Open Access Articles

Cool, Pathogen-Free Refuge Lowers Pathogen-Associated Prespawn Mortality of Willamette River Chinook Salmon

The Faculty of Oregon State University has made this article openly available.
Please share how this access benefits you. Your story matters.

Citation	Benda, S. E., Naughton, G. P., Caudill, C. C., Kent, M. L., & Schreck, C. B. (2015). Cool, Pathogen-Free Refuge Lowers Pathogen-Associated Prespawn Mortality of Willamette River Chinook Salmon. Transactions of the American Fisheries Society, 144(6), 1159-1172. doi:10.1080/00028487.2015.1073621
DOI	10.1080/00028487.2015.1073621
Publisher	Taylor & Francis
Version	Version of Record
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsofuse

Transactions of the American Fisheries Society 144:1159–1172, 2015
© American Fisheries Society 2015
ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2015.1073621

ARTICLE

Cool, Pathogen-Free Refuge Lowers Pathogen-Associated Prespawn Mortality of Willamette River Chinook Salmon

Susan E. Benda*
Oregon Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA

George P. Naughton and Christopher C. Caudill
Department of Fish and Wildlife Sciences, University of Idaho, Moscow, Idaho 83844-1136, USA

Michael L. Kent
Department of Microbiology, Oregon State University, 204 Nash Hall, Corvallis, Oregon 97331, USA

Carl B. Schreck
U.S. Geological Survey, Oregon Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA

Downloaded by [Oregon State University] at 07:59 15 January 2016

Abstract

Spring Chinook Salmon *Oncorhynchus tshawytscha* are transported above dams in the Willamette River to provide access to blocked spawning habitat. However, 30–95% of these transplants may die before spawning in some years. To varying degrees, salmon in other tributaries—both blocked and unblocked—have similar prespawn mortality (PSM) rates. Our study determined whether holding fish in constant temperature, pathogen-free conditions prior to spawning increased survival through spawning in 2010 through 2012. In addition, we evaluated pathogens as a potential cause of PSM. To monitor survival we captured adult Chinook Salmon early and late in the season from the lower Willamette River and upper tributaries and held them until spawning in 13°C, pathogen-free water. Samples were collected at the time of transport, from moribund or dead fish throughout the summer, and after spawning in the autumn. Prespawn mortalities and postspawned fish from river surveys on holding and spawning reaches above traps were also sampled. Necropsies were performed on all fish, and representative organs were processed for histopathological analysis. Using multiple logistic regression odds ratio analysis, fish that were held were up to 12.6 times less likely to experience PSM than fish that were outplanted to the river. However, *Aeromonas salmonicida* and *Renibacterium salmoninarum* were more prevalent in held fish that had PSM than in outplanted fish with PSM, suggesting that fish that were held were more susceptible to these bacteria. Spawned held fish were more likely to have *Myxobolus* sp. brain infections and less likely to be infected with the kidney myxozoan, *Parvicapusla minibicornis*, than were spawned outplanted fish. The equal likelihood of other pathogens for held fish and outplanted spawned fish suggests interactive effects determine survival and that holding Chinook Salmon at 13°C prevented expression of lethal pathogenesis. Overall, holding could be a viable method to reduce PSM, but issues of transport stress, proliferative disease, and antibiotics remain.

The historical levels of prespawn mortality (PSM), defined here as mortalities that occur after adult salmon enter into freshwater but prior to the expression of gametes during spawning, are not well documented. Given the physiological and environmental challenges of migration, it is natural for some death to occur before spawning (Gauthreaux 1980;

*Corresponding author: sbenda14@gmail.com
Received February 19, 2015; accepted July 13, 2015

Downloaded by [Oregon State University] at 07:59 15 January 2016

Quinn 2005). However, the chance of a self-sustaining population decreases with as little as 10% PSM (Keefer et al. 2010; Spromberg and Scholz 2011). With the goal of managing salmon populations effectively, it is necessary to determine the cause of PSM and evaluate potential management tactics.

Exposure to warm water temperatures and the accumulation of degree-days (DD) have been correlated with PSM (Crossin et al. 2008; Keefer et al. 2010; Jeffries et al. 2012). Temperatures outside the acceptable range of 13.9–20°C for Chinook Salmon *Oncorhynchus tshawytscha* (McCullough et al. 2001) can cause a change in energy requirements for basal metabolic activity (McCullough 1999). This leads to a depletion of energy that could have been used for migration, reproduction, and immune responses (McCullough 1999) and is also thought to cause direct physiological stress (Richter and Kolmes 2005). Elevated temperature can also create a more suitable environment for pathogens, either through increasing host susceptibility, increasing transmission rates, or shortening temperature-dependent life cycles (Rucker et al. 1954). Further, warm temperatures can affect survival to spawning as well as progeny survival (McCullough 1999; Beer and Anderson 2001; Quinn 2005).

Pathogens have been associated with PSM, e.g., *Parvicapsula minibicornis* in Sockeye Salmon *O. nerka* of the Fraser River (Raverty et al. 2000) or ichthyophoniasis in Chinook Salmon of the Yukon River (Kocan et al. 2004, but see Hamazaki et al. 2013). Pacific salmon are exposed to a suite of proliferative and nonproliferative freshwater pathogens during their migration and holding on spawning grounds. Concurrently, during upstream migration and spawning, Pacific salmon develop high levels of circulating cortisol, a known immunosuppressant, leaving fish with a potentially increased susceptibility to pathogens (Schreck 1996). As a result, death after spawning is generally from disease or energy depletion (Schreck et al. 2001).

Spring Chinook Salmon returning to the Willamette River in Oregon can experience 90% PSM in the upper reaches (Schroeder et al. 2007; Roumasset 2012). These fish are also listed under the U.S. Endangered Species Act (ESA) and the population is now composed largely of hatchery-origin individuals (NMFS 2008). Dams have contributed to population declines through blocked passage and altered temperature and flow regimes (NMFS 1999). In an effort to both create self-sustaining runs and provide access to spawning habitat blocked by dams, salmon are transported above impassible dams into upstream tributaries (these fish are hereafter referred to as outplanted fish). Transporting both adult and juvenile fish is not an unusual practice (Zimmerman and Duke 1995; Mosser et al. 2013), and PSM occurs in both transported and nontransported populations (Roumasset 2012). In addition, many adult Chinook Salmon in the Willamette River encounter water temperatures in the 18–22°C range and accumulate as many as ~1,500 DD (~700–900 DD, on average; Keefer et al. 2015).

The primary focus of our study was to determine whether Chinook Salmon held in a pathogen-free, constant temperature environment had lower PSM than those in the river. We hypothesized that salmon held in a constant, cool temperature facility without exposure to additional pathogens would have higher survival to spawn than outplanted fish (hypothesis H_1). Of particular interest was the relationship between survival and the duration of holding on adult survival as this would influence future management recommendations. We also hypothesized that salmon taken earlier from the river, both spatially and temporally, would have higher survival to spawn than fish taken later (H_2).

The second aim of our study was to determine whether there was an association of parasites with survival to spawning by examining the suite of pathogens infecting adult Chinook Salmon under different conditions (e.g., held versus outplanted fish, held and outplanted fish with PSM versus live fish sampled at trapping facilities, and outplanted fish with PSM versus outplanted spawned fish). We used a histological approach to meet this aim because it allowed examination at the tissue level for known and unknown pathogens and provided insight into the disease status of infected fish as explained by Kent et al. (2013). We expected spawned fish to have higher pathogen burdens than fish collected earlier in the season irrespective of spawning success, due to the longer exposure to pathogens, longer time for proliferative pathogens to multiply in the fish host, increased thermal loads, increased susceptibility with senescence, and because adult salmonids generally die from pathogens after spawning. Therefore, if fish that experienced PSM died due to pathogen-associated mortality, we predicted that they would have similar pathogen burdens as observed in fish that spawned successfully (H_3). When we compared outplanted fish and fish held at the holding facility (considering PSM and spawned fish separately), we expected to find lower pathogen burdens for held fish because they were removed from pathogen exposure and because we expected they would have lower thermal loads (H_4).

METHODS

Field sites and holding facility.—Adult, hatchery-origin Chinook Salmon were collected from as far downstream in the Willamette River system as possible at Willamette Falls, located 32.6 river kilometers (rkm) from the mouth of the Willamette River or 206 rkm from the mouth of the Columbia River. Fish were also collected en route to spawning tributaries upstream at Foster Dam (418 rkm from the mouth of the Columbia River), Dexter Dam (491 rkm), and Fall Creek Dam (493 rkm) (Figure 1). In addition, salmon carcasses were collected from tributaries upstream from traps on the North Fork Middle Fork of the Willamette River, Fall Creek, and South Santiam River.

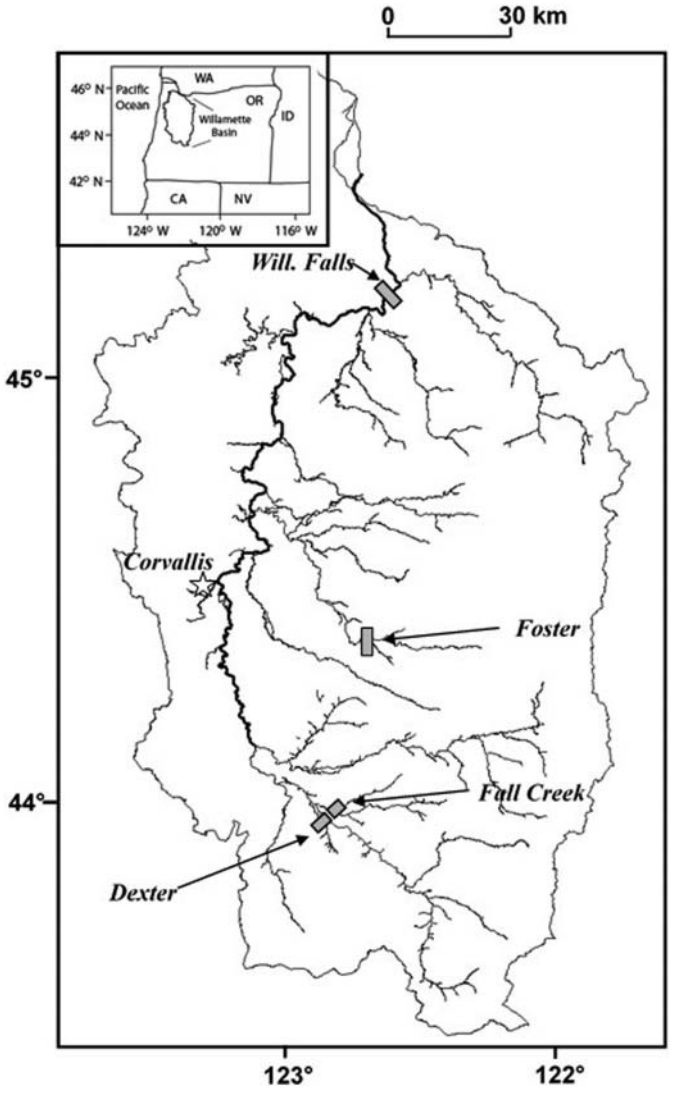


FIGURE 1. The Willamette River basin, Oregon. Willamette (Will.) Falls, Foster Dam, Fall Creek Dam and Dexter Dam traps are shown as bars, the OSU Fish Performance and Genetics Laboratory is indicated by the star.

Fish were transported to the Fish Performance and Genetics Laboratory (hereafter referred to as the holding facility) of Oregon State University (OSU), Corvallis, Oregon, for holding in cool, pathogen-free water. This facility uses pathogen-free well water, and the flow-through system ensures a constant temperature of 13°C (±1°C seasonal variation). Fish were held in six outdoor tanks (measuring 3 m in diameter and having a water depth of 1 m). Tanks were covered with black screen to provide shade (80%) and prevent fish from escaping.

All work was done in conformance with OSU Institutional Animal Care and Use Committee (ACUP 4438). The use of AQUI-S 20E (AquaTactics Fish Health and Vaccines, Kirkland, Washington) was conducted under investigational new animal drug (INAD) protocol 11–741 (from Aquatic Animal Drug Approval Partnership of the U.S. Fish and Wildlife Service). Adult salmon were sampled under ESA take permits W1-10-UI200, W1-11-UI200, and W1-12-UI200 issued by NOAA Fisheries and appropriate state scientific collection permits issued by Oregon Department of Fish and Wildlife (ODFW).

Transport and holding.—Adult Chinook Salmon from the collection sites described above were transported to the holding facility. A total of 182 fish were transported over the 3-year study period (35 from Willamette Falls, 100 from Dexter Dam, 17 from Fall Creek Dam, and 30 from Foster Dam). Transport dates and sample sizes for each location and year are listed in Table 1. We attempted to obtain fish over a time period that spanned the timing of the run as closely as feasible. Chinook Salmon enter freshwater beginning around December, pass Willamette Falls from mid-March to July, and arrive at upper tributaries in April through September. Procedures at each trapping facility varied slightly and are described below.

At Willamette Falls, fish were diverted from the fish ladder into a trap. Fish had to voluntarily ascend a Denil fish ladder leading to an anesthetic bath. In 2011, fish were anesthetized using 50 mg/L tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate to a pH of 7.0. The anesthetic AQUI-S 20E at a dose of 22 ppm was used in 2012. The first fish to arrive were collected for transport; once the transport quota was met, fish were euthanized on site for later pathogen assessment. For transport, anesthetized fish were placed into individual cylinders filled with oxygenated water. These cylinders were placed in a secondary tote of oxygenated water to move fish to a transport truck located about 15 min away. Total collection and holding time prior to transport did not exceed 3 h to minimize holding mortality.

TABLE 1. Transport dates to holding facility and respective sample sizes (*N*) of Chinook Salmon for each location and year.

Location	Year	Date	*N*
Willamette Falls	2011	May 18	2
		Jun 5	5
		Jun 7	8
	2012	May 16	6
		May 23	5
		Jun 13	9
Dexter Dam	2010	Jun 8	10
		Jul 14	20
	2011	May 26	20
		Jul 20	20
	2012	Jun 6	15
		Aug 3	15
Fall Creek Dam	2010	Jun 7	10
		Jul 12	7
Foster Dam	2012	Jun 5	15
		Aug 2	15

Downloaded by [Oregon State University] at 07:59 15 January 2016

Downloaded by [Oregon State University] at 07:59 15 January 2016

Fish returning to Dexter and Foster dams ascended a fish ladder and entered raceways where they were held for a period of up to several days. They were then crowded into a bath containing carbon dioxide (CO_2), which sedated the fish sufficiently to allow them to be handled and lifted to a processing table. From there they were randomly assigned to be either euthanized for necropsy or individually netted into a transport truck.

At Fall Creek, fish were crowded from the ladder into a holding tank and anesthetized with 60 ppm eugenol. They were then randomly assigned to be either necropsied and sampled for parasites or individually netted into a transport truck.

Fish recovered from the anesthetic in the transport vehicles. It took about 2 h to transport fish from Willamette Falls, Dexter Dam, and Fall Creek Dam traps and 1 h to transport fish from Foster Dam trap. Fish were transported by U.S. Army Corps of Engineers and ODFW personnel in various transport tanks or trucks. Tank volumes ranged from 1.89 to 5.68 m^3. In all cases water (temperatures of 13–15°C) was used from either the holding facility or the collection site and was oxygenated throughout the transport period. Upon arrival at the holding facility the fish were anesthetized with buffered MS-222 (see above) to minimize effects of netting stress and then stocked into holding tanks.

Due to permit restrictions, we were not able to transport fish from Willamette Falls in 2010. While we aimed for two collection times per site, Willamette Falls required three sampling events to obtain the minimum number of fish necessary. The Chinook Salmon run at Fall Creek comprises primarily fish of natural origin and this tributary was not sampled after 2010. Foster Dam was added in 2012.

At the holding facility each transport group was placed into individual outdoor tanks. No more than 15 fish were held in a single tank. Because we had more than two collection dates at Willamette Falls (and a limited number of tanks), fish from this site were individually PIT-tagged and divided equally into two tanks. With the exception of a few Dexter Dam groups (see Table 1), we did not have replicate tanks for each location and collection date within a year. This was due in part to limited space at the holding facility (at most six tanks were available) and by the number of fish we were permitted to take. We did not cohabitate fish from different locations or collection dates out of concern for pathogen transmission and potentially losing all of the fish to disease. Because of these restraints, we repeated the study for multiple years (2010–2012). While data from multiple years are not true replicates, it did allow us to examine interannual variation.

Hydrogen peroxide was administered every 2 weeks as an external treatment for *Saprolegnia* because we were interested in pathogen infections that occurred prior to the fish arriving at our facility. Fish were checked at least twice a day (more often towards the spawning season) for mortalities. Dead fish were individually placed in plastic bags, put on ice, stored in a cold room, and necropsied before any further deterioration could take place.

Spawning and progeny.—Starting in September, fish were checked weekly. "Ripe" female salmon were characterized as having loose eggs in the body cavity and ripe males by the expression of sperm. Ripe females were euthanized with a blow to the head and eggs were removed following standard hatchery procedures. Males were live-spawned until all females had spawned; milt was collected in Whirl-Paks, oxygenated, and kept on ice. Between 500 and 1,000 fertilized eggs per female were placed into Heath trays for incubation to determine whether progeny were viable. All dead spawned fish were stored as described above for necropsy.

River samples.—River surveys of outplanted Chinook Salmon conducted by ODFW and the University of Idaho provided "fresh" mortalities (i.e., a fish with pink gills), which were placed individually in plastic bags and kept on ice for later necropsy. Spawned fish were also sampled at the Willamette Hatchery every year in September. Willamette Hatchery is located above Dexter Dam (Figure 1). A total of 149 fish were collected: 19 from Fall Creek Dam, 40 from Dexter Dam, 9 from Foster Dam, and 81 from the Willamette Hatchery. Information on sample size for outplanted fish collected from river surveys and spawned fish collected at Willamette Hatchery is provided in Table 2.

To provide a baseline of pathogen burden, 15 fish were euthanized and sampled for parasite burden at each collection site each year. Because we did not know whether fish sampled at trapping facilities would have naturally died before or after spawning, we referred to them as live fish. In total 90 fish were sampled: 39 from Willamette Falls, 11 from Fall Creek Dam, 13 from Foster Dam, and 27 from Dexter Dam. We attempted to obtain fish over a time period that spanned the timing of the run as closely as feasible (Table 3).

Tissue processing.—A complete necropsy was performed on each fish. External and internal conditions were noted, as well as fork length, sex, percentage of eggs spawned (for females), percentage of fish with *Saprolegnia* present, and presence of an adipose fin clip to identify whether the fish was of wild or hatchery origin. Pieces of gill, kidney, spleen, heart, brain, pyloric ceca, lower intestine, and liver were stored in 10% buffered formalin. After fixing for at least 7 d, tissues were trimmed and placed in cassettes by our laboratory.

TABLE 2. Sample sizes for pathogen burden of PSM and spawned Chinook Salmon outplanted above dams for each location and year.

Location	Year	PSM	Spawned
Fall Creek Dam	2010	8	11
Dexter Dam	2010	19	6
	2011	13	2
Foster Dam	2012	3	6
Willamette Hatchery	2010	0	26
	2011	0	28
	2012	0	27

TABLE 3. Sample sizes (*N*) of Chinook Salmon sampled for pathogen burden at each collection site by year and month.

Location	Year	Month	*N*
Willamette Falls	2011	May	9
		June	7
		July	8
	2012	May	9
		June	6
Fall Creek Dam	2010	June	5
		August	6
Foster Dam	2012	June	7
		August	6
Dexter Dam	2010	June	3
		July/Aug	6
	2011	May	7
		July	4
	2012	June	5
		August	2

Cassettes were embedded and sectioned in our laboratory in 2010 and at the Veterinary Diagnostic Laboratory at OSU in 2011 and 2012. Slides were stained with hematoxylin and eosin in our laboratory.

Pathogens and associated lesions present in each tissue sample were then identified. Histology allowed us to detect a suite of pathogens that can infect Willamette River spring Chinook Salmon: both proliferative (*Ceratonova shasta* [formerly *Ceratomyxa shasta*], a species of *Myxobolus* of the central nervous system, *Parvicapsula minibicornis*, *Renibacterum salmoninarum*, and *Aeromonas salmonicida*), and nonproliferative (*Nanophyetus salmincola*, *Apophallus* sp., and *Echinochasmus milvi*) pathogens using the methods described by Kent et al. (2013). We defined proliferative pathogens as those that multiply within the fish host (i.e., microparasites), whereas nonproliferative pathogens do not (i.e., macroparasites).

Statistical analysis.—Estimates of percent PSM for outplanted fish was supplied by ODFW and were based only on females because the degree of spawning in males cannot be determined in the field. Therefore, comparisons of percent PSM between fish at the holding facility and outplanted fish were made using only females. Conversely, comparisons within the holding facility of percent PSM used both males and females. Multiple logistic regression (R Core Team 2012) was used to determine associations between PSM and year, length of holding, and location as follows,

$$\text{PSM (Y/N)} = \text{year} + \text{holding} + \text{location},$$

to compare outplanted and held females, where yes (Y) or no (N) indicates occurrence or absence of PSM, respectively. When considering only held fish, sex was added as a variable to the model. In both models the variable "holding" indicates whether the fish were outplanted, collected early and held, or collected late and held. All analyses were conducted at the alpha = 0.05 level.

We were unable to examine interactions between location, year, or length of holding because we did not have replicate tanks for each location and collection date within a single year. Further, May and June collections were designated as "Early" and July and August were considered "Late" collection dates, reflecting relatively cool and warm periods, respectively.

All fish from Willamette Falls were coded as an early collection date because collections at Willamette Falls were "early" relative to upstream collection dates and this did not significantly change the comparison between early and late collections (see Table 5).

We also assessed presence or absence of pathogens in live fish, fish that had PSM, and spawned fish using two separate logistic regression models implemented in R (R Core Team 2012). A multiple logistic regression model was used to compare all (both held and outplanted) fish that experienced PSM and spawned fish (see Table 6). The general model used was

$$\text{PSM (Y/N)} = \text{P1} + \text{P2} \ldots + \text{P7} + \text{location} + \text{year} + \text{holding},$$

where P1 to P7 indicate the presence or absence of (1) *P. minibicornis*, (2) *R. salmoninarum*, (3) *C. shasta*, (4) *N. salmincola*, (5) *Myxobolus* sp., (6) *A. salmonicida*, and (7) *Apophallus* sp./*E. milvi*. Location included Fall Creek, Foster and Dexter dams. Data were used from years 2010–2012 and arrival indicates whether collection was "early," "late," or from the river (outplanted fish); this comparison did not include fish from Willamette Falls.

Fish collected from Willamette Falls were not included in the model above for two reasons. First, we did not have complete groups to compare; e.g., there was no outplanted group to compare with early and late held fish. Secondly, there was complete separation of data for most pathogens when comparing held fish and fish sampled at the falls (see Figure 2). Specifically, while fish sampled at Willamette Falls show low prevalence of infection with pathogens, these infections develop with time (i.e., pathogens are more prevalent and severe in spawned fish).

For comparisons involving live fish (i.e., fish sampled directly at a trapping facility), the following multinomial logistic regression models were used:

$$(\text{PSM}^{\text{H}}, \text{PSM}^{\text{O}}, \text{live}) = \text{P1} + \text{P2} \ldots + \text{P6} + \text{location} + \text{year}$$

and

$$(\text{spawn}^{\text{H}}, \text{spawn}^{\text{O}}, \text{live}) = \text{P1} + \text{P2} \ldots + \text{P6} + \text{location} + \text{year},$$

Downloaded by [Oregon State University] at 07:59 15 January 2016

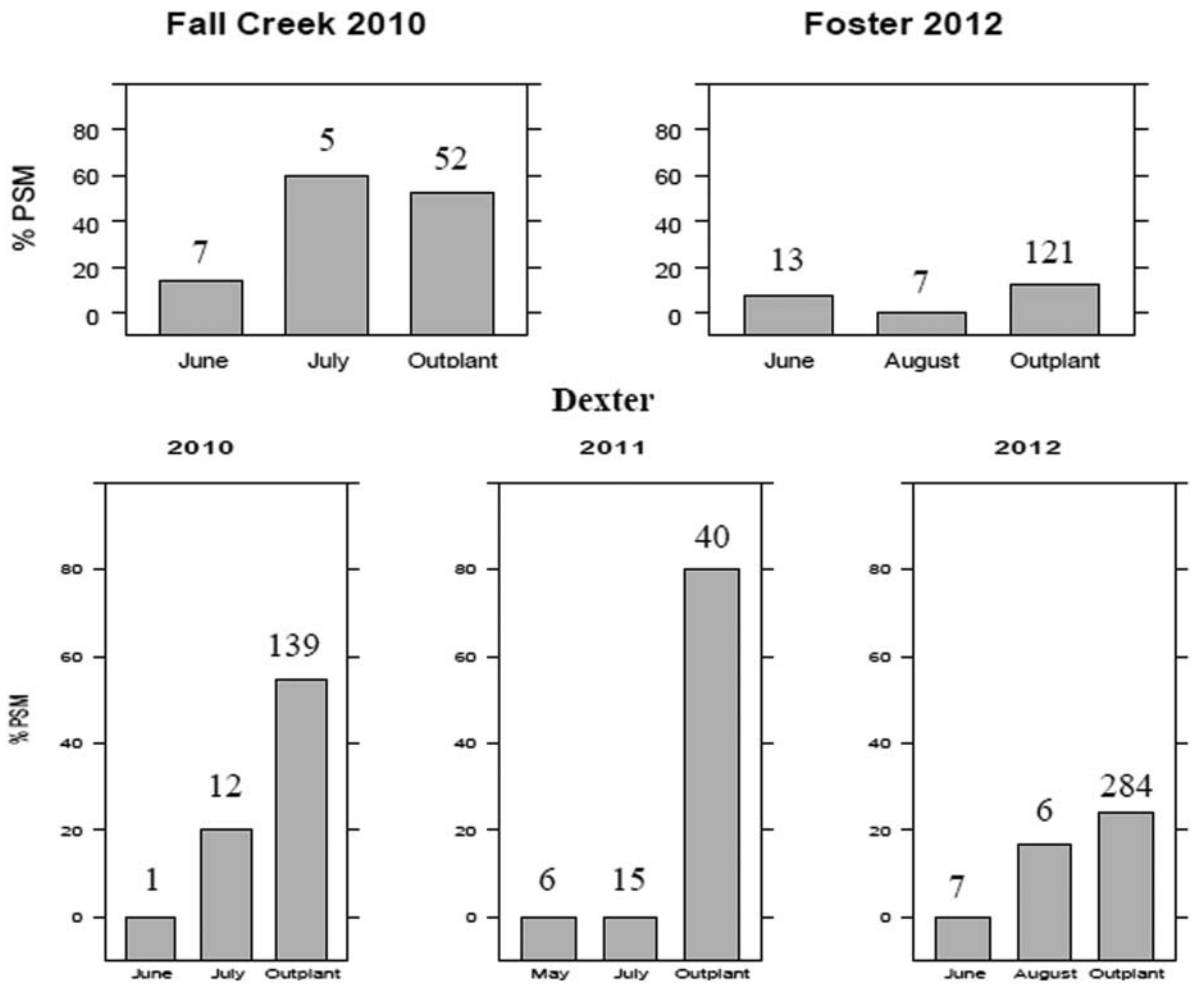


FIGURE 2. Percent PSM for only female Chinook Salmon for each location and year. Sample size is indicated above each bar. Months indicate collection times for fish transported to the holding facility, and Outplant indicates fish outplanted above traps.

where P1 to P6 indicates presence or absence of pathogens listed above except for *A. salmonicida*, and the superscripts ^{H} and ^{O} denote whether fish were held or outplanted, respectively. This allowed us to compare live fish to PSM held (PSM^{H}) and PSM outplanted (PSM^{O}) fish (see Table 7) or live fish to spawned held ($spawn^{H}$) and spawned outplanted ($spawn^{O}$) fish (see Table 8). *Aeromonas salmonicida* was excluded; there was complete separation in the model as *A. salmonicida* was not detected in the live group. Further, arrival was not characterized for these two models because outplanted fish could not be categorized as either early or late, and live fish were not grouped as either outplanted or held (leading to complete separation in the model). Other than these changes, the general model is the same as the one described above for PSM and spawned comparisons.

RESULTS

The percent PSM for held female Chinook Salmon was generally less than 20%, (with the exception of one group at Fall Creek Dam), while the percent PSM for outplanted females varied from less than 20% up to 80% (Figure 3). Using multiple logistic regression, we modeled the odds of PSM as a function of collection groups (early held, late held, and outplanted), location (Foster, Fall Creek, and Dexter dams), and year (2010–2012) for female fish (Table 4). With this model, PSM was 12.6 times more likely to occur in outplanted female fish than in early held female fish, after accounting for location and year. Prespawn mortality was 7.9 times more likely in outplanted female fish than late female fish that were held. Fall Creek female salmon were 2.3 times more likely to have PSM than those at the Foster Dam location. Similarly, female fish from Dexter Dam were 2.1 times more likely to have PSM than those at the Foster Dam location. Finally PSM in outplanted female fish was more likely to occur in 2010 than in 2012.

Percent PSM at the holding facility (for both males and females) was 20% or less (with the exception of one group each from Willamette Falls and Fall Creek Dam) (Figure 4). There was no mortality directly associated with the capture or

Downloaded by [Oregon State University] at 07:59 15 January 2016

TABLE 4. Likelihood of PSM for female Chinook Salmon based on multiple logistic regression of collection groups, location, and year. The odds ratio applies to the first group in the comparison, e.g., PSM is 12.6 times more likely in the outplanted group versus the early held group. Statistical significance is indicated as follows: $P \leq 0.001$***; $P \leq 0.01$**; $P \leq 0.05$*; $P \leq 0.1^{\dagger}$.

Comparison	Odds ratio (95% CI)
Outplant versus early	12.6 (3.4–81.4)***
Outplant versus late	7.9 (3.3–22.6)***
Fall Creek versus Foster	2.3 (1.0–5.6)†
Fall Creek versus Dexter	1.1 (0.6–2.0)
Dexter versus Foster	2.1 (1.2–4.0)*
2010 versus 2011	0.5 (03–1.1)†
2010 versus 2012	3.8 (2.5–5.9)***
2011 versus 2012	7.1 (3.7–14.2)***

transport process. The point estimate of the odds ratio indicated a salmon from a late collection date was 3.1 times more likely to experience PSM than a fish from an early collection date, though this was not significant at alpha = 0.05 (Table 5). Based on the model, held fish from Willamette Falls were 10 times more likely to experience PSM than held fish from Dexter Dam; we point out here that these samples were likely from different populations and that sources of mortality between the two groups are probably different, as discussed later. There were no differences in the likelihood of PSM at the holding facility between the upper Willamette River locations. Regarding differences between years, a fish was more likely to experience PSM in 2010 than 2012 (Table 5). Held fish produced viable offspring, regardless of location or collection time (>58% survival to hatch across all groups).

For all salmon (held and outplanted), fish that experienced PSM were 5.0 times more likely to have *R. salmoninarum* than spawned fish (Table 6). Conversely, spawned fish were 7.0 times more likely to have *C. shasta* than fish having PSM (Table 6). There was no difference between spawned fish and fish that had PSM for the remaining pathogens.

Bacterial kidney disease was substantially more prevalent in PSM^{H} groups than in other groups. The PSM^{H} fish were 36 times more likely to be positive for *R. salmoninarum* than were live fish, after accounting for year and location (Table 7). Likewise, PSM^{H} fish were 32 times more likely to be *R. salmoninarum* positive than were PSM^{O} fish. Live fish were about three times more likely to be positive for *Myxobolus* sp. than were PSM^{O} fish.

$Spawn^{H}$ fish were 3.6 times more likely to be positive for *R. salmoninarum* than live fish after accounting for year and location (Table 8). $Spawn^{H}$ fish were also about four times more likely to be infected with *C. shasta* than were live fish. Conversely, live fish were about three times more likely to be positive for *P. minibicornis* than $spawn^{H}$ fish.

Similar to $spawn^{H}$ fish, $spawn^{O}$ fish were more likely to have *C. shasta* than were live fish (Table 8). However, live fish were more likely to have both *Myxobolus* sp. brain infection and *Apophallus* sp./*E. milvi* gill infections than were $spawn^{O}$ fish. Comparison of the two categories of spawned fish revealed that $spawn^{H}$ fish were much more likely to be infected with *Myxobolus* sp. than were $spawn^{O}$ fish (Table 8). Conversely, $spawn^{O}$ fish were more likely to have *P. minibicornis* than were $spawn^{H}$ fish.

DISCUSSION

The primary focus of this study was to determine whether Chinook Salmon held in a pathogen-free, constant temperature environment have lower PSM than those in the river (H_1). We found that both early and late female fish that were held were less likely to experience PSM than outplanted female fish, after accounting for location and year. Our study design did not allow us to test this hypothesis for males, but holding males would likely experience a decrease in PSM than would outplanted males because sex did not significantly affect the likelihood of PSM in held fish. We also found that early held fish are less likely to experience PSM than late held fish (H_2).

As percent PSM varies by year, the effectiveness of holding does as well; i.e., in years where percent PSM is higher in outplants there is comparatively less PSM at the holding facility, but when percent PSM is low in outplants, it is also low at the holding facility. As a management tactic, holding salmon would be most effective in years where high PSM is expected and less helpful in years with low PSM. Development of management plans for years with different anticipated river conditions and other variables could be used to ensure minimum impacts to outplanted fish while balancing biological benefits and economic costs (e.g., Schreck et al. 2013). If we could predict good versus bad PSM years using environmental forecasts, one could hold fish as needed.

While our study cannot directly attribute lower PSM at the holding facility to the cool constant temperatures and a parasite-free environment, those are the most plausible explanations for the results. The higher PSM in outplants in 2010 versus 2012 is correlated to temperatures being ~2°C warmer in 2010 than in 2012 (USGS 2015). Other studies provide evidence of the correlation between elevated temperatures and PSM (Crossin et al. 2008; Keefer et al. 2010). Furthermore, Jeffries et al. (2012) found an upregulation in genes involved in immunity in Sockeye Salmon held at 19°C. They indicated that while this could be due to temperature stress, it could also be a response to higher virulence of pathogens that progresses in a temperature-dependent manner.

With the exception of one fish, salmon that experienced PSM at our holding facility exhibited a high prevalence and severity of *R. salmoninarum* or *A. salmonicida.* We speculate that the stresses experienced by the fish prior to our collection and/or the collection and transportation process itself may have resulted in proliferation of these pathogens. This was suggested when we examined percent prevalence and severity

Downloaded by [Oregon State University] at 07:59 15 January 2016

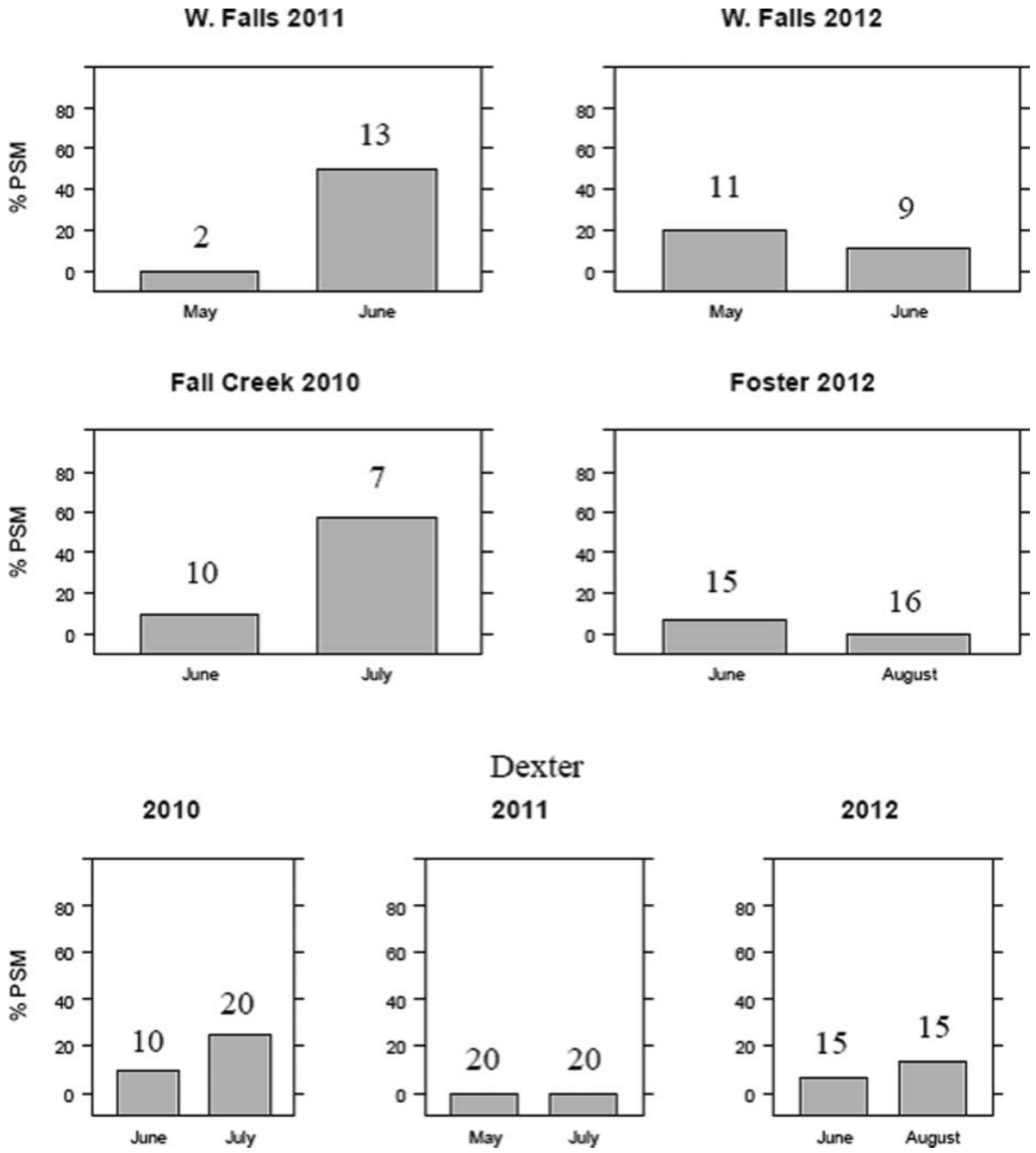


FIGURE 3. Percent PSM for male and female Chinook Salmon at the holding facility for each collection date, location, and year. Sample size is indicated above each bar.

of bacterial infections of fish sampled at the trapping facilities, the holding facility, and outplant locations. Fish at the trapping facilities had little or no histological evidence of *R. salmoninarum* or *A. salmonicida* infections, and prevalence remained low in outplanted fish (Benda 2014). Meanwhile, fish having PSM at the holding facility showed a high prevalence of infection with these pathogens.

The observed higher prevalence of bacterial infection at the holding facility is not surprising. *Renibacterum salmoninarum* has long been a major problem for cultured salmon (Fryer and Sanders 1981) and the infection is very prevalent in wild Chinook Salmon in the Pacific Northwest (Banner et al. 1986; Pascho and Mulcahy 1987; Arkoosh et al. 2004; Rhodes et al. 2006). *Renibacterum salmoninarum* is a slow-growing bacterium; it takes weeks before causing fatality in the fish, and horizontal transmission is similarly slow (Murray et al. 1992; McKibben and Pascho 1999). Therefore, many of the fish collected as adults from the Willamette River may have had

Downloaded by [Oregon State University] at 07:59 15 January 2016

TABLE 5. Likelihood of PSM for male and female held Chinook Salmon based on multiple logistic regression of collection date, location, and year. The odds ratio applies to the first group in the comparison. Statistical significance is indicated as follows: $P \leq 0.001$***; $P \leq 0.01$**; $P \leq 0.05$*; $P \leq 0.1^{\dagger}$.

Comparison	Odds ratio (95% CI)
Late versus early	3.1 (0.9–12.9)†
Dexter versus Willamette Falls	0.1 (0.0–0.35)**
Fall Creek versus Willamette Falls	0.2 (0.0–1.3)†
Foster versus Willamette Falls	0.1 (0.0–0.6)*
Fall Creek versus Dexter	2.3 (0.5–10.5)
Foster versus Dexter	0.9 (0.0–9.5)
Foster versus Fall Creek	0.4 (0.0–6.4)
2011 versus 2010	0.2 (0.0–1.1)†
2012 versus 2010	0.2 (0.0–0.8)*
2011 versus 2012	1.5 (0.4–5.9)
Female versus male	0.9 (0.4–2.5)

subclinical infections that became clinical several weeks after capture or being subjected to other stressors.

With *A. salmonicida*, one highly infective fish can spread the pathogen to other individuals. The density-dependent transmission of *A. salmonicida* makes it an especially important consideration for holding facilities, where fish would presumably be held in close quarters (Ogut and Reno 2004). The Willamette Falls fish held in 2011 are the best example of this, where 100% of PSM fish were moderately or severely infected with *A. salmonicida*. These fish died within a month of transport, most within the first 2 weeks. Not only does this emphasize the importance of this disease, it calls attention to the importance of reducing transport and handling stress and the potential negative aspect to a collect-and-hold management tactic. Bacterial infection could also explain why held salmon from Willamette Falls were more likely to experience PSM than salmon collected upstream (Table 5). It is possible that fish experience pathogen-associated mortality between Willamette Falls and the trapping facilities, which would result in an underestimation of total freshwater mortality. Alternatively, the higher mortality may have been related to greater handling and transport time required for collection at Willamette Falls.

Both *R. salmoninarum* and *A. salmonicida* are common to hatcheries, which usually treat for bacterial infections with antibiotics. *Aeromonas salmonicida* was not detected histologically in spawned fish from Willamette Hatchery (fish treated with antibiotic), while we found residual levels of infection in held spawned fish (not treated with antibiotics) (Benda 2014). The infection rate with *R. salmoninarum* was equal to or less than that of spawned fish at the holding facility, and the severity of infection was generally lower at Willamette Hatchery.

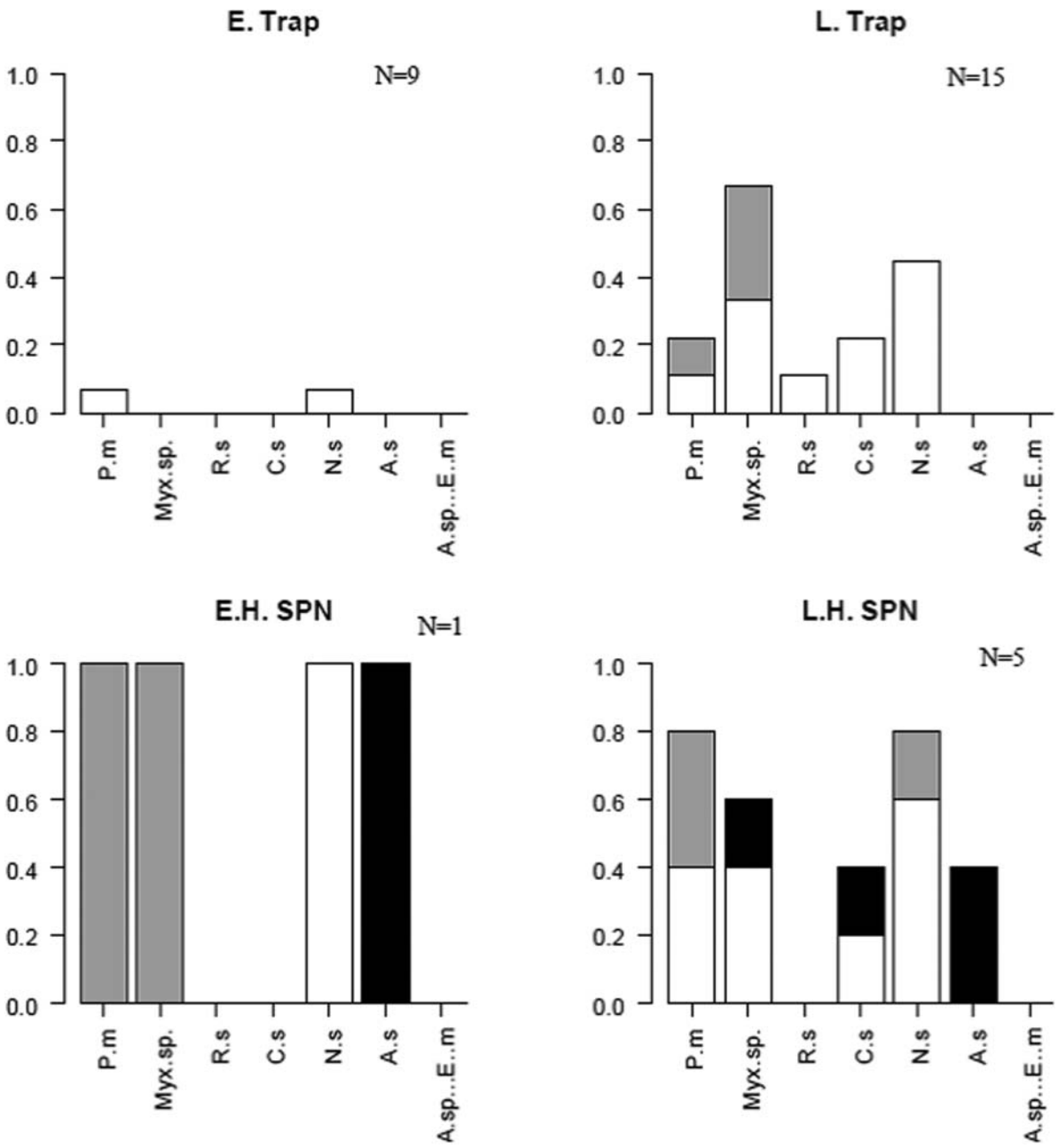
Our held fish were not treated with antibiotics to ensure they were similar to outplanted fish. If holding were used as a management tactic, fish would be held for a period of time and then released to spawn. Based on the prevalence of bacterial disease in held fish, careful consideration will have to be given to the use of antibiotics. We know that fish in a held setting can experience pathogen-associated mortality from these diseases. There is also a possibility that stress (e.g., handling and transport to release site) and lack of treatment could increase the chances of death before spawning. Additionally, the ODFW fish health management policy (ODFW 2003) is designed to prevent the holding and transportation of fish that will either incubate or release high levels of bacteria into the environment.

While antibiotics would help to alleviate the issues described in the previous paragraph, this approach has certain concerns. Antibiotic use could lead to the selection of drug-resistant bacteria, which would affect terrestrial livestock and humans using the same antibiotics (Bell et al. 1988; Kemper 2008; Rhodes et al. 2008). There is also a risk of human exposure to antibiotics after fish are released, although fish are only released where or when harvest is illegal in the Willamette River. The application of antibiotics would therefore be limited, which could help create a balance between the release of bacteria and/or antibiotics and the survival of held fish.

The second aim of this study was to determine whether Chinook Salmon that experienced PSM and spawned fish had similar pathogen burdens (H_3) and whether held fish had lower pathogen burdens than outplanted fish (H_4). When we compared all PSM fish to all spawned fish (H_3), we found that fish were likely to have similar pathogen burdens with only two exceptions. Fish that had PSM were more likely to have *R. salmoninarum* and spawned fish were more likely to have *C. shasta* (Table 6). As discussed previously, most of the held PSM fish died from bacterial infection, meaning this result reflects more on holding than causes of PSM. Further, it is very likely that all fish (live, PSM, or spawned fish at both the holding facility or outplanted) are infected with *R. salmoninarum* and that subclinical infections are hard to detect with histology (Kent et al. 2013). *Ceratonova shasta* is a proliferative pathogen that has a degree-day dependent life cycle and infections are initiated after water temperatures rise in the late spring or early summer (Johnson 1975; Chiaramonte 2013). Because spawned Chinook Salmon spend the most time in the river (fish spawn in September, and live and PSM fish were collected May through August), it follows that we would be more likely to detect this pathogen in spawned fish than in either live or PSM fish.

The PSM of fish with pathogen burdens similar to that of a spawned fish could be explained several ways. First, it is possible that a PSM fish is more susceptible to pathogenesis than a successful spawner for a given burden. The specific cause of this increased susceptibility (e.g., individual genotype, fish stock, year-class, ocean conditions, previous life history, stress, pathogen burden, a weakened immune system, senescence, thermal experience) cannot be determined from this study. Alternatively, similar pathogen burdens could indicate that fish that experience PSM spent more time in freshwater, which could affect such factors as

Downloaded by [Oregon State University] at 07:59 15 January 2016



Downloaded by [Oregon State University] at 07:59 15 January 2016

FIGURE 4. Pathogen prevalence and severity for Chinook Salmon sampled at Willamette Falls early (E. Trap) and late (L.Trap) and held spawned fish collected from Willamette Falls early (E.H. SPN) and late (L.H. SPN) in 2011. Sample size (*N*) is indicated in top right corner of each panel. Shading indicates the severity of infection: white = low, gray = moderate, and black = severe. Pathogens: P.m = *Parvicapsula minibicornis*; Myx.sp. = *Myxobolus* sp.; R.s = *Renibacterium salmoninarum*; C.s = *Ceratonova shasta*; N.s = *Nanophyetus salmincola*; A.s = *Aeromonas salmonicida*; and A.sp. . .E..m = *Apophallus* sp. and *Echinochasmus milvi*.

the timing of senescence, the amount of pathogen exposure and burden, the degree-days acquired, the amount of time fish are held below trapping facilities (dams) and on spawning grounds, and the amount of energy reserves available for migration.

When we compared held versus outplanted fish (H_4), there were few differences between groups. For fish that experienced PSM, those that were held were more likely to have *R. salmoninarum* than those that were outplanted(discussed previously). For spawned fish, held fish were more likely to have *Myxobolus* sp. and less likely to have *P. minibicornis* than were outplanted fish; this can be explained by the pathogens' respective life cycles. The latter myxozoan uses the freshwater polychaete, *Manayunkia speciosa*, as an

Downloaded by [Oregon State University] at 07:59 15 January 2016

TABLE 6. Odds ratios from binomial logistic regression model comparing spawned versus PSM (for both outplanted and held Chinook Salmon) as a function of pathogens (presence [+] or absence [−]), location, year, and collection time. This would be read as: a spawned fish was seven times more likely to have C. shasta than a PSM fish (95% CI, 2.6–21.6); spawned fish are 1.8 times more likely than PSM fish at Foster Dam than Fall Creek Dam (95% CI, 0.1–32.9); spawned fish are 1.2 times more likely than PSM fish in 2012 than in 2010 (95% CI, 0.1–27.9). Pathogens: *Parvicapsula minibicornis*, *Renibacterium salmoninarum*, *Ceratonova shasta*, *Nanophyetus salmincola*, *Aeromonas salmonicida*, *Myxobolus* sp., *Apophallus* sp., and *Echinochasmus milvi*. Statistical significance is indicated as follows: $P \leq 0.001$***; $P \leq 0.01$**; $P \leq 0.05$*; $P \leq 0.1^{\dagger}$.

Pathogen or comparison	Odds ratio (95% CI)
P. minibicornis +	1.2 (0.4–3.9)†
R. salmoninarum +	0.2 (0.0–0.7)***
C. shasta +	7.0 (2.6–21.6)**
N. salmincola +	0.9 (0.2–4.5)
A. salmonicida+	1.4 (0.4–4.8)
Myxobolus sp. +	2.0 (0.7–6.4)
Apophallus sp./*E. milvi* +	0.8 (0.3–2.3)
Foster versus Fall Creek	1.8 (0.1–32.9)
Dexter versus Fall Creek	0.5 (0.1–1.6)
Foster versus Dexter	3.9 (0.1–57.8)
2011 versus 2010	0.5 (0.1–2.2)
2012 versus 2010	1.2 (0.1–27.9)
2012 versus 2011	2.4 (0.2–55.4)
Early versus outplant	159.1 (26.2–1,747.2)***
Late versus outplant	24.4 (7.2–105.7)***

alternate host (Bartholomew et al. 2006), and thus it is likely that salmon do not become infected until they return to freshwater to spawn. In this case, removing adult salmon from the river early in the summer may reduce the prevalence of infection. Conversely, based on our knowledge of other neurotropic *Myxobolus* species infecting salmon, the Chinook Salmon in our study are very likely infected with *Myxobolus* sp. as juveniles in freshwater and the infections persist to adulthood (Kent et al. 1993, 1994; Ferguson et al. 2008). The lower likelihood of *Myxobolus* sp. in spawned outplanted fish than in either live fish at traps or spawned held fish suggests that outplanted fish with this infection are dropping out of the population and holding may help survival. However, live fish were also more likely to have *Myxobolus* sp. infections than outplanted fish that experienced PSM, which does not support the hypothesis of parasite-associated mortality. This could be due to a lack of detection by histological examination or an inadequate sample size of fish that experienced PSM.

The similar prevalence of infection between held and outplanted spawned fish for several of the pathogens in this study was not surprising as they were taken from the same population and two groups were likely exposed to the same pathogens before capture. Moreover, there could be differences that we were not able to reveal due to statistical limitations, such as relatively small sample sizes and replicates, and several confounding factors such as time and location in the river and variability in PSM and temperatures between years.

Nevertheless, similar severity of infection could indicate several factors: there is some stress associated with holding,

TABLE 7. Multinomial logistic regression of PSM^{H}, PSM^{O}, and live Chinook Salmon as a function of pathogens (presence [+] or absence [−]), location, and year. Odds ratio and 95% CI are listed for each comparison. This would be read as: PSMH fish are 36.1 times more likely to have R. salmoninarum than live fish (95% CI, 4.8–268.8); PSMH fish are 1.9 times more likely than live fish at Dexter Dam compared with those at Fall Creek Dam (95% CI, 0.2–16.0); PSMH fish are 0.2 times less likely than live fish to occur in 2011 than in 2010 (95% CI, 0.0–4.7). Pathogens: *Parvicapsula minibicornis*, *Renibacterium salmoninarum*, *Ceratonova shasta*, *Nanophyetus salmincola*, *Myxobolus* sp., *Apophallus* sp., and *Echinochasmus milvi*. Statistical significance is indicated as follows: $P \leq 0.001$***; $P \leq 0.01$**; $P \leq 0.05$*; $P \leq 0.1^{\dagger}$.

Pathogen or comparison	PSM^{H} versus live	PSM^{O} versus live	PSM^{H} versus PSM^{O}
P. minibicornis +	0.2 (0.0–1.8)	0.5 (0.1–1.6)	0.5 (0.1–3.5)
R. salmoninarum +	36.1 (4.8–268.8)***	1.1 (0.2–6.4)	32.1 (5.0–205.3)***
C. shasta +	0.5 (0.1–4.1)	0.7 (0.3–2.0)	0.7 (0.1–6.1)
N. salmincola +	2.3 (0.1–51.6)	1.3 (0.2–7.3)	1.8 (0.1–30.4)
Myxobolus sp. +	0.7 (0.1–6.2)	0.3 (0.1–0.9)*	2.1 (0.2–20.3)
Apophallus sp./*E. milvi* +	0.2 (0.0–1.3)	0.3 (0.1–1.0)†	0.6 (0.1–3.8)
Foster versus Fall Creek	6.8×10^{6} ($1.5 \times 10^{6} - 3.1 \times 10^{7}$)***	5.6 (0.3–108.9)	9.7×10^{5} ($2.0 \times 10^{5} - 4.7 \times 10^{6}$)***
Dexter versus Fall Creek	1.9 (0.2–16.0)	2.9 (0.7–11.7)	0.7 (0.1–4.7)
Foster versus Dexter	2.8×10^{6} ($3.1 \times 10^{5} - 2.7 \times 10^{7}$)***	2.0 (0.1–26.5)	1.9×10^{6} ($2.0 \times 10^{5} - 1.9 \times 10^{7}$)***
2011 versus 2012	4.0×10^{6} ($5.0 \times 10^{5} - 3.1 \times 10^{7}$)***	9.9 (0.9–106.9)†	5.2×10^{5} ($6.6 \times 10^{4} - 4.1 \times 10^{6}$)***
2011 versus 2010	0.2 (0.0–4.7)	0.4 (0.1–1.9)	0.5 (0.0–9.7)
2012 versus 2010	4.8×10^{-8} ($1.1 \times 10^{-8} - 2.2 \times 10^{-7}$)***	0.0 (0.0–0.5)*	1.3×10^{-6} ($2.8 \times 10^{-7} - 6.5 \times 10^{-6}$)***

TABLE 8. Multinomial logistic regression of spawn^H, spawn^O, and live Chinook Salmon as a function of pathogens (presence [+] or absence [−]), location, and year. Odds ratio and 95% CI are listed for each comparison. This would be read as: spawnH fish are 3.6 times more likely to have R. salmoninarum than live fish (95% CI, 1.1–12.5); spawnH fish are 2.4 times more likely than live fish at Dexter Dam compared with Fall Creek Dam (95% CI, 0.7–8.7); spawnH fish are 1.1 times more likely than live fish to occur in 2011 than in 2010 (95% CI, 0.3–3.8). Pathogens: *Parvicapsula minibicornis*, *Renibacterium salmoninarum*, *Ceratonova shasta*, *Nanophyetus salmincola*, *Myxobolus* sp., *Apophallus* sp., and *Echinochasmus milvi*. Statistical significance is indicated as follows: $P \leq 0.001$***; $P \leq 0.01$**; $P \leq 0.05$*; $P \leq 0.1^{\dagger}$.

Pathogen or comparison	Spawn^H versus live	Spawn^O versus live	Spawn^H versus spawn^O
P. minibicornis +	0.3 (0.1–0.9)*	1.5 (0.4–6.0)	0.2 (0.1–0.8)*
R. salmoninarum +	3.6 (1.1–12.5)*	0.7 (0.1–8.0)	5.1 (0.6–46.8)
C. shasta +	4.1 (1.9–8.8)***	5.8 (1.7–19.7)**	0.7 (0.2–2.3)
N. salmincola +	0.6 (0.1–2.3)	0.9 (0.1–7.4)	0.6 (0.1–4.0)
Myxobolus sp. +	0.8 (0.4–1.7)	0.1 (0.0–0.5)**	5.6 (1.6–20.6)**
Apophallus sp./E. milvi +	0.5 (0.2–1.1)†	0.2 (0.0–0.6)**	3.3 (0.9–11.7)†
Foster versus Fall Creek	1.6 (0.3–9.0)	4.8×10^6	4.1×10^{-7}
		$(2.3 \times 10^6 - 1.0 \times 10^7)$***	$(1.4 \times 10^{-7} - 1.2 \times 10^{-6})$***
Dexter versus Fall Creek	2.4 (0.7–8.7)	0.8 (0.2–3.9)	3.0 (0.7–12.6)
Foster versus Dexter	0.7 (0.2–2.1)	9.1×10^6	1.3×10^{-7}
		$(2.8 \times 10^6 - 3.0 \times 10^7)$***	$(3.9 \times 10^{-8} - 4.1 \times 10^{-7})$***
2011 versus 2012	0.7 (0.2–2.4)	2.0×10^6	6.5×10^{-7}
		$(5.0 \times 10^5 - 7.7 \times 10^6)$***	$(1.6 \times 10^{-7} - 2.6 \times 10^{-6})$***
2011 versus 2010	1.1 (0.3–3.8)	0.1 (0.0–0.6)*	14.0 (2.1–93.9)**
2012 versus 2010	1.5 (0.4–5.9)	6.4×10^{-8}	2.0×10^7
		$(3.0 \times 10^{-8} - 1.4 \times 10^{-7})$***	$(7.6 \times 10^6 - 5.1 \times 10^7)$***

Downloaded by [Oregon State University] at 07:59 15 January 2016

fish are senescing, and proliferative pathogens multiply regardless of location. It also suggests that interactive effects are important for causing mortality (e.g., both pathogens and warm temperatures are needed before significant mortality is evident). Crossin et al. (2008) held Sockeye Salmon at 10°C and 18°C and found more pathogen-related mortality at the higher temperature. Whereas we used higher temperatures as an example of interaction with pathogens, there could also be an interaction with low flow, holding below trapping facilities, transport stress, or other factors. For instance, a low-flow environment could be favorable to intermediate hosts of pathogens or the pathogens themselves, increase the density of holding salmon, and thus increase the exposure of salmon to pathogens (Leniham et al. 1999; Hallett and Bartholomew 2008; Bjork and Bartholomew 2009). Holding fish below trapping facilities at higher densities could allow for the horizontal transmission of certain pathogens, e.g., *A. salmonicida* (Ogut and Reno 2004), and stress would make salmon more susceptible to disease (Schreck 1996).

In conclusion, fish held in pathogen-free, constant temperature water had lower PSM than fish that were outplanted, indicating that holding could be a viable method to increase survival to spawn. The majority of the mortality in held fish that experienced PSM was due to infections of *R. salmoninarum* and *A. salmonicida*, both of which are exacerbated by stress and holding. Interestingly, unlike most of the parasites in our study, these bacteria are well recognized to be important causes of disease in salmonid fishes held in captivity. Therefore, careful consideration should be given not only to the transport and handling methods, but also to the possible use of antibiotics in held fish. Holding as a management tactic would involve the release of held fish into spawning tributaries, which our study did not examine. Future work should hold and release fish and account for antibiotics as well as the timing of collection and release. Fish that undergo PSM are dying from the presence of pathogens, based on the similar pathogen burdens of PSM fish and spawned fish. The next step would be to determine whether this is simply a function of exposure time to the pathogens (e.g., early returning fish) or whether PSM fish are inherently more susceptible to infection. Finally, an examination of the prevalence and cause of PSM in Chinook Salmon between Willamette Falls and upstream tributaries would not only broaden our understanding of PSM but potentially identify other management tactics to increase the survival of fish to spawn.

ACKNOWLEDGMENTS

Cameron Sharpe, ODFW, assisted with data collection, fish transport, and organized crews to collect PSM fish. Rob Chitwood contributed to transport, holding, and spawning of fish at Fish Performance and Genetics Laboratory at Oregon State University (OSU). Julia Unrein and Courtney Danley of OSU assisted data collection and histology. James Peterson, OSU,

provided statistical guidance. Steven R. Lee, Mark Morasch, Matt Knoff, Adrienne Roumasset, and Eric Powell of the University of Idaho (UI) helped with field work. Karen Johnson, UI, assisted with logisitics. Matt Keefer, UI, provided the map in Figure 1. Mike Jepson, UI, was responsible for obtaining permits. Greg Taylor, Doug Gartletts, Chad Helms, Todd Pierce, and Greg Gauthier of the U.S. Army Corps of Engineers (USACE) provided access and support during sampling at Fall Creek and Dexter dams. Brett Boyd, ODFW, provided access and logistical support at Foster Dam. David Griffith administered the contracts for the USACE. Publication of this paper was supported, in part, by the Thomas G. Scott Publication Fund.

Downloaded by [Oregon State University] at 07:59 15 January 2016

REFERENCES

Arkoosh, M. R., E. Clemons, A. N. Kagley, C. Stafford, A. C. Glass, K. Jacobson, P. Reno, M. S. Myers, E. Casillas, F. Loge, L. L. Johnson, and T. K. Collier. 2004. Survey of pathogens in juvenile salmon *Oncorhynchus* spp. migrating through Pacific Northwest estuaries. Journal of Aquatic Animal Health 16:186–196.

Banner, C. R., J. J. Long, J. L. Fryer, and J. S. Rohovec. 1986. Occurrence of salmonid fish infected with *Renibacterium salmoninarum* in the Pacific Ocean. Journal of Fish Diseases 9:273–275.

Bartholomew, J. L., S. D. Atkinson, and S. L. Hallett. 2006. Involvement of *Manayunkia speciosa* (Annelida: Polychaeta: Sabellidae) in the life cycle of *Parvicapsula minibicornis*, a myxozoan parasite of Pacific salmon. Journal of Parasitology 92:742–748.

Beer, N. W., and J. J. Anderson. 2001. Effect of spawning day and temperature on salmon emergence: interpretations of a growth model for Methow River Chinook. Canadian Journal of Fisheries and Aquatic Sciences 58:943–949.

Bell, G. R., G. S. Traxler, and C. Dworschak. 1988. Development in vitro and pathogenicity of an erythromycin-resistant strain of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmonids. Diseases of Aquatic Organisms 4:9–25.

Benda, S. E. 2014. Cool, pathogen free refuge lowers pathogen associated prespawn mortality of Willamette River Chinook Salmon (*Oncorhynchus tshawytscha*). Master's thesis. Oregon State University, Corvallis.

Bjork, S. J., and J. L. Bartholomew. 2009. The effects of water velocity on the *Ceratomyxa shasta* infectious cycle. Journal of Fish Diseases 32:131–142.

Chiaramonte, L. V. 2013. Climate warming effects on the life cycle of the parasite *Ceratomyxa shasta* in salmon of the Pacific Northwest. Master's thesis. Oregon State University, Corvallis.

Crossin, G. T., S. G. Hinch, A. P. Farrell, D. A. Higgs, A. G. Lotto, J. D. Oakes, and M. C. Healey. 2008. Exposure to high water temperature influences the behavior, physiology, and survival of Sockeye Salmon during spawning migration. Canadian Journal of Zoology 86:127–140.

Ferguson, J. A., S. D. Atkinson, C. M. Whipps, and M. L. Kent. 2008. Molecular and morphological analysis of *Myxobolus* spp. of salmonid fishes with the description of a new *Myxobolus* species. Journal of Parasitology 94:1322–1334.

Fryer, J. J., and J. E. Sanders. 1981. Bacterial kidney disease of salmonid fish. Annual Review of Microbiology 35:273–298.

Gauthreaux, S. A., editor. 1980. Animal migration, orientation, and navigation. Academic Press, New York.

Hallett, S. L., and J. L. Bartholomew. 2008. Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. Parasitology 135:371–384.

Hamazaki, T., E. Kahler, B. M. Borba, and T. Burton. 2013. Impact of Ichthyophonus infection on spawning success of Yukon River Chinook Salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms 106:207–215.

Jeffries, K. M., S. G. Hinch, T. Sierocinski, T. D. Clark, E. J. Eliason, M. R. Donaldson, S. Li, P. Pavlidis, and K. M. Miller. 2012. Consequences of high temperatures and premature mortality on the transcriptome and blood physiology of wild adult Sockeye Salmon (*Oncorhynchus nerka*). Ecology and Evolution 2:1747–1764.

Johnson, K. A. 1975. Host susceptibility, histopathologic, and transmission studies on *Ceratomyxa shasta*, a myxosporidan parasite of salmonid fish. Doctoral dissertation. Oregon State University, Corvallis.

Keefer, M. L., T. S. Clabough, M. A. Jepson, G. P. Naughton, T. J. Blubaugh, D. C. Joosten, and C. C. Caudill. 2015. Thermal exposure of adult Chinook Salmon in the Willamette River basin. Journal of Thermal Biology 48:11–20.

Keefer, M. L., G. A. Taylor, D. F. Garletts, G. A. Gauthier, T. M. Pierce, and C. C. Caudill. 2010. Prespawn mortality in adult spring Chinook Salmon outplanted above barrier dams. Ecology of Freshwater Fish 19:361–372.

Kemper, N. 2008. Review: Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators 8:1–13.

Kent, M. L., S. Benda, S. St-Hilaire, and C. B. Schreck. 2013. Sensitivity and specificity of histology for diagnoses of four common pathogens and detection of nontarget pathogens in adult Chinook Salmon (*Oncorhynchus tshawytscha*) in freshwater. Journal of Veterinary Diagnostic Investigation 25:341–351.

Kent, M. L., L. Margolis, and J. O. Corliss. 1994. The demise of a class of protists: taxonomic and nomenclatural revisions proposed for the protest phylum Myxozoa Grassé, 1970. Canadian Journal of Zoology 72:932–937.

Kent, M. L., D. J. Whitaker, and L. Margolis. 1993. Transmission of *Myxobolus arcticus* Pugachev and Khokhlov, 1979, a myxosporean parasite of Pacific salmon, via a triactinomyxon from the aquatic oligochaete *Stylodrilus heringianus* (Lumbriculidae). Canadian Journal of Zoology 71:1207–1211.

Kocan, R., P. Hershberger, and J. Winton. 2004. Ichthyophoniasis: an emerging disease of Chinook Salmon in the Yukon River. Journal of Aquatic Animal Health 16:58–72.

Leniham, H. S., F. Micheli, S. W. Shelton, and C. H. Peterson. 1999. The influence of multiple environmental stressors on susceptibility to parasites: an experimental determination with oysters. Limnology and Oceanography 44:910–924.

McCullough, D. A. 1999. A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook Salmon. U.S. Environmental Protection Agency, Water Resources Assessment, Seattle.

McCullough, D. A., S. Spalding, D. Sturdevant, and M. Hicks. 2001. Summary of technical literature examining the physiological effects of temperature on salmonids. U.S. Environmental Protection Agency, EPA-910-D-01-005, Seattle.

McKibben, C. L., and R. J. Pascho. 1999. Shedding of *Renibacterium salmoninarum* by infected Chinook Salmon *Oncorhynchus tshawytscha*. Diseases of Aquatic Organisms 38:75–79.

Mosser, C. M., L. C. Thompson, and J. S. Strange. 2013. Survival of captured and relocated adult spring-run Chinook Salmon *Oncorhynchus tshawytscha* in a Sacramento River tributary after cessation of migration. Environmental Biology of Fishes 96:405–417.

Murray, C. B., T. P. T. Evelyn, T. D. Beacham, L. W. Barner, J. E. Ketcheson, and L. Prosper-Porta. 1992. Experimental induction of bacterial kidney disease in Chinook Salmon by immersion and cohabitation challenges. Diseases of Aquatic Organisms 12:91–96.

NMFS (National Marine Fisheries Service). 1999. Endangered and threatened species: threatened status for three Chinook Salmon evolutionarily significant units (ESUs) in Washington and Oregon, and endangered status for one Chinook Salmon ESU in Washington. Federal Register 64:56(24 March 1999):14308–14328.

NMFS (National Marine Fisheries Service). 2008. Endangered Species Act section 7(a)(2) consultation biological opinion and Magnuson–Stevens Fishery Conservation and Management Act essential fish habitat consultation. NMFS, Portland, Oregon.

ODFW (Oregon Department of Fish and Wildlife). 2003. Fish health management policy. Oregon Department of Fish and Wildlife, Bend.

Ogut, H., and P. Reno. 2004. Prevalence of furunculosis in Chinook Salmon depends on density of the host exposed by cohabitation. North American Journal of Aquaculture 66:191–197.

Pascho, R. J., and D. Mulcahy. 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease. Canadian Journal of Fisheries and Aquatic Sciences 44:183–191.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. American Fisheries Society, Bethesda, Maryland and University of Washington Press, Seattle.

R Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available: http://www.R-project.org/. (September 2015).

Raverty, S., D. Kieser, J. Bagshaw, and S. St-Hilaire. 2000. Renal infestation with *Parvicapsula minibicornis* in wild Sockeye Salmon from the Harrison and Adams rivers in British Columbia. Canadian Veterinary Journal 41:317–318.

Rhodes, L. D., C. Durkin, S. L. Nance, and C. A. Rice. 2006. Prevalence and analysis of *Renibacterium salmoninarum* infection among juvenile Chinook Salmon *Oncorhynchus tshawytscha* in north Puget Sound. Diseases of Aquatic Organisms 71:179–190.

Rhodes, L. D., O. T. Nguyen, R. K. Deinhard, T. M. White, L. W. Harrell, and M. C. Roberts. 2008. Characterization of *Renibacterium salmoninarum* with reduced susceptibility to macrolide antibiotics by a standardized antibiotic susceptibility test. Diseases of Aquatic Organisms 80:173–180.

Richter, A., and S. A. Kolmes. 2005. Maximum temperature limits for Chinook, Coho, and Chum salmon, and steelhead trout in the Pacific Northwest. Reviews in Fisheries Science 13:23–49.

Roumasset, A. 2012. Prespawn mortality of upper Willamette River spring Chinook Salmon: associations with stream temperature, watershed attributes and environmental conditions on the spawning grounds. Master's thesis. University of Idaho, Moscow.

Rucker, R. R., B. J. Earp, and E. J. Ordal. 1954. Infectious diseases of Pacific salmon. Transactions of the American Fisheries Society 83:297–312.

Schreck, C. B. 1996. Immunomodulation: endogenous factors. Fish Physiology 15:311–337.

Schreck, C. B., W. Contreras-Sanchez, and M. S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3–24.

Schreck, C. B., M. L. Kent, M. E. Colvin, S. Benda, C. Sharpe, J. T. Peterson, and B. Dolan. 2013. Potential causes and management of prespawn mortality in adult upper Willamette River spring Chinook. Annual Report to the U.S. Army Corps of Engineers, Portland District, Portland, Oregon.

Schroeder, R. K., K. R. Kenaston, and L. K. McLaughlin. 2007. Spring Chinook Salmon in the Willamette and Sandy rivers. Oregon Department of Fish and Wildlife, Portland.

Spromberg, J. A., and N. L. Scholz. 2011. Estimating the future decline of wild Coho Salmon populations resulting from early spawner die-offs in urbanizing watersheds of the Pacific Northwest, USA. Integrated Environmental Assessment and Management 7:648–656.

USGS (U.S. Geological Survey). 2015. National Water Information System data available on the World Wide Web (USGS water data for the nation). Available: http://waterdata.usgs.gov/nwis/wys_rpt?wys_water_yr=2012&site_no=14150000&agency_cd=USGS&adr_water_years=2006%2C2007%2C2008%2C2009%2C2010%2C2011%2C2012%2C2013%2C2014&referred_module=. (June 2015).

Zimmerman, B. C., and B. B. Duke. 1995. Trapping and transportation of adult and juvenile salmon in the lower Umatilla River in northeast Oregon—Umatilla River basin trap and haul program October 1994–September 1995. Annual Progress Report to the Bonneville Power Administration, Project 198802200, Portland, Oregon.

Downloaded by [Oregon State University] at 07:59 15 January 2016