mN) is located on the north bank of the North Fork of the Clearwater River, 2.4 rkm downstream from Dworshak Dam, in Ahsahka (Clearwater County), Idaho, and is approximately 811 rkm upstream of the mouth of the Columbia River. The North Fork of the Clearwater River (Dworshak Reservoir) is the water source for this hatchery. Adult salmon were not released into the water source to spawn volitionally.

Methods

Fish Culture Methods

McCall Hatchery.—Eggs were collected, fertilized, and then transported from the South Fork trap on the Salmon River to McCall Hatchery, where they were immediately loaded into Heath trays. Trays were loaded with the eggs of two females, and water flows were set at 19 L/min per stack. Once ELISA-based culling and segregation were instituted, ELISA values were communicated to the hatchery staff, and the appropriate eyed eggs were culled or placed into segregation. Eggs were treated daily with 1,667 (L/L formalin for 15 min to control fungal growth. This treatment was continued until just before hatching. The eggs eyed at 600 temperature units (TU) and were then shocked to identify dead eggs for discard. This practice is necessary to limit fungal growth on the egg mass. Incubation temperatures ranged from 3–7°C. Fry were set out into the 14 indoor concrete raceways at about 1,700 TU. These raceways were loaded with 30,000–55,000 fry, and flow rates were set at a minimum of 304 L/min. As density index (DI) reaches approximately 0.45 in mid-June, these fish were moved outside into the two concrete final rearing ponds after they were fin-clipped, coded-wire-tagged, and enumerated. The temperature range for indoor rearing ranged from 3–8°C. The fish remained in the rearing ponds until the following March. Prior to release, DI typically reached 0.23. Water temperatures during final rearing were 7–12°C. The fish were then transported to the South Fork of the Salmon River and released to migrate to the Pacific Ocean.

Rapid River Hatchery.—Eggs were collected in similar fashion as at McCall Hatchery at the South Fork trap and at Sawtooth Hatchery. Eggs were placed in Heath stacks and were shocked at 500 TU by pouring the eggs from the trays into well water. A portion of the eggs, up to 1.5 million, were transported as green eggs to Oxbow Hatchery to facilitate single-family incubation. Incubation of these eggs is described in the Oxbow Hatchery section. Once ELISA-based culling and segregation were instituted, ELISA values were communicated to the hatchery staff, and the appropriate eggs were culled or placed into segregation at Clearwater Hatchery. The dead eggs were removed and the remaining live eggs were enumerated using a Jensorter. The eggs were then returned to clean trays. At 760, 1,000, and 1,500 TU, eggs were picked to remove dead ones. Formalin was applied to eggs at a rate of 1,667 (L/L for 15 min three times per week to control fungal growth until 800 TU. Fry were placed into 10 concrete raceways for early rearing at 1,700 TU. As DI reached the maximum of 0.8, fry were fin-clipped, coded-wire-tagged, enumerated, and moved to the final rearing ponds. This transfer occurred during June when fish averaged 3.8 g. The final rearing ponds were disinfected with chlorine (200 mg/L of water) before fish were introduced. Fish were reared in these ponds until the following March. Before release, DI in the ponds typically reached 0.23. Water temperatures in final rearing were 0–17°C. Portions of the population were released in the Snake River below Hells Canyon Dam, a portion in the Little Salmon River, and the rest of the fish were released at the hatchery.

Oxbow Hatchery.—Approximately 1.5×10^6 green eggs were transported from Rapid River Hatchery to Oxbow Hatchery on an annual basis to facilitate single-family incubation of eggs. Upon arrival they were disinfected with iodophor (100 mg/L of water) for 30 min. Eggs from females with high ELISA values were culled or placed in segregation. Flows for incubation were set for 19 L/min for each stack of Heath trays. Incubation water temperature was approximately 7°C. These eggs were treated with formalin at 1,667 (g/L for 15 min, three times per week. Eggs were incubated in well water until 500 TU; dead eggs were then removed after shocking and live eggs enumerated. The eggs

were returned to Rapid River Hatchery as eyed eggs to complete incubation and rearing. In years of segregation, 1994–1997, eggs from high-ELISA-value females were transported to Clearwater Hatchery as eyed eggs for segregation rearing. Clearwater Hatchery culture details are described in the Clearwater Hatchery section.

Sawtooth Hatchery.—Eggs were collected, fertilized, and transported in similar fashion as at South Fork trap and Rapid River Hatchery. After disinfection with iodophor (100 mg/L of water) for 30 min, eggs were loaded into Heath trays with the eggs of two females per tray. Thus, if one female's eggs were to be culled or segregated, both sets of eggs in that particular tray would be subject to that management action. Flows were set at 19 L/min per stack. Incubation water temperatures were 4–10°C. Eggs were treated three times per week with 1,667 (g/L formalin for 15 min. The eggs were incubated in well water, and once ELISA results were communicated and culling or segregation actions were taken, eggs were shocked, dead eggs removed, and live eggs enumerated. Fry were loaded into the indoor raceways and well water flows were set to 76 L/min. Indoor early incubation temperatures were 4–8°C. As the fish grew, flows were increased to a maximum of 418 L/min to accommodate a maximum DI of 0.14. By May, the fish reached 7 cm in length and were adipose fin-clipped and coded-wire-tagged before being placed in the outdoor raceways. The fish remained in the outdoor raceways until the first week of the following April. Final rearing temperatures were 0–21°C. The majority of the fish were released at the hatchery into the Salmon River to migrate to the Pacific Ocean. Smaller portions of the fish were released at various alternate sites off the hatchery.

Clearwater Hatchery.—Fish with high BKD values were segregated into groups and transported from Rapid River and Oxbow hatcheries to Clearwater Hatchery as eyed eggs for brood years 1994–1997. These eggs were disinfected with iodophor (100 mg/L of water) for 30 min. The eyed eggs were then loaded into Heath trays and flows were set to 19 L/min per stack. At 500 TU, the eggs were shocked, dead eggs removed, and live eggs enumerated. Fry were loaded into the 60 indoor raceways, and flows were set up to 456 L/min; flow indices were kept below 0.5, and DI was kept below 0.3. By May, fish were moved from the indoor raceways to 11 Chinook salmon outdoor raceways. The fish were reared in these raceways until release the following March. Water temperatures during the final rearing period typically were 2–15°C. Each final rearing raceway received an estimated 2,000 L/s of water, and the final DI was 0.28. The high-BKD segregation groups were transported to either below Hells Canyon Dam for direct release into the Snake River or to Rapid River Hatchery for release into the Rapid River.

BKD Management Program

The management of BKD in IDFG Chinook salmon hatcheries commenced as returning adult fish were trapped. During the initial handling, each fish received an intraperitoneal injection of erythromycin at a dose of 10–20 mg/kg body weight, and fish were placed into holding ponds until spawning. Eggs were collected, fertilized, and water hardened in iodophor (100 mg/L of water) for 30 min. During the spawning process, a 1-g piece of kidney tissue was collected from each female, bagged, labeled, and chilled during transport to the Eagle Fish Health Laboratory at Eagle, Idaho, where each female was tested via ELISA for *R. salmoninarum* antigens to obtain an optical density (OD) value. From 1993 onward, 100% of spawned Chinook salmon females were analyzed by ELISA, and the eggs from high risk females were placed into segregation groups and isolated from the general population until they were released as smolts or culled. Originally, IDFG segregation groups consisted of negative-lows (ELISA OD, <0.25), moderate-highs (0.25–0.4), and highs (>0.4). Beginning in 1997, most IDFG Chinook salmon programs began culling eggs from females with OD values above 0.249. The results of early ELISA screening of adult fish and implementation of broodstock segregation procedures suggested that use of this ELISA OD cutoff value would remove eggs from the majority of females at risk for vertical transmission of the bacterium, without severely jeopardizing fish production goals. If an excess of eggs still existed within a hatchery's broodstock, then the hatchery staff culled the eggs of the female with the highest kidney tissue ELISA OD value and continued to the next highest ELISA OD value until the hatchery reached rearing capacity. If adult returns were below broodstock requirements, eggs from females with kidney ELISA OD values above 0.249 were kept, and the culling point was then set at an OD value of approximately 0.6. The eggs from these higher-risk females were reared in segregation. The progeny of fish with OD values greater than 0.6 were culled, even in years with low adult returns, because of the likelihood of vertical and horizontal transmission of *R. salmoninarum*. Once the fish were ponded and fed a commercial diet, they received two applications of erythromycin feed (100 mg/kg of body weight) for 28 d, to treat the possibility of an insidious infection of *R. salmoninarum*. This treatment regimen was established before ELISA-based segregation and culling was

instituted, and continued after the ELISA-based program was introduced. Usually, the first feeding was applied in June, and the second feeding of erythromycin medicated feed was applied in August and September.

Preliberation Health Examination

At 30–45 d before liberation, 60 Chinook salmon from each stock from each facility were sampled to determine the prevalence of *Myxobolus cerebralis*, *Renibacterium salmoninarum*, and viral pathogens. Beginning in 1995 (brood year 1993), the sample size was reduced to a random sample of 20 fish. A direct fluorescent antibody test (DFAT) for *R. salmoninarum* was the preferred technique for individual fish because ELISA sampling required more kidney tissue than was available in individual juvenile Chinook salmon at the time of sampling. Preliberation ELISA testing was performed on pools of kidney tissue sampled from five fish.

Monthly Inspections

Monthly inspections of Chinook salmon were completed on 10 fish from each stock from each facility. Moribund fish were targeted for these samples, because of the higher likelihood of detecting pathogens within the particular stock of fish. If 10 moribund fish could not be acquired, the remainder of the sample was composed of randomly selected fish. This pathogen surveillance program included DFAT for *R. salmoninarum*.

Techniques for Detecting R. salmoninarum

Direct fluorescent antibody test.—Specific goat antibody against *R. salmoninarum*, conjugated with fluorescein isothiocyanate, was obtained from Kirkegaard and Perry Laboratories, Inc. (Gaithersburg, Maryland). The conjugated anti-*R. salmoninarum* antibody, diluted to 1:40 (volume : volume), was used to stain individual kidney tissue smears on a 12-well slide. Fixation and staining of the tissue smears were performed as described by Pascho et al. (1987). A Nikon Optiphot microscope was used to examine 30 microscope fields (1,000× magnification) by epifluorescence on each well for the presence of *R. salmoninarum*. In the EFHL, a low positive detection of *R. salmoninarum* by direct fluorescent antibody test is equated to an ELISA OD value of 0.6. Bullock et al. (1980) reported that about 10^4 *R. salmoninarum* cells per kidney smear were needed for a positive DFAT, and Meyers et al. (1993a) determined that *R. salmoninarum* detections by DFAT in kidney samples were rare for samples with ELISA OD values of 0.173 or less, inconsistent for samples with values greater than 0.173 but less than 0.978, and consistent for samples with values of 0.978 or more.

Enzyme-linked immunosorbent assay.—Beginning in 1991, a polyclonal antibody ELISA, performed with commercially produced reagents and buffers (Kirkegaard and Perry Laboratories, Inc.) as described by Pascho et al. (1991) in ELISA II procedure, was used as the preferred technique for broodstock evaluation for *R. salmoninarum* in IDFG Chinook salmon hatchery programs. For all years of adult fish testing, kidney tissue samples with ELISA OD values less than 0 0.10 were considered to be ELISA negative (below detection limits for *R. salmoninarum* antigens), samples with values of 0.10–0.249 were considered to be low-positive for *R. salmoninarum* antigens, and samples with values of 0.25 or more were considered to be high-positive for *R. salmoninarum* antigens. Polyclonal antibody ELISAs have been reported to detect *R. salmoninarum* antigen levels as low as 20 ng/mL in kidney homogenates (Meyers et al. 1993b) and bacterial concentrations as low as 10^3 cells/g of kidney tissue (Jansson et al. 1996).

Mortality and Survival Measurements

Mortality data were collected from each of the study hatcheries to determine effects of these ELISA-based segregation and culling practices. Differences in percent mortality of fish reared under the different BKD management strategies were determined and used to estimate changes in survival associated with each strategy. Total mortality for a given brood year was determined by subtracting inventory number at release from inventory number at initial ponding. This number, expressed as percent, was total mortality for that brood year and was verified by daily mortality counts. Mortality was determined for years in the study period before ELISA-based management, during ELISA-based segregation, and during ELISA-based culling for BKD. Percent mortality was computed for all the hatcheries combined and for individual hatcheries.

Statistical Analysis

A log-linear model (Feinberg 1977) was used to test for differences among hatcheries and years of occurrence of BKD (as indicated by elevated ELISA OD values) in spawning female Chinook salmon. The ELISA OD values for returning adults reared prior to ELISA-based BKD management were compared with those of adults reared after establishment of ELISA-based BKD management. We coded OD as 0 if the ELISA value was less than 0.25 and 1 if it was 0.25 or greater. Letting $Y = 0$–1 for coded OD value and $X_1 =$ location, $X_2 =$ year, we tested for the effects of location and year on the proportion of ELISA OD values greater

than or equal to 0.25. We followed the log-linear analysis by a nonparametric Jonckheere–Terpstra test (Higgins 2004) for each hatchery to test for trends in ELISA value proportions greater than 0.25 over years. All analyses were run in SAS (SAS 2005).

For data from each hatchery, Fisher's exact test (InStat3, Graph Pad) was used to compare proportions of juvenile fish testing positive for *R. salmoninarum* by DFAT in brood years that ELISA-based culling was not used against brood years that ELISA-based culling was used. In cases where zero values were encountered (i.e., no DFAT-positive fish), the program added 0.5 to each value to make calculations possible.

Analysis of variance (ANOVA; Ott and Longnecker 2001) was used to test for differences in presmolt mortality in brood years that ELISA-based culling was not used against brood years that ELISA-based culling was used. Because the mortality data consist of proportions mostly less than 0.2, an arcsine square root transformation was used to normalize the data prior to testing by ANOVA. All analyses were run in SAS (Proc Mixed; SAS 2005).

Results

The success of the project was beyond our expectations. Regardless of rearing circumstances, pond or raceway, secured water source or anadromous adults released into water source to spawn volitionally, or climate fluctuations, ELISA-based management of hatchery Chinook salmon broodstocks reduced prevalence, intensity of infection, and mortality due to *R. salmoninarum* in juveniles and broodstock.

Mortality of Juvenile Fish during Hatchery Rearing

When fish mortality from each study hatchery was combined, mortality averaged 5.0% in years before ELISA-based management was applied to salmon culture. In years that ELISA-based segregation was applied to the salmon cultured at these hatcheries, mortality averaged 4.0%, whereas in the years that ELISA-based culling was used to manage BKD, mortality averaged 2.1%. Data for years in which segregation was practiced were too limited to include in statistical analysis. However, ANOVA testing revealed significantly lower mortality rates ($P < 0.0001$) at each of the three hatcheries in years that ELISA-based culling was used compared with years that ELISA-based BKD management was not practiced.

Mortality at McCall Hatchery.—Mortality averaged 5% in the 6 years of the study before ELISA management of BKD was applied to Chinook salmon (Table 1). In the 3 years that ELISA-based segregation was used to manage BKD in the Chinook salmon reared at this hatchery, overall mortality averaged 4%. However, during this 3-year period, the high-BKD segregation groups were reared in the small indoor raceways, and mortality in these groups was 8% when calculated separately from the rest of production. In the 10 years that ELISA-based culling was practiced during this study, mortality dropped to 3%.

Mortality at Rapid River Hatchery.—Mortality averaged 4% in the 7 years of the study period before ELISA management of BKD was applied to the Chinook salmon reared at this hatchery (Table 2). In the 4 years that ELISA-based segregation was used to manage BKD, mortality averaged 0.1%. During this 4-year period, the high-BKD segregation groups were reared at Clearwater Hatchery. Mortality measured only in the high-BKD segregation groups was 3%. In the 9 years that ELISA-based culling was practiced, mortality averaged 1% at this hatchery.

Mortality at Sawtooth Hatchery.—Mortality averaged 6% in the 4 years of the study period before ELISA management of BKD was applied to the Chinook salmon reared at this hatchery (Table 3). In the 5 years that ELISA-based segregation was used to manage BKD, mortality averaged 21%. In 1992, during a segregation management year, 48% of the juvenile production was lost to external mycosis caused by *Saprolegnia parasitica*. If this 1 year is removed from the segregation results, then the average mortality for this management practice is 1%. In the 10 years that ELISA-based culling was practiced, mortality averaged 5%. Mortality in juvenile Chinook salmon during culling management was attributed to infectious hematopoietic necrosis (IHN) in brood year (BY) 2000 (approximately 6,000), and to erythromycin toxicity in BY 2004 (approximately 50,000), whereas *Ichthyophthirius multifilis* has been a constant summer problem at this hatchery since the year 2000.

Detections of R. salmoninarum in Juveniles via DFAT

The DFAT data from all of the hatcheries combined showed that *R. salmoninarum* was detected by this test in 8% of juvenile Chinook salmon examined before the implementation of ELISA-based BKD management, but in only 0.4% of juvenile fish examined after the institution of ELISA-based culling. Analysis of data from individual hatcheries showed significant decreases in DFAT detections of *R. salmoninarum* in juvenile fish at McCall Hatchery ($P < 0.0001$), Rapid River Hatchery ($P = 0.0041$), and Sawtooth Hatchery ($P < 0.0001$) during the years of ELISA-based culling compared with years that no ELISA-based BKD management was practiced.

McCall Hatchery.—In the 6 years prior to implementation of an ELISA-based program, *R. salmonina-*

TABLE 1.—Mortality of Chinook salmon juveniles in years before ELISA-based management and with ELISA-based segregation and ELISA-based culling at McCall Hatchery. Mortality of the high-bacterial-kidney-disease (BKD) segregation group was calculated separately from those for McCall Hatchery production.

Brood year	Fish ponded	Mortality	Mortality (%)	SE
Before ELISA management				
1987	1,082,420	107,420	10	0.0003
1988	1,070,111	37,611	4	0.0002
1989	728,845	20,245	3	0.0002
1990	942,469	40,969	4	0.0002
1991	620,754	13,600	2	0.0002
1992	1,139,383	79,220	7	0.0002
Total	5,583,982	299,065	5	0.00009
With ELISA-based segregation of production ponds				
1994	586,306	19,877	3	0.0002
1995	233,188	12,477	5	0.0005
1996	397,554	10,612	3	0.0003
Total	1,217,048	42,966	4	0.0002
With high-BKD segregation vats				
1994	21,556	2,331	11	0.002
1995	18,623	967	5	0.002
1996	7,602	672	9	0.003
Total	47,781	3,970	8	0.001
With ELISA-based culling				
1993	1,132,045	40,552	4	0.0002
1997	1,259,433	76,822	6	0.0002
1998	1,074,199	34,269	3	0.0002
1999	1,207,904	42,673	4	0.0002
2000	1,085,366	21,116	2	0.0001
2001	1,098,171	44,511	4	0.0002
2002	1,118,454	29,644	3	0.0002
2003	1,070,839	23,309	2	0.0001
2004	1,123,680	27,550	3	0.0001
2005	1,109,448	22,278	2	0.0001
Total	11,279,539	362,724	3	0.00005

rum was detected by DFAT in 9% of sampled fish, whereas during the 3 years of ELISA-based segregation programs *R. salmoninarum* was detected by DFAT in 12% of sampled fish in the high-BKD segregation groups but in none of the fish from the low-BKD groups (Table 4). Clinical signs of BKD were also observed in the high-BKD segregation groups during 1994 and 1996. In the 10 years that ELISA-based culling was performed, only a single fish was DFAT positive for *R. salmoninarum* (Table 4), and no clinical signs of BKD were observed. That detection occurred during BY 1997, when the culling ELISA OD point was elevated to 0.4 to meet hatchery production goals.

Rapid River Hatchery.—In the 7 years before ELISA-based BKD management was begun in BY 1993, *R. salmoninarum* was detected by DFAT in 4% of fish examined (Table 5). During the 4 years of high-BKD segregation, no *R. salmoninarum* was detected by DFAT in the low-negative groups reared at Rapid River Hatchery, whereas 17% of fish sampled in the high-BKD segregation groups reared at Clearwater Hatchery were *R. salmoninarum* positive by DFAT. Clinical BKD and elevated mortality occurred in the high-BKD segregation group in 1996. When ELISA-based culling was used to manage BKD, DFAT detections dropped to 1% of fish tested during a 9-year period (1993, 1998–2005; Table 5).

Sawtooth Hatchery.—In the 6 years prior to 1991, *R. salmoninarum* was detected by DFAT in 11% of sampled fish (Table 6). During the 5 years of high-BKD segregation, *R. salmoninarum* was detected in 11% of sampled fish from the high-BKD group (Table 6), but there were no detections of the fish sampled from the low-negative groups. There were no detections of *R. salmoninarum* in the 10 brood years managed by ELISA-based culling (Table 6).

Adult ELISA Sampling

Most of the returning adult Chinook salmon in any given year of the study were age 4. The ELISA OD values of kidney tissues from returning adult females declined in each of the three study hatcheries after the 1993 brood year, once the ELISA-based BKD management strategy was implemented. From the

TABLE 2.—Mortality of Chinook salmon juveniles in years before ELISA-based management and with ELISA-based segregation and ELISA-based culling at Rapid River Hatchery. Mortality of the high-BKD segregation groups was calculated separately from those for Clearwater Hatchery production.

Brood year	Fish produced	Mortality	Mortality (%)	SE
Before ELISA management				
1987	3,200,000	380,500	12	0.00018
1988	3,370,944	49,334	2	0.00007
1989	3,205,000	40,000	1	0.00006
1990	3,185,000	69,000	2	0.00008
1991	2,299,023	38,428	2	0.00005
1992	3,141,954	213,808	7	0.0001
Total	18,401,921	791,070	4	0.00005
During ELISA-based segregation				
1994	379,865	698	0.2	0.00007
1995	86,072	232	0.3	0.0002
1996	869,953	783	0.1	0.00003
1997	3,134,835	3,135	0.1	0.00002
Total	4,470,725	4,848	0.1	0.00001
High-BKD segregation groups reared at Clearwater Hatchery				
1994	68,960	1,142	2	0.0005
1995	15,323	1,853	12	0.003
1996	168,252	8,513	5	0.0005
1997	223,347	3,097	1	0.0003
Total	475,882	14,605	3	0.0003
With ELISA-based culling				
1993	3,300,696	14,241	0.4	0.00003
1998	2,465,267	2,922	0.1	0.00002
1999	739,042	2,441	0.3	0.00006
2000	3,473,011	3,322	0.1	0.00002
2001	2,881,797	51,486	2	0.00008
2002	3,608,831	46,677	1	0.00006
2003	3,468,082	22,729	0.7	0.00004
2004	3,146,971	16,443	0.5	0.00004
2005	3,028,097	43,725	1	0.00007
Total	26,111,794	203,986	0.8	0.00002

log-linear analysis of coded ELISA OD values from adult females, we found both location and year to have significant ($P < 0.05$) effects on the proportion of ELISA OD values greater than 0.25. The chi-square values suggested that the year was the dominant significant term. Location was significant because the levels of *R. salmoninarum* were different at the three hatcheries. Given that both location and year were significant, we then ran the Jonckheere–Terpstra test to determine the effect of year at each location. The proportion of ELISA OD values less than 0.25 increased over time, and the proportion of ELISA OD values greater than 0.25 decreased over time for each of the three hatcheries (Monte Carlo P-value $<$ 0.0001 in each case), suggesting a positive effect of the ELISA-based BKD management program on reducing *R. salmoninarum* levels of adult females returning to each hatchery. After culling was established as a standard operating procedure (SOP) at each hatchery, the proportions of returning adults with ELISA OD values greater than 0.25 decreased 56–85% for fish reared as juveniles during the years that culling was practiced compared with fish reared in the years prior to culling, whereas the proportions of ELISA-negative fish increased 55–58% (Figure 2).

McCall Hatchery.—In 1997, ELISA-based culling became an SOP at McCall Hatchery. Most adults returning to the South Fork trap from 1991 to 2000 were progeny of adult females not subject to a culling program. During this period, 37.1% of sampled females were considered negative for *R. salmoninarum* antigens by ELISA testing, whereas 7.3% of the returning females had high ELISA OD values ($\geq$0.25), indicative of high *R. salmoninarum* antigen levels (Figure 2A). From 2002 to 2006, most adults spawned at the South Fork trap were progeny from ELISA-based culling programs. During this period, 57.4% of the adult females were considered negative for *R. salmoninarum*, and only 3.2% tested by ELISA were highly positive for *R. salmoninarum*.

Rapid River Hatchery.—In 1998, ELISA-based culling became SOP at this facility. Most adults

TABLE 3.—Mortality of Chinook salmon juveniles in years before ELISA-based management and with ELISA-based segregation and ELISA-based culling at Sawtooth Hatchery.

Brood year	Fish produced	Mortality	Mortality (%)	SE
		Before ELISA-based management		
1987	2,487,500	149,256	6	0.0002
1988	2,818,312	276,812	10	0.0002
1989	667,900	15,300	2	0.0002
1990	1,300,290	26,890	2	0.0001
Total	7,274,002	468,258	6	0.00002
		With ELISA-based segregation		
1992	409,970	213,830	48	0.0008
1993	341,252	6,939	2	0.0002
1995	4,859	103	2	0.0002
1997	228,613	5,073	2	0.0003
1998	124,810	1,385	1	0.0003
Total	1,109,504	227,330	21	0.0004
		With ELISA-based culling		
1991	793,908	19,325	2	0.0002
1994	25,938	932	4	0.001
1996	44,478	1,298	3	0.0008
1999	58,139	1,005	2	0.0005
2000	397,273	11,512	3	0.0003
2001	1,222,271	117,102	10	0.0003
2002	873,847	52,432	6	0.0003
2003	136,605	1,836	1	0.0003
2004	1,678,677	126,133	8	0.0002
2005	1,001,468	6,206	0.6	0.00008
Total	6,232,604	337,781	5	0.00009

returning to this hatchery from 1991 to 2002 were progeny of adult females not subject to a culling program. During this period, 30.3% of returning adults had negative ELISA OD values, and 14.7% had high OD values (Figure 2B). In contrast, from 2003 through 2006, 47.9% of returning females tested ELISA negative, and 5.9% had high OD values (Figure 2B).

Sawtooth Hatchery.—In 1999, ELISA-based culling became SOP at this hatchery. Adults returning to this hatchery from 1991 to 2003 were progeny of adult females not consistently subject to a culling program. During this period, 42.5% of the female adults sampled were ELISA negative, and 13.7% had high ELISA OD values (Figure 2C). From 2004 to 2006, most adults spawned at this hatchery were progeny from ELISA-based culling programs. During this period, 66.3% of the females were ELISA negative; only 2% had high ELISA OD values (Figure 2C).

Discussion

Bacterial kidney disease, similar to most other disease manifestations in aquaculture, is a product of the interaction of the host, environment, and pathogen. Although ELISA-based management may not completely eliminate *R. salmoninarum* or BKD from a population, the results of this study indicate that it may (1) reduce the overall prevalence and intensity of *R. salmoninarum* infection and thereby control disease outbreaks during hatchery rearing, (2) reduce disease and horizontal transmission during emigration and ocean residence, (3) reduce prespawning BKD mortality of adult fish upon return to native streams, and (4) reduce prevalence and intensity of *R. salmoninarum* infection in adult fish at spawning. Such a disease management program may have positive impacts other than the obvious direct benefits to the hatchery population subjected to the program. For example, a model of *R. salmoninarum* dynamics in Lake Michigan suggests that even short-term failures of hatchery procedures that result in release of high proportions of *R. salmoninarum*-infectious fish can lead to a drastic increase in *R. salmoninarum* prevalence and potential decreases in stock size of both wild and hatchery salmon (Fenichel et al. 2009). Conversely, hatchery releases of low proportions of *R. salmoninarum*-infectious fish may have beneficial effects on the health and size of wild and hatchery fish populations. A key to the success of the ELISA-based BKD management is reduction of the risk of vertical transmission of *R. salmoninarum*. A risk assessment model for vertically transmitted pathogens applied to *R. salmoninarum* demonstrated that a significant decrease in the risk of vertical transmission of this pathogen could be achieved through broodstock testing, even if faced with a worst case scenario of 30% infection prevalence and a poor screening test (Peeler et al.

TABLE 4.—Detection of *Renibacterium salmoninarum* by the direct fluorescent antibody test (DFAT) in kidney tissue smears from juvenile Chinook salmon at McCall Hatchery. Data were collected in years before ELISA-based BKD management was established, after segregated rearing of progeny from females with high kidney tissue optical density values (>0.249) was implemented, and after culling of eggs from females with high kidney tissue values was implemented.

Brood year	Number of fish examined	Number of fish positive	Percent positive
Detection before ELISA-based management			
1987	348	65	19
1988	202	4	2
1989	201	0	0
1990	100	0	0
1991	148	13	9
1992	54	14	13
Total	1,053	96	9
Detection in high-BKD segregation groups after implementation of ELISA-based segregated rearing[a]			
1994	94	18[b]	19
1995	100	0	0
1996	83	15[b]	18
Total	277	33	12
Detection after implementation of ELISA-based culling			
1993	70	0	0
1997	96	1[c]	1
1998	60	0	0
1999	54	0	0
2000	50	0	0
2001	63	0	0
2002	70	0	0
2003	70	0	0
2004	40	0	0
2005	80	0	0
Total	653	1	2

[a] No *R. salmoninarum* was detected by DFAT in negative–low-BKD segregation groups reared at McCall Hatchery during these same years.
[b] Clinical BKD was detected in some juvenile fish in the high-BKD segregation groups.
[c] Eggs were culled from female fish with ELISA optical density values ≥0.4 during 1997. No clinical disease was associated with the DFAT detection of *R. salmoninarum*.

TABLE 5.—Detection of *Renibacterium salmoninarum* by the direct fluorescent antibody test (DFAT) in kidney tissue smears from juvenile Rapid River Chinook salmon. See Table 4 for more information.

Brood year	Number of fish examined	Number of fish positive	Percent positive
Detection before ELISA-based BKD management			
1986	152	4	3
1987	271	29	12
1988	120	0	0
1989	180	0	0
1990	101	0	0
1991	152	0	0
1992	48	5	10
Total	1,024	38	4
Detection in high-BKD segregation groups reared at Clearwater Hatchery after ELISA-based segregated rearing[a]			
1994	30	6	25
1995	30	0	0
1996	47	16[b]	52
1997	20	0	0
Total	127	22	17
Detection after implementation of ELISA-based culling			
1993	115	1	1
1998	55	0	0
1999	70	0	0
2000	40	0	0
2001	50	0	0
2002	46	0	0
2003	67	6	10
2004	54	0	0
2005	60	0	0
Total	557	7	1

[a] No *R. salmoninarum* was detected by DFAT in negative–low-BKD segregation groups reared at Rapid River Hatchery during these same years.
[b] Clinical BKD and elevated mortality occurred in the high-BKD segregation group.

2005). The current research findings supported the results of this model, and data from our study could be used to develop more robust models. It is possible that screening and disease management programs similar to the one described here may prove successful for reducing the impact of certain other vertically transmitted fish pathogens, Before ELISA-based management of BKD at IDFG Chinook salmon hatcheries, survey samples of 60 adults per stock were tested for *R. salmoninarum* by DFAT. During that period, BKD management consisted of (1) injection of returning adult Chinook salmon with erythromycin to reduce prespawning BKD mortality and limit vertical transmission of *R. salmoninarum*, (2) iodophor topical disinfection of green eggs, and (3) prophylactic medicated feed treatments of progeny to restrict horizontal transmission of *R. salmoninarum*. Despite an active BKD control program, epidemics of BKD still caused substantial losses in Chinook salmon populations reared at IDFG hatcheries. The work of Pascho et al. (1991) demonstrated that ELISA-based segregation could reduce the risk of vertical transmission of *R. salmoninarum* to Chinook salmon progeny and, thus, might provide a tool to manage BKD at the population scale.

Significant reductions in DFAT detections of *R. salmoninarum*, clinical signs of BKD, and mortality due to BKD in progeny of females with low-negative ELISA OD values were observed upon implementation of the ELISA-based segregation and culling programs at IDFG Chinook salmon hatcheries. Initially, segregation based on female ELISA OD values was tested by IDFG at Chinook salmon hatcheries in an effort to minimize horizontal transmission of the pathogen from the progeny of females with high ELISA OD values to the progeny of females with negative-low ELISA OD

TABLE 6.—Detection of *Renibacterium salmoninarum* by the direct fluorescent antibody test (DFAT) in kidney tissue smears from juvenile Chinook salmon at Sawtooth Hatchery. See Table 4 for more information.

Brood year	Number of fish examined	Number of fish positive	Percent positive
Detection before ELISA-based BKD management			
1985	79	32	41
1986	316	32	10
1987	120	32	27
1988	455	42	9
1989	182	0	0
1990	116	6	5
Total	1,268	144	11
Detection in high-BKD segregation groups after implementation of ELISA-based segregated rearing[a]			
1992	152	0	0
1993	120	9	8
1995	39	0	0
1997	62	0	0
1998	58	39	67
Total	431	48	11
Detection after implementation of ELISA-based culling			
1991	130	0	0
1994	70	0	0
1996	44	0	0
1999	40	0	0
2000	90	0	0
2001	101	0	0
2002	60	0	0
2003	50	0	0
2004	50	0	0
2005	40	0	0
Total	675	0	0

[a] No *R. salmoninarum* was detected by DFAT in negative–low-BKD segregation groups reared at Sawtooth Hatchery during these same years.

values. We accomplished this by rearing all progeny of females with ELISA OD values above 0.249 in isolated aggregates. During this initial period, all DFAT detections and clinical outbreaks of BKD were limited to high-BKD segregation groups, leading us to question whether rearing and releasing these high-BKD segregation groups created an unnecessary risk to the progeny from brood females with lower ELISA OD values, which composed the majority of IDFG salmon production, and whether release of these fish presented a risk to wild salmon. These high-BKD segregation groups contained much smaller numbers of fish than the negative-low ELISA groups and, thus, were not capable of significantly contributing to adult returns. Outbreaks of clinical BKD were observed in negative-low production groups only after periods of high stress, such as overcrowding, turbid water events, and outbreaks of other diseases.

When nonspecific mortality throughout rearing was examined, significantly lower mortality rates in progeny fish were realized for brood years in which

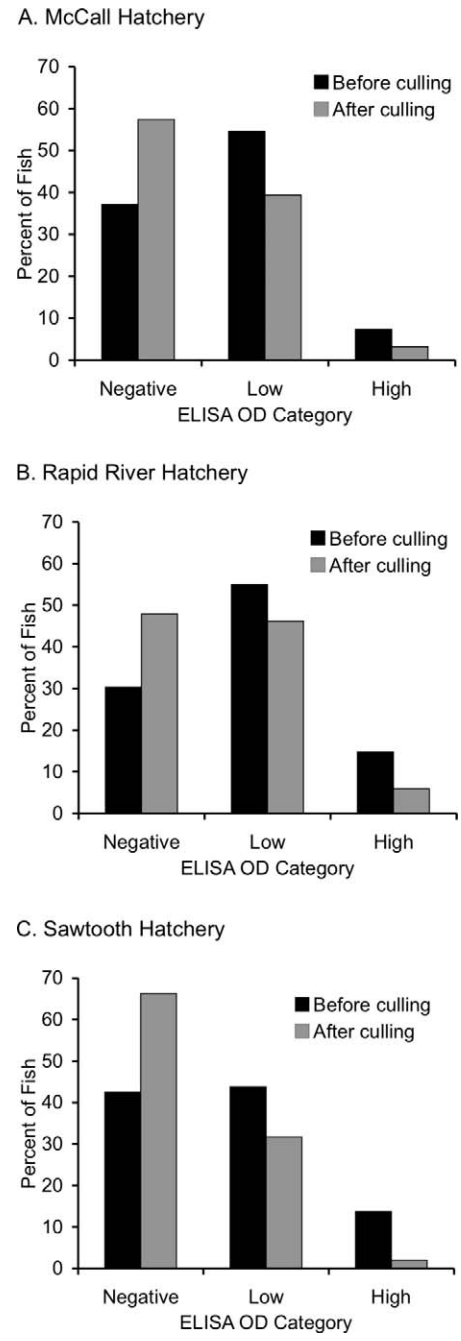

FIGURE 2.—Results of testing kidney tissues from returning adult female Chinook salmon for detection of the *Renibacterium salmoninarum* antigen by enzyme-linked immunosorbent assay (ELISA) at **(A)** McCall Hatchery, **(B)** Rapid River Hatchery, and **(C)** Sawtooth Hatchery. The percentages of females in each of three optical density (OD) categories (negative, <0.10; low, 0.10–0.249; and high, ≥ 0.250) are shown for years before and after the implementation of ELISA-based culling.

ELISA-based culling was practiced. This significant increase in survival during hatchery rearing allowed us to decrease the number of eggs taken during spawning to meet hatchery production objectives. Thus, fewer adult fish had to be trapped and held, allowing more adult fish to spawn naturally or to be left in the river for sport or tribal fisheries.

At IDFG hatcheries, the management of BKD evolved into a risk management strategy using a combination of ELISA-based culling and segregation. When adult salmon returns were so few that production goals could not be met by culling eggs from females with ELISA OD values $\geq$0.25, we reared the progeny of females with ELISA OD values between 0.25 and 0.6 in segregation but culled the eggs of females with ELISA OD values greater than 0.6. This practice removed the eggs of highest risk females and limited vertical transmission of BKD. The progeny groups reared in segregation were typically maintained at lower densities and were usually given a third treatment of erythromycin medicated feed. This procedure allowed us to produce more fish while reducing the risk of vertical and horizontal transmission of *R. salmoninarum*. Further benefits of the ELISA-based BKD management program were demonstrated by the reduced ELISA OD values, indicative of reduced *R. salmoninarum* infection levels, in returning adult females originating from the brood years that culling was the SOP. In addition, the proportion of fish categorized as *R. salmoninarum* negative by ELISA testing increased after ELISA-based culling became routine practice.

Adult Chinook salmon returning to IDFG hatcheries have had a lower prevalence of infection and lower intensity of infection, as measured by ELISA, since application of ELISA-based management of BKD. The success of this management application has allowed us to reevaluate the continued use of the previous BKD management strategies, specifically prophylactic erythromycin-medicated feed treatments. Development of erythromycin resistance is a serious concern for IDFG and all the salmon culture community. Reduction in sensitivity to macrolide antibiotics such as erythromycin and azithromycin has been observed in *R. salmoninarum* (Bell et al. 1988; Rhodes et al. 2008). The *R. salmoninarum* genome contains genes encoding antibiotic resistance (Wiens et al. 2008); these authors determined that the expression of resistance genes was increased upon exposure of *R. salmoninarum* to erythromycin or azithromycin. This finding may partially explain why treatment with macrolide antibiotics does not eliminate *R. salmoninarum* from infected fish populations. Currently, IDFG Chinook salmon hatcheries are investigating the reduction or elimination of prophylactic medicated feed treatments. McCall Hatchery, which does not have spawning Chinook salmon in its water source, has completed its first year of testing. Half of the Chinook salmon reared at this hatchery did not receive an erythromycin medicated feed treatment, while the other half received only one treatment. The ELISA and DFAT testing demonstrated virtually identical negative results (data not shown). Minimizing erythromycin medicated feed treatments reduces the risk of development of bacterial resistance to this chemotherapeutant and decreases the amount of antibiotic released into the environment. Because of concerns that agricultural use of antibiotics may contribute to the generation of bacterial strains that cause disease in both humans and food animals (Shea et al. 2004; Gilchrist et al. 2007), reduction or elimination of antibiotic use in fish hatcheries is desirable. Additionally, IDFG and its funding sources would be able to save thousands of dollars per year if the agency was able to eliminate erythromycin prophylactic treatments.

Genetic impacts of a BKD culling program have been a source of concern for hatchery managers because selective culling of hatchery broodstock based on ELISA OD values could possibly result in reduced BKD resistance in descendents over generations. Concerns have also been raised about the possible effects of such programs on natural fish that interact with hatchery-origin salmon, such as in hatchery–wild integrated programs. The results of Hard et al. (2006) suggest that selective culling of the eggs of females with high ELISA OD values would not have deleterious effects on the ability of their progeny to resist BKD because *R. salmoninarum* levels in adult females were best explained by environmental sources and not by genetic susceptibility. Furthermore, because IDFG Chinook hatchery programs do not cull spawning males on the basis of *R. salmoninarum* levels, the male contributions probably help to maintain genetic variation of these populations in BKD resistance or in other traits that might be affected by any low-level culling of females.

We conclude that ELISA-based management of IDFG hatchery-origin Chinook salmon has had several positive effects. This management strategy has demonstrated significantly lower levels of *R. salmoninarum* infection during hatchery rearing of progeny from female Chinook salmon with negative and low ELISA OD values. In addition, significant reductions in ELISA OD values have been observed in returning adults, further reducing *R. salmoninarum* load in the overall population and requiring the culling of fewer adults. Only after extended stressful events have we been able to detect *R. salmoninarum* in hatchery

juveniles, and these clinical outbreaks have been contained by application of medicated feed. This program is allowing IDFG Chinook hatcheries to consider reductions in prophylactic medicated feed treatments and develop BKD and *R. salmoninarum* surveillance programs to determine if and when treatments should be applied. Based on the results of this multiyear study, we recommend ELISA-based management as a possible method to control BKD.

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