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ARTICLE

Survey of Pathogens in Hatchery Chinook Salmon with Different Out-Migration Histories through the Snake and Columbia Rivers

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Abstract

The operation of the Federal Columbia River Power System (FCRPS) has negatively affected threatened and endangered salmonid populations in the Pacific Northwest. Barging Snake River spring Chinook salmon *Oncorhynchus tshawytscha* through the FCRPS is one effort to mitigate the effect of the hydrosystem on juvenile salmon out-migration. However, little is known about the occurrence and transmission of infectious agents in barged juvenile salmon relative to juvenile salmon that remain in-river to navigate to the ocean. We conducted a survey of hatchery-reared spring Chinook salmon at various points along their out-migration path as they left their natal hatcheries and either migrated in-river or were barged through the FCRPS. Salmon kidneys were screened by polymerase chain reaction for nine pathogens and one family of water molds. Eight pathogens were detected; the most prevalent were *Renibacterium salmoninarum* and infectious hematopoietic necrosis virus. Species in the family Saprolegniaceae were also commonly detected. Pathogen prevalence was significantly greater in fish that were barged through the FCRPS than in fish left to out-migrate in-river. These results suggest that the transmission of infectious agents to

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susceptible juvenile salmon occurs during the barging process. Therefore, management activities that reduce pathogen exposure during barging may increase the survival of juvenile Chinook salmon after they are released.

The completion of the Federal Columbia River Power System (FCRPS) on the Snake and Columbia rivers has resulted in a decline in the number of returning adult spring Chinook salmon *Oncorhynchus tshawytscha*. In addition to restricting access to adult salmon spawning habitat (Raymond 1988), the FCRPS contributes to losses of juvenile stocks during their out-migration (Raymond 1979). Key factors influencing the loss of juvenile anadromous salmonids during out-migration include the following: mortality associated with turbine passage (Raymond 1988), the timing and duration of peak flows (Raymond 1988; Smith et al. 2002, 2003), and the delay of juvenile out-migration caused by the creation of slow-moving reservoirs above each dam (Budy et al. 2002). Prolonged out-migration increases predation pressure and associated mortality (Raymond 1979, 1988; Rieman et al. 1991) and causes stress that can lead to mortality after the fish leave the hydropower system (Budy et al. 2002). Juvenile salmon stocks with in-river out-migration histories in Lower Snake River must navigate up to eight dams and reservoirs before reaching the Columbia River estuary.

Two strategies employed to mitigate the effects of the hydropower system on juvenile salmon survival are (1) the transportation of juvenile fish by barge from Snake River dams to below Bonneville Dam, the final dam on the Columbia River, and (2) the construction of juvenile fish-passage facilities (bypasses) at many of the dams to help mitigate the direct mortality associated with turbine passage (Ebel 1980; Raymond 1988). Mitigation strategies themselves may induce levels of stress that exacerbate delayed health effects in salmon. For example, barging induces stress in fish associated with handling and crowding, and passage through the bypass system can cause mechanical injuries such as bruising and descaling (NRC 1996; Budy et al. 2002; Sandford and Smith 2002). Despite ongoing mitigation efforts, Pacific salmon populations have not reached the levels observed before the rivers' impoundment (Waples 1991; Waterstrat 1997; Schaller and Petrosky 2007). Thirteen stocks, or evolutionarily significant units (ESUs), from this region are threatened or endangered under the U.S. Endangered Species Act (NRC 1996; NOAA 2005).

Transported juvenile Chinook salmon have a significantly greater survival rate through the FCRPS than do in-river out-migrants (Ward et al. 1997). However, the relative proportion of transported juveniles returning as adults has been unexpectedly low compared with the adult returns of in-river out-migrants (Williams et al. 2005). The proportionally unequal return rates indicate that different rates of mortality occur between transported and in-river out-migrants after they pass Bonneville Dam (hereafter referred to as differential delayed mortality). Several hypotheses have been proposed to explain this differential de-

layed mortality including disrupted smoltification (Budy et al. 2002), the stress and crowding associated with barging (Congleton et al. 2000), impairment of the auditory or olfactory system during barging (Halvorsen et al. 2009), increased straying of adults due to poor imprinting during barging (Keefer et al. 2008), altered timing of ocean entry (Muir et al. 2006), and lost growth opportunity (Muir et al. 2006). In addition, we propose that juvenile salmon exposed to the stressors of prolonged in-river out-migration or the handling and crowding associated with barging may be more susceptible to pathogens. An increase in disease can cause an increase in mortality directly, or may decrease the fitness of a population by increasing predation, reducing reproductive potential, or both (Sindermann 1990; Congleton et al. 2000; Schreck et al. 2006). Knowledge of the spatial and temporal variation of pathogen prevalence is critical to understanding the ecology of infectious disease and ultimately the population dynamics of both barged Chinook salmon and salmon left in river to navigate the FCRPS.

The following pathogens were selected for this study based on the likelihood of distribution in Pacific Northwest watersheds and the availability of polymerase chain reaction (PCR) detection assays in the literature: *Aeromonas hydrophila* (etiological agent of motile aeromonas septicemia), *A. salmonicida* (etiological agent of furunculosis disease), *Flavobacterium psychrophilum* (etiological agent of coldwater disease), *Listonella anguillarum* (formerly *Vibrio anguillarum*; one of the etiological agents of vibriosis), *Renibacterium salmoninarum* (the etiological agent of bacterial kidney disease, or BKD), *Yersinia ruckeri* (etiological agent of enteric redmouth disease), infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), and viral hemorrhagic septicemia virus (VHSV). A family-specific PCR assay was developed for members of the Saprolegniaceae, commonly known as water molds.

Few studies have examined pathogen prevalence in juvenile spring Chinook salmon out-migrants in the Snake and Columbia rivers. Earlier studies have mainly focused on *R. salmoninarum*, which is commonly detected in hatcheries in the Columbia River basin (Maule et al. 1996; VanderKooi and Maule 1999), is very difficult to treat (Fryer and Lannan 1993), and has contributed significantly to mortality in both hatchery (Fryer and Sanders 1981; Fryer and Lannan 1993) and wild salmon (Evelyn et al. 1973). Saprolegniasis, the infection of salmonids by members of the family Saprolegniaceae, has also been observed in hatchery salmon in the Columbia River basin (Mueller and Whisler 1994; Waterstrat 1997). Pathogenic Saprolegniaceae species create a chronic, fungal-like infection in freshwater fish, often acting as secondary invaders in lesions or abrasions caused by other

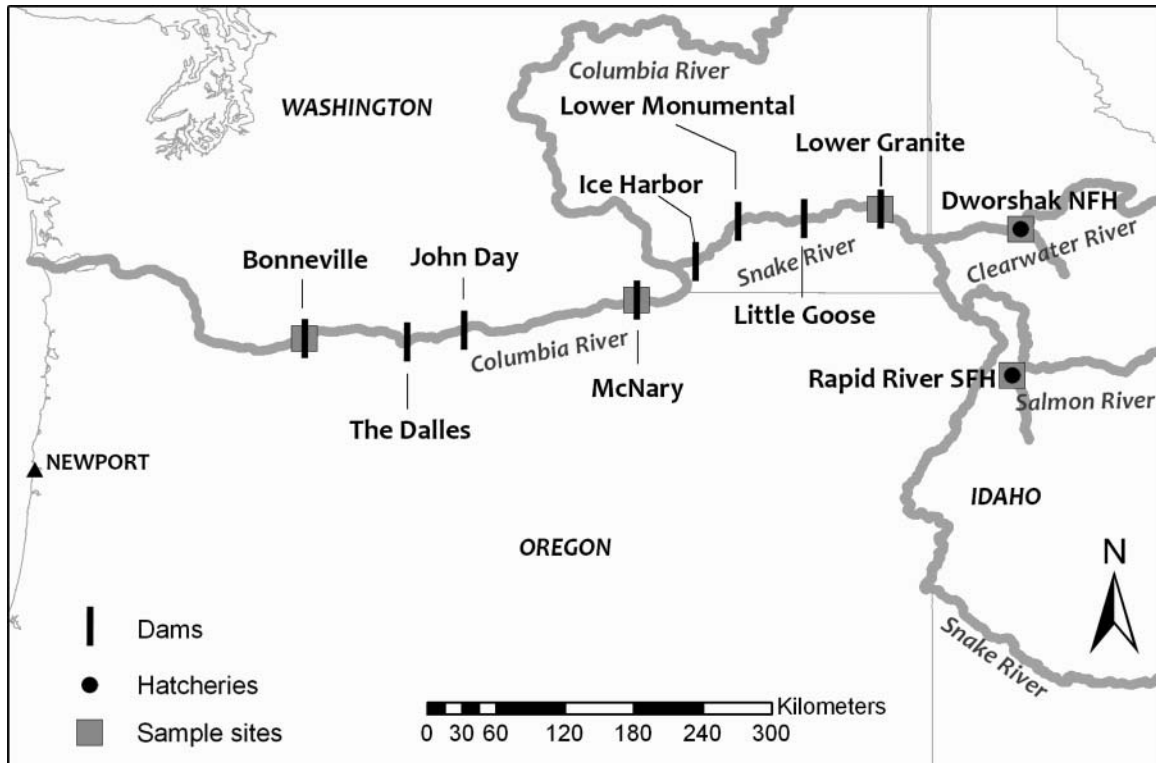


FIGURE 1. Migration corridor in the Snake and Columbia rivers and sites of collection of juvenile Chinook salmon originating from Dworshak National Fish Hatchery (NFH) and Rapid River State Fish Hatchery (SFH).

pathogens, excessive handling, or poor environmental conditions (Tiffney 1939; Meyer 1991; Mueller and Whisler 1994; Bruno and Wood 1999; Roberts 2001). However, the only study, to our knowledge, of saprolegniasis in the Columbia River basin focused on the presence and diversity of Saprolegniaceae isolates taken from salmonids, but did not report on the prevalence of infection (Mueller and Whisler 1994). Similarly, while some work has been done on isolating and defining strains of IHNV throughout the Columbia River basin (Garver et al. 2003), this is the first study to investigate the prevalence of IHNV in the hydropower system. Infectious hematopoietic necrosis virus is a serious and economically significant disease infecting salmonids throughout the Pacific Northwest, including the Columbia River basin. Leong et al. (1995) estimated that 70 million fish and eggs (an economic loss of at least US\$350 million) have been destroyed since 1981 in Columbia River hatcheries due to IHNV infection. Epizootics of IHNV are generally limited to fry and juvenile salmonids, though evidence suggests that older salmonids can act as carriers of the disease (LaPatra et al. 1989; St-Hilaire et al. 2001).

Herein we report on the first comprehensive survey of the occurrence and distribution of salmonid pathogens in out-migrating in-river and barged Snake River spring Chinook salmon from their hatchery of origin through the Snake and Columbia rivers to Bonneville Dam. A suite of 10 pathogens was surveyed in juvenile salmon kidney tissue and water sam-

ples by means of PCR. The main objectives of this study were to (1) survey and report on the pathogen prevalence and diversity in hatchery-reared spring Chinook salmon at locations along the FCRPS migration corridor and (2) compare the pathogen prevalence of juvenile Chinook salmon with different out-migration histories (barged and in-river).

METHODS

Project Area Description

The study area includes the 2007 spring Chinook salmon migration routes from Dworshak National Fish Hatchery (NFH) and Rapid River State Fish Hatchery (SFH), Idaho, to Bonneville Dam on the lower Columbia River (Figure 1). Both hatcheries are located on Snake River tributaries in Idaho. From Dworshak NFH (Clearwater River kilometer 66), the migration route is approximately 578 river kilometers (rkm) to Bonneville Dam, compared with 740 rkm from Rapid River SFH (Salmon River kilometer 151). The 460-rkm migration through the FCRPS begins at Lower Granite Dam and includes eight hydroelectric projects (Figure 1): four on the lower Snake River, including Lower Granite (Snake River kilometer (SRkm) 173), Little Goose (SRkm 113), Lower Monumental (SRkm 67), and Ice Harbor (SRkm 15.6); and four on the Columbia River, including McNary (Columbia River kilometer (CRkm) 470), John Day

(CRkm 347), The Dalles (CRkm 308), and Bonneville (CRkm 235).

Water temperatures in the Snake and Columbia rivers increased over the course of our study, ranging from 9°C in mid-April to 15°C in late May at Bonneville Dam (USACE 2010). Water temperatures in the barge hold generally mirrored the river and varied from 9°C to 14°C during transport of the “barged” treatment. Flows measured at Lower Granite Dam during our sampling period ranged from 1,274 to 2,860 m³/s (USACE 2010). Average flow at Lower Granite Dam was 1,441 m³/s during the out-migration season (defined here as 1 February to 1 June) and peak flows were between 29 April and 26 May. For comparison, the 10-year average flow at Lower Granite Dam for the years 2000 to 2010 during the defined out-migration season was 1,631 m³/s (USACE 2010).

Tagging and Field Collection

Juvenile spring Chinook salmon (referred to as “juvenile salmon” or “fish” hereafter) were implanted with passive integrated transponder (PIT) tags (BioMARK; Boise, Idaho) at Dworshak NFH ($n = 70,102$) and Rapid River SFH ($n = 69,865$) approximately 1 month before their 2007 release from the hatchery.

Hatcheries.—Juvenile salmon were sampled approximately 2 weeks before the start of release at Dworshak NFH and Rapid River SFH (Figure 1). The mean \pm SD fork length (FL) and weight of sampled fish from Dworshak NFH were 126 \pm 9 mm and 30.6 \pm 5.3 g, and from Rapid River SFH were 124 \pm 7 mm and 26.3 \pm 4.1 g. Fish were released from Dworshak NFH directly into the Clearwater River on 28 and 29 March. Fish at Rapid River SFH out-migrated voluntarily from the hatchery’s rearing pond from 15 March until 27 April when the remaining fish were forced out. Fish sampled at the hatcheries (“prerelease” fish) are considered separately from the FCRPS out-migrants because the samples were taken before the start of out-migration.

Dam sampling.—PIT-tagged fish were automatically separated from the population passing through the dams’ juvenile bypass systems with a PIT tag sort-by-code system. At Lower Granite, McNary, and Bonneville dams, a specified number of fish on each collection day were diverted from the river system into temporary holding tanks, rescanned for the presence of PIT tags, and necropsied. The majority of fish collected at Lower Granite Dam and McNary Dam were held in temporary tanks for 24–48 h, and the majority of fish collected at Bonneville Dam were held for 48–72 h before necropsy. Fish were also collected at Lower Granite Dam for barging to and subsequent sampling at Bonneville Dam.

For the purpose of this survey Lower Granite Dam is considered the beginning of the FCRPS out-migration and Bonneville Dam is considered the endpoint. Therefore, the entire group of fish sampled at Lower Granite Dam before out-migration is compared with two different groups of fish (“post-in-river”

and “postbarged”) sampled at Bonneville Dam at the end of out-migration.

In-river treatment.—The in-river treatment includes fish kidneys sampled from Lower Granite, McNary, and Bonneville dams; samples collected from these fish are referred to as Lower Granite Dam, McNary Dam, and post-in-river samples, respectively. Fish were sampled on three occasions at Lower Granite Dam on 26 April, 2 May, and 14 May 2007, and at McNary Dam on 3, 15, and 23 May 2007. Post-in-river fish were sampled on nine occasions at Bonneville Dam on the following dates in 2007: 9, 11, 12, 14, 19, 21, 22, and 25 May and 1 June. The mean \pm SD FL and weight of sampled fish at Lower Granite Dam from Dworshak NFH were 133 \pm 14 mm and 22.8 \pm 4.7 g, and from Rapid River SFH were 136 \pm 8 mm and 24.2 \pm 4.8 g. Fish sampled at McNary Dam from Dworshak NFH had a mean FL of 139 \pm 9 mm and mean weight of 24.6 \pm 5.0 g; fish from Rapid River SFH had a mean FL of 140 \pm 8 mm and mean weight of 24.7 \pm 4.6 g. Dworshak NFH fish sampled at Bonneville Dam were an average of 143 \pm 8 mm FL and weighed 25.3 \pm 5.3 g. Rapid River fish sampled at Bonneville Dam had a mean FL of 145 \pm 8 mm and mean weight of 26.2 \pm 5.1 g. Fish within the post-in-river treatment experienced one to seven bypass facilities and zero to seven spillways or turbines.

Barging and the barged treatment.—Eight barges were loaded with our study fish at Lower Granite Dam and transported to Bonneville Dam on the following dates in 2007: 21 April (barge 1), 26 April (barge 2), 2 May (barge 3), 4 May (barge 4), 6 May (barge 5), 12 May (barge 6), 14 May (barge 7), and 16 May (barge 8). After separation in the sort-by-code system at Lower Granite Dam, but before barging, PIT-tagged fish were held for a minimum period of 12 h and an average of 24–48 h in tanks supplied with flow-through river water at an approximate density of 0.5–1 fish/L. After the recovery period, study fish were loaded into 1-m \times 1-m \times 1.2-m net-pens, at a density less than 1 fish/L, that were suspended in the barge hold of a U.S. Army Corp of Engineers (USACE) 8000 series barge. The transport barges were also loaded by USACE with out-migrating run-of-the-river populations (primarily wild and hatchery steelhead *O. mykiss* and spring Chinook salmon) at Lower Granite, Little Goose, and Lower Monumental dams, with the exception of barge 1. Barge transit times from Lower Granite Dam to Bonneville Dam ranged from 32 to 44 h. Fish within the postbarged treatment experienced one dam bypass and 460 km in a barge hold shared with other out-migrating populations.

The barged treatment group includes fish sampled from Lower Granite and Bonneville dams; kidney samples collected from these fish are referred to as prebarged and postbarged samples, respectively. Prebarged fish were necropsied on 26 April, 2 May, and 14 May 2007 at Lower Granite Dam on the same day we transported fish on barges 2, 3, and 7, respectively. Prebarged fish were not sampled on any other dates or before any other barges. Postbarged fish were necropsied at Bonneville Dam at the conclusion of all eight barging events.

TABLE 1. Out-migration timing of PIT-tagged spring juvenile Chinook salmon originating from Dworshak NFH and Rapid River SFH in 2007. Passage cohorts were calculated using the entire detected population passing each dam, not just the sampled population.

Hatchery	Dam	Passage cohort		
		Early	Middle	Late
Dworshak NFH	Lower Granite	Apr 2–16	Apr 17–May 2	May 3–21
	McNary	Apr 17–May 4	May 5–13	May 14–Jun 1
	Bonneville	Apr 26–May 10	May 11–16	May 17–Jun 2
Rapid River SFH	Lower Granite	Mar 26–May 2	May 3–9	May 10–17
	McNary	Apr 20–May 8	May 9–17	May 18–26
	Bonneville	Apr 19–May 12	Apr 13–May 20	May 21–28

Temporal treatments.—Fish in the barged and in-river treatments were further subdivided based on their passage date at individual dams (Table 1). Passage cohorts were defined by the entire population of PIT-tagged juvenile Chinook salmon detected at a dam's juvenile bypass (i.e., not just our sampled population) and classified as “early,” “middle,” or “late” to represent the first 25%, middle 50%, and last 25% of the out-migrating population, respectively. Postbarged fish were binned into passage cohorts by the date collected at Lower Granite Dam. Differences in the timing of juvenile salmon release and out-migration distance resulted in different passage cohort assignments for Dworshak NFH and Rapid River SFH. Despite efforts to collect fish from all passage cohorts (i.e., early, middle, and late), the following cohorts were not collected: the early cohort of Dworshak NFH fish from Lower Granite Dam and subsequent postbarged treatment, the middle cohort of Dworshak NFH fish from McNary Dam, and the middle cohort of Rapid River SFH fish from Lower Granite Dam.

Sample Collection and Preparation

Water samples.—One 20-L grab sample of water was collected at each of the following sites: Lower Granite, McNary, and Bonneville dams, and the barge hold before and after barging on the dates listed in Table 2. The samples were taken to the Newport Research Station, Newport, Oregon, and stored at 4°C before being processed within 48 h. The samples were filtered and concentrated to an approximate volume of 70 mL using a hollow fiber ultrafiltration system, and the resulting samples were stored at –20°C before nucleic acid recovery and PCR analyses (Rajal et al. 2007b). Each water sample was spiked with a benign surrogate virus before filtration (bacteriophage PP7) to calculate recovery efficiency by plaque assay and to determine the extent of PCR inhibition by compounds present in the samples that interfere with PCR (Rajal et al. 2007a).

Juvenile salmon kidney samples.—At the time of necropsy, a 20–30-mg piece of anterior kidney necessary for DNA and RNA extraction (described in the next section) was collected in duplicate from each fish by means of sterile technique. Each

sample was placed into 96-well plates containing a nucleic acid preservative (25 mM sodium citrate, 10 mM EDTA, 700 g/L ammonium sulfate; pH of 5.2), and stored at –20°C before nucleic acid extraction.

PCR Primer Design and Selection

Nine salmonid pathogens were screened with PCR using primers from the literature described in Table 3. A family-specific PCR assay was developed for Saprolegniaceae for this study. Vector NTI (Invitrogen, Carlsbad, California) was used for sequence alignment and PCR primer design in the ITS1 region (internal transcribed spacer) of the 18S rRNA gene. GenBank accession numbers for aligned sequences included the following for *Saprolegnia parasitica*: AY455777.1, AM228725.1, GQ183896.1, FJ545238.1, AB219393.1 and the following for *S. diclina*: EU240070.1, EU240107.1. The designed PCR assay

TABLE 2. Locations and dates of collections of 20-L water samples from the Snake and Columbia rivers in 2007 and detection of *R. salmoninarum* by polymerase chain reaction (present [+] or absent [-]). Each date represents one water sample.

Sampling location	Date and detection of <i>R. salmoninarum</i>		
Lower Granite Dam			
Raceway ^a	Apr 26 (–)	May 2 (–)	May 14 (–)
Tailrace	Apr 26 (–)	May 2 (–)	May 14 (–)
McNary Dam			
Forebay	May 15 (–)	May 23 (–)	
Bonneville Dam			
Bypass ^b	May 24 (–)		
Tailrace	May 24 (–)		
Barge holds			
Loading	Apr 26 (–) ^c	May 2 (–) ^d	May 16 (–) ^e
Unloading	Apr 28 (+) ^c	May 3 (–) ^d	May 17 (–) ^e

^aSamples obtained from the raceway prior to barge loading.

^bSamples obtained from the juvenile bypass system at the sort-by-code holding tanks.

^cSamples obtained from barge 2.

^dSamples obtained from barge 3.

^eSamples obtained from barge 4.

TABLE 3. Pathogen descriptions and primer sequences utilized in polymerase chain reaction detection assays.

Pathogen Type	Target	Primer name	Primer sequence (5'–3')	Source
Gram-negative bacterium				
	<i>Aeromonas hydrophila</i>	AH1:	GAA AGG TTG ATG CCT AAT ACG TA	Dorsch et al. (1994)
		AH2:	CGT GCT GGC AAC AAA GGA CAG	
	<i>Listonella anguillarum</i>	Van-ami8:	ACA TCA TCC ATT TGT TAC	Hong et al. (2007)
		Van-ami417:	CCT TAT CAC TAT CCA AAT TG	
	<i>Flavobacterium psychrophilum</i>	FP1:	GTT AGT TGG CAT CAA CAC	Urdaci et al. (1998)
		FP2:	TCG ATC CTA CTT ACT TGC GTA G	
	<i>Yersinia ruckeri</i>	YER8:	GCG AGG AGG AAG GGT TAA GTG	Altinok et al. (2001)
		YER10:	GAA GGC ACC AAG GCA TCT CTG	
	<i>Aeromonas salmonicida</i>	PAAS1:	CGT TGG ATA TGG CTC TTC CT	Byers et al. (2002)
		PAAS2:	CTC AAA ACG GCT GCG TAC CA	
Gram-positive bacterium				
	<i>Renibacterium salmoninarum</i>	RS1:	CAA CAG GGT GGT TAT TCT GCT TTC	Powell et al. (2005)
		RS2:	CTA TAA GAG CCA CCA GCT GCA A	
Virus				
	Infectious hematopoietic necrosis virus	IHN3:	GTT CAA CTT CAA CGC CAA CAG G	Williams et al. (1999)
		IHN4:	TGA AGT ACC CCA CCC CGA GCA TCC	
	Infectious pancreatic necrosis virus	WB1:	CCG CAA CTT ACT TGA GAT CCA TTA TGC	Williams et al. (1999)
		WB2:	CGT CTG GTT CAG ATT CCA CCT GTA GTG	
	Viral hemorrhagic septicemia virus	VHS3:	CGG CCA GCT CAA CTC AGG TGT CC	Williams et al. (1999)
		VHS4:	CCA GGT CGG TCC TGA TCC ATT CTG TC	
Oomycete				
	Saprolegniaceae	SAP435:	ATT TAA AGG TAT GCC TGC GC	This study
		SAP651:	GAA TTC CCA ATT TGC CTC CA	

was tested and optimized with purified DNA from *S. parasitica* (ATCC 90213) and *S. diclina* (ATCC 90215). Owing to the lack of available sequence data at the time, the PCR assay cannot distinguish between pathogenic (*S. parasitica* and *S. diclina*) and nonpathogenic species.

Nucleic Acid Extraction

Water samples.—The DNA and RNA from a 10-mL subsample of each concentrated water sample was purified with the QIAamp Viral RNA kit (Qiagen, Valencia, California) according to Rajal et al. (2007b). The purified DNA extracts were diluted 1:2 and 1:10 in RNase-free water. The RNA was immediately converted to cDNA with the High Capacity cDNA Archive kit (Applied Biosystems, Foster City, California). Nucleic acid and the resultant dilutions were stored at -20°C before analyses by PCR.

Kidney samples.—The DNA and RNA were extracted from a maximum of 25 mg (DNA) or 30 mg (RNA) of kidney tissue in 96-well format following the manufacturers' directions for animal tissues (DNeasy 96 Blood and Tissue kit, RNeasy 96 kit; Qiagen). Four negative controls (no sample) were placed on each 96-well nucleic acid extraction plate to control for

contamination. To ensure the purity and integrity of RNA extracted from the kidney samples, 4% of the samples from each extraction were analyzed with an Agilent 2100 Bioanalyzer and the RNA 6000 Nano Total RNA kit (Agilent, Santa Clara, California). The purity and integrity of RNA was evaluated by means of the RNA integrity number (RIN) as per Schroeder et al. (2006) and was calculated with the Agilent 2100 Bioanalyzer software. Purified RNA was immediately converted to cDNA with the High Capacity cDNA Archive kit (Applied Biosystems). The DNA and cDNA were diluted 1:10 with RNase-free water. Purified nucleic acids were stored at 4°C before analyses by PCR.

PCR and RT-PCR Reaction Conditions

The water and kidney tissue samples were screened by means of similar methods for each pathogen listed in Table 3. Bacterial pathogens and Saprolegniaceae were screened with conventional PCR and viral pathogens were screened with reverse-transcriptase PCR (RT-PCR). Kidney tissues were screened in 10- μL reaction volumes on a 384-well platform; water samples were screened in 25- μL reaction volumes in 0.2-mL thin-walled tubes. Cocktails for PCR and RT-PCR contained the following:

1.5–5 mM MgCl₂, 10× Buffer II, 800 nM each primer, 800 μM deoxynucleotide triphosphates (dNTPs), 0.5 unit (U) of AmpliTaq Gold DNA polymerase (Applied Biosystems), and 3 μL DNA or cDNA for kidney tissues or 5 μL for water samples. Nucleic acids were diluted to control for PCR inhibition (Wilson 1997) and each sample was run in two separate reactions; one reaction with nondiluted DNA or cDNA and one reaction with a 1:10 dilution made with RNase-free water. Water samples were run with an additional 1:2 DNA or cDNA dilution. All PCR runs were accompanied by positive controls (genomic DNA specific to each assay), no-template controls (RNase-free water), and negative DNA extraction controls. Semiautomated high-throughput processing of the kidney tissues (only) was performed with a Biomek FX 8-channel robot (Beckman Coulter, Fullerton, California). Amplification was performed with a GeneAmp 9700 thermal cycler (Applied Biosystems) according to the referenced literature in Table 3 and optimized protocol. Amplified kidney DNA and cDNA were stored at 4°C before fragment analysis.

We optimized a conventional PCR to detect *R. salmoninarum* using primer sequences originally developed by Powell et al. (2005) for quantitative PCR. Conventional PCR was carried out in a 10-μL volume for kidney samples and a 25 μL volume for water samples under the following reaction conditions: 0.3 μM primer (each), 0.2 mM of each nucleotide, 5.0 mM MgCl₂, 1 or 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), and 3 or 5 μL template DNA. Thermal cycling was done under the following conditions: initial denaturation at 95°C for 5 min, followed by 38 rounds of amplification (denaturing at 95°C for 15 s, annealing and extension at 60°C for 1 min), and a final extension at 72°C for 7 min.

Analysis of PCR Results

Water samples.—The PCR products were visualized with ethidium bromide on 1.5% agarose gel in 0.5× tris-acetate-EDTA buffer. A water sample was considered positive if any replicate or nucleic acid dilution generated a PCR product of appropriate length in base pairs.

Kidney samples.—High-throughput fragment analyses were performed on an Applied Biosystems DNA analyzer 3730xl. Up to four fluorescently labeled PCR products were robotically added to a sequencing cocktail consisting of GeneScan size standard (Applied Biosystems) and HiDi Formamide (Applied Biosystems) on a 384-well platform and prepared for analysis according to the manufacturer's instructions. Raw data output from the DNA analyzer was imported into GeneMapper (version 3.7, Applied Biosystems) software and analyzed for the presence of length-specific peaks, which represent positive PCR products of target pathogens. A fish kidney sample was considered positive if any replicate or nucleic acid dilution generated a PCR product of appropriate length.

Statistic analysis.—Chi-square analysis was used to determine whether the prevalence of a target differed between hatchery of origin, sampling site, and passage cohort for the following:

R. salmoninarum, Saprolegniaceae, and IHNV. The remaining pathogens in this study were not statistically analyzed owing to their low prevalence. The *P*-values from all two-way and multi-way analyses were determined from the two-tailed Fisher exact test and Pearson's chi-square test, respectively (SYSTAT 12; Systat Software, Chicago, Illinois). The level of significance (α) was set at 0.05 for all analyses.

RESULTS

Pathogens in River and Barge Water

Renibacterium salmoninarum was detected in one post-barged water sample (Table 2). Saprolegniaceae was not targeted in water samples because nonpathogenic species in the family Saprolegniaceae are ubiquitous in river water. No other pathogens were detected in any water samples. The mean recovery of the seeded PP7 virus was 46 ± 8% for each sample, which falls within the range of expected recovery efficiency for ultrafiltration of 25.1% to 89.7% (Rajal et al. 2007a).

Detection of Pathogens in Fish Kidneys

All of the pathogens targeted in this study, with the exception of VHSV, were detected in at least one sampled fish (Figures 2, 3; Table 4). *Renibacterium salmoninarum* (402 positives detected out of 1,517 fish sampled, or 26.5%) and Saprolegniaceae (85 out of 1,517, or 5.6%) were the most commonly observed targets (Figure 3). Infectious hematopoietic necrosis virus was the most commonly detected virus (25 out of 1,472, or 1.7%; Figure 3). Infrequently detected bacterial and viral pathogens (0.0–1.0%) included *Aeromonas hydrophila*, *A. salmonicida*, *Flavobacterium psychrophilum*, IPNV, *Listonella anguillarum*, and *Yersinia ruckeri* (Table 4).

Pathogen Prevalence in Juvenile Salmon at the Hatcheries

Only *R. salmoninarum* and Saprolegniaceae were detected in fish sampled at Dworshak NFH and Rapid River SFH before their release into the river system (Figure 2a, b; Table 4). The *R. salmoninarum* prevalence was not significantly different between the two hatcheries (*P* = 0.075); fish sampled at Dworshak NFH had a 33.9% prevalence and those sampled at Rapid River SFH had a 29.3% prevalence (Figure 2a). Saprolegniaceae was not detected in fish from Dworshak NFH. Although the Saprolegniaceae prevalence at Rapid River SFH was 6.9%, the difference from Dworshak NFH was not significant (*P* = 0.119; Figure 2b).

Pathogen prevalence was greater in prerelease juvenile Chinook salmon than in fish collected at the first FCRPS location (Lower Granite Dam). Specifically, the prevalence of *R. salmoninarum* was greater in the prerelease fish from each hatchery than in the fish collected at Lower Granite Dam from each respective hatchery (*P* = 0.007 for both hatcheries). Furthermore, the Saprolegniaceae prevalence was greater in prerelease fish from Rapid River SFH than in postrelease Rapid River fish collected at Lower Granite Dam (*P* = 0.041).

TABLE 4. Prevalence of infrequently detected pathogens in the anterior kidneys of spring juvenile Chinook salmon from Dworshak NFH and Rapid River SFH. The values are the number of positive juvenile salmon/the total number of juvenile salmon analyzed; positives are denoted by bold italics, with percentages in parentheses. Abbreviations are as follows: IPNV = infectious pancreatic necrosis virus; VHSV = viral hemorrhagic septicemia virus.

Site	Pathogen						
	IPNV	VHSV	<i>Aeromonas hydrophila</i>	<i>Aeromonas salmonicida</i>	<i>Flavobacterium psychrophilum</i>	<i>Listonella anguillarum</i>	<i>Yersinia ruckeri</i>
Dworshak NFH							
Prerelease	0/58	0/58	0/56	0/56	0/56	0/56	0/56
Lower Granite Dam ^a	0/178	0/178	0/178	0/178	0/178	<i>1/178 (0.6)</i>	<i>1/178 (0.6)</i>
McNary Dam	<i>1/178 (0.6)</i>	0/178	0/178	0/178	0/178	<i>1/178 (0.6)</i>	0/178
Post-in-river	<i>1/236 (0.4)</i>	0/236	<i>2/235 (0.9)</i>	0/235	0/235	0/235	0/235
Postbarged	<i>1/146 (0.7)</i>	0/146	0/144	0/144	0/144	0/144	<i>1/144 (0.7)</i>
Rapid River SFH							
Prerelease	0/58	0/58	0/58	0/58	0/58	0/58	0/58
Lower Granite Dam ^a	<i>1/142 (0.7)</i>	0/142	<i>1/165 (0.6)</i>	<i>2/165 (1.2)</i>	<i>1/165 (0.6)</i>	0/165	0/165
McNary Dam	0/170	0/170	<i>1/168 (0.6)</i>	0/168	0/168	0/168	0/168
Post-in-river	0/197	0/197	0/193	<i>1/193 (0.5)</i>	0/193	0/193	<i>1/193 (0.5)</i>
Postbarged	0/144	0/144	0/142	0/142	0/142	0/142	0/142

^aThese samples are also considered prebarged.

Pathogen Prevalence in Juvenile Chinook Salmon within the River System

Co-infection.—Our results showed that 14.7% of all fish that tested positive for *R. salmoninarum* and 62.4% of the fish that tested positive for Saprolegniaceae were also infected with another pathogen. Altogether, 25% of the fish that screened positive for *A. hydrophila* or IPNV, 33.3% of the fish that tested positive for *A. salmonicida* or *Y. ruckeri*, and 44% of the fish that were positive for IHNV were also positive for *R. salmoninarum*. Similarly, 25% of the fish that screened positive for *A. hydrophila*, 33% of the fish screened positive for *Y. ruckeri*, and 28% of the fish positive for IHNV also tested positive for Saprolegniaceae. Additionally, 57.6% of those fish testing positive for Saprolegniaceae in this survey also tested positive for *R. salmoninarum*. Finally, 56% of fish positive for IHNV were also positive for *R. salmoninarum*, Saprolegniaceae, or both.

Comparison of hatchery of origin.—The prevalence of *R. salmoninarum*, Saprolegniaceae, and IHNV in fish originating from Dworshak NFH was not significantly different from that in fish originating from Rapid River SFH at any specific FCRPS sampling location (Figure 2). Given the lack of statistical difference in the prevalence of commonly detected pathogens between the hatcheries at individual sites, we pooled sample results from both hatchery populations at each location for further comparison between FCRPS locations (Figure 3). However, when fish from a given hatchery were pooled across all FCRPS sites, the Saprolegniaceae prevalence was significantly greater in fish

originating from Dworshak NFH (7.1%) than in fish originating from Rapid River SFH (4.3%; $P = 0.030$). Similarly, *R. salmoninarum* prevalence in fish originating from Dworshak NFH (27.6%) was greater than in fish originating from Rapid River SFH (24.4%), but the difference was not significant ($P = 0.181$) when fish from a given hatchery were pooled across FCRPS sites. Prevalence of IHNV was equivalent in fish originating from Dworshak NFH and Rapid River SFH when fish from a given hatchery were pooled across FCRPS locations (1.8%; data not shown).

Pathogen prevalence increases with out-migration distance.—Over the course of the out-migration season, the prevalence of *R. salmoninarum*, Saprolegniaceae, and IHNV increased with distance traveled in-river in the FCRPS. In-river fish collected at a particular FCRPS location were pooled across both hatcheries and the out-migration season. Prevalence of *R. salmoninarum* increased from 14.3% at Lower Granite Dam to 18.2% at McNary Dam (Figure 3a), although the increase was not significant ($P = 0.181$). The prevalence of *R. salmoninarum* increased significantly ($P = 0.007$) from McNary Dam to Bonneville Dam, with a prevalence of 26.4% in the post-in-river treatment. Likewise, the prevalence of Saprolegniaceae (Figure 3b) increased from Lower Granite Dam (0.9%) to McNary Dam (1.7%), although the increase was not significant ($P = 0.505$). The prevalence of Saprolegniaceae increased significantly ($P < 0.001$) from McNary Dam to Bonneville Dam, with a value of 7.7% in the post-in-river group. Finally, the prevalence of IHNV

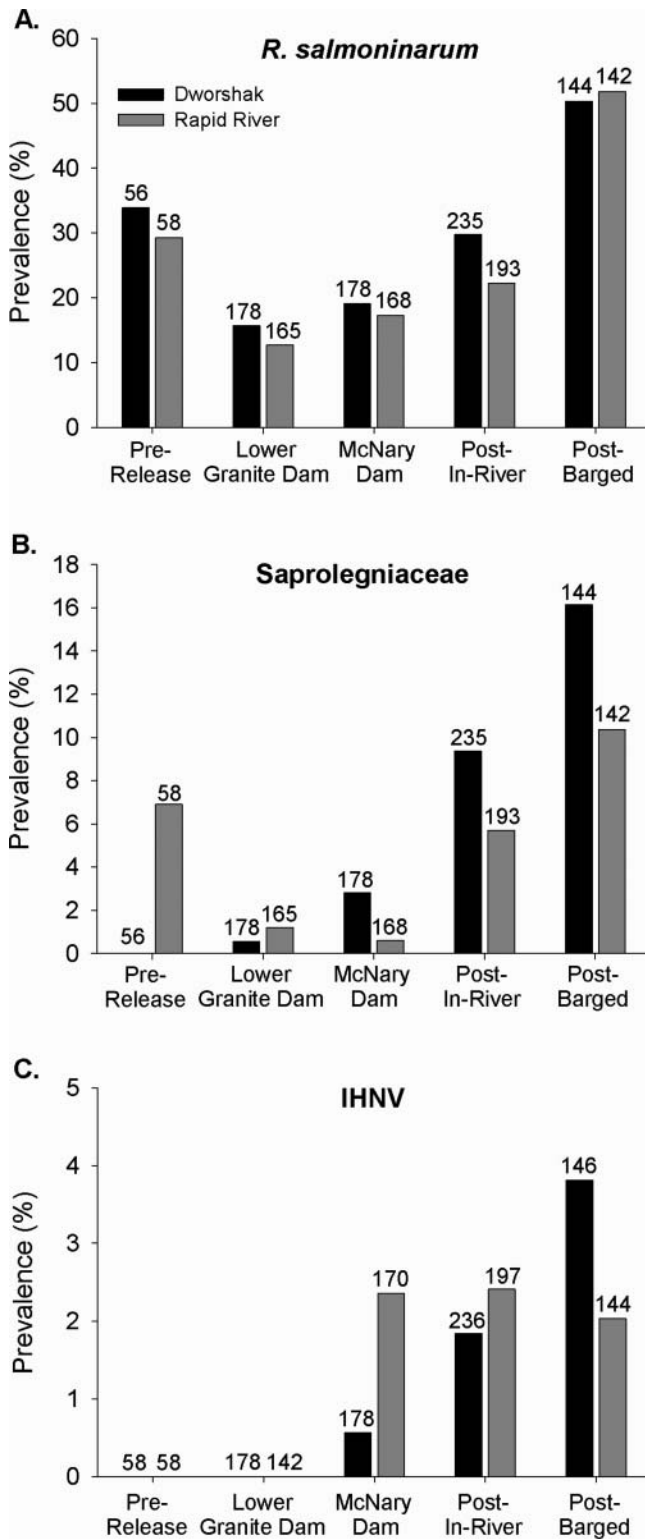


FIGURE 2. Distribution of (A) *Renibacterium salmoninarum*, (B) Saprolegniaceae, and (C) infectious hematopoietic necrosis virus (IHNV) detected in spring juvenile Chinook salmon pooled across the out-migration season. No significant differences in pathogen prevalence were detected between hatcheries. Sample sizes are indicated above the bars. Note the differences in scale of the y-axes.

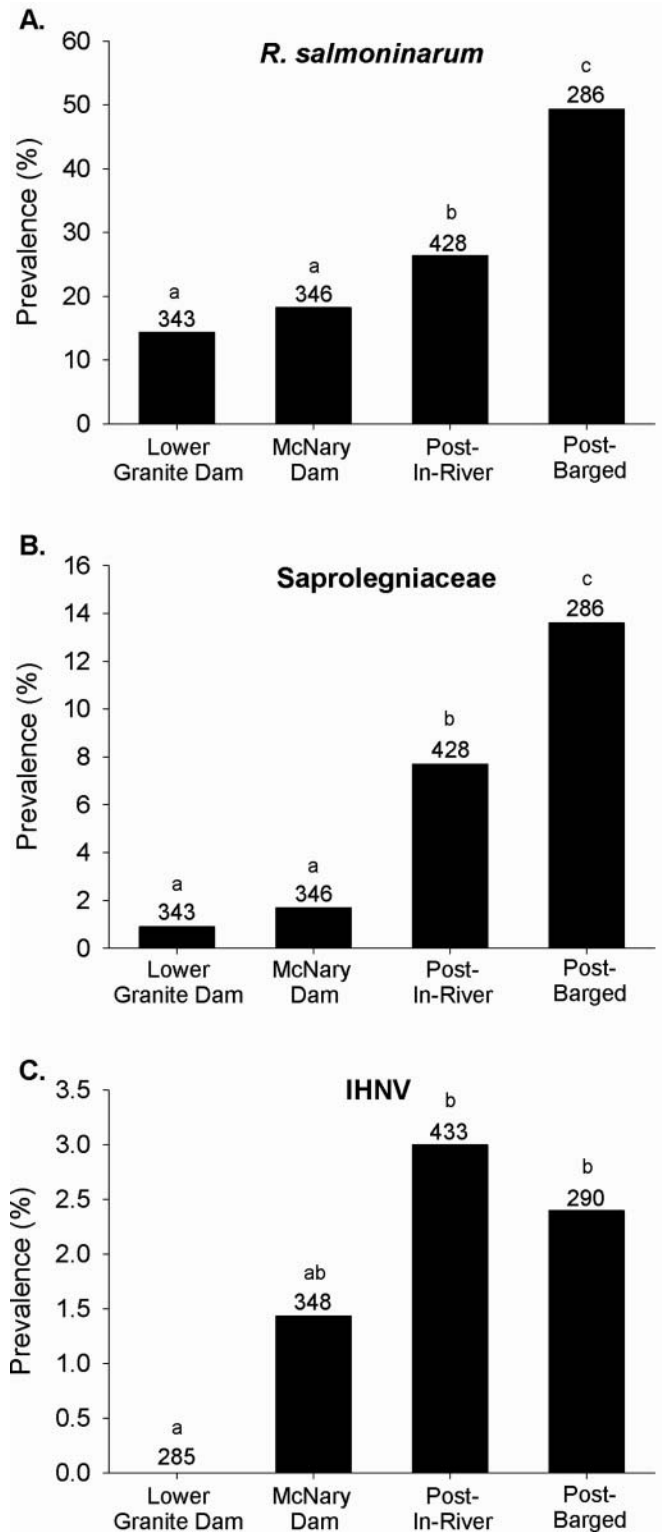


FIGURE 3. Distribution of (A) *Renibacterium salmoninarum*, (B) Saprolegniaceae, and (C) infectious hematopoietic necrosis virus (IHNV) detected in hatchery spring juvenile Chinook salmon pooled across both the hatcheries and the out-migration season. Means with different letters are significantly different (Fisher's exact test: $P < 0.05$). Sample sizes are indicated above the bars. Note the differences in scale of the y-axes.

increased from nondetectable levels in fish collected at Lower Granite Dam to 1.4% at McNary Dam ($P = 0.068$) and then to 3.0% at Bonneville Dam (in the post-in-river group; $P = 0.160$). Notably, the increase in IHNV prevalence from Lower Granite Dam to Bonneville Dam was significant ($P < 0.001$).

Pathogen prevalence increases after barging.—The prevalence of the three most common pathogens in prebarged fish pooled from barge 2, barge 3, and barge 7 ($n = 343$ for bacteria and Saprolegniaceae, $n = 285$ for viruses) was less than in pooled postbarged samples collected from all eight barging trips ($n = 286$ for bacteria, $n = 290$ for viruses; Figure 4, data not shown for viruses). *Renibacterium salmoninarum* prevalence increased more than threefold from the time of barge loading (14.3%) to the conclusion of barging (49.3%; $P < 0.001$). Similarly, the Saprolegniaceae prevalence increased over 15-fold from barge loading (0.9%) to the conclusion of barging (13.6%; $P < 0.001$). Finally, the IHNV prevalence for barged fish collected and loaded at Lower Granite Dam significantly increased from 0.0% to 2.4% by the conclusion of barging ($P = 0.015$).

The prebarged and postbarged pathogen prevalence from each individual barge varied depending on the pathogen of interest (Figure 4). The prevalence of *R. salmoninarum* increased significantly from 9.3% to 51.1% on barge 2, from 21.2% to 60.5% on barge 3, and from 11.9% to 47.4% on barge 7 ($P < 0.001$ for each of the three barges). Similarly, Saprolegniaceae prevalence increased significantly from 0.0% to 31.6% on barge 3 and from 1.7% to 15.8% on barge 7 ($P \leq 0.003$). There was no significant change ($<1\%$; $P = 1.0$) in the prevalence of Saprolegniaceae on barge 2. Infectious hematopoietic necrosis virus was not detected on any of the three barges that we sampled before barge loading. However, all seven cases of IHNV observed in postbarged fish were detected from barge 5 (16.7% of 42 fish sampled from barge 5; data not shown).

Pathogen prevalence over the out-migration season.—In general, *R. salmoninarum* prevalence decreased over time for fish sampled at a particular location in the FCRPS, while there were no statistically significant differences in Saprolegniaceae or IHNV prevalence (Table 5; data not shown for IHNV).

Fish reared at Dworshak NFH had lower *R. salmoninarum* prevalence over the out-migration season at specific river sampling locations (Table 5). The prevalence of *R. salmoninarum* at Lower Granite Dam decreased from the middle cohort to the late cohort ($P = 0.668$), though not significantly. The prevalence at Lower Granite Dam decreased from the middle cohort to the late cohort ($P = 0.668$), though not significantly. The prevalence at McNary Dam significantly decreased from the early cohort to the late cohort ($P < 0.001$). Among post-in-river fish, prevalence significantly decreased from the early cohort to that observed in the middle cohort ($P = 0.005$), and from the middle cohort to the late cohort ($P = 0.039$). Finally in the postbarged treatment, the prevalence was significantly decreased from the middle cohort to the late cohort ($P = 0.019$).

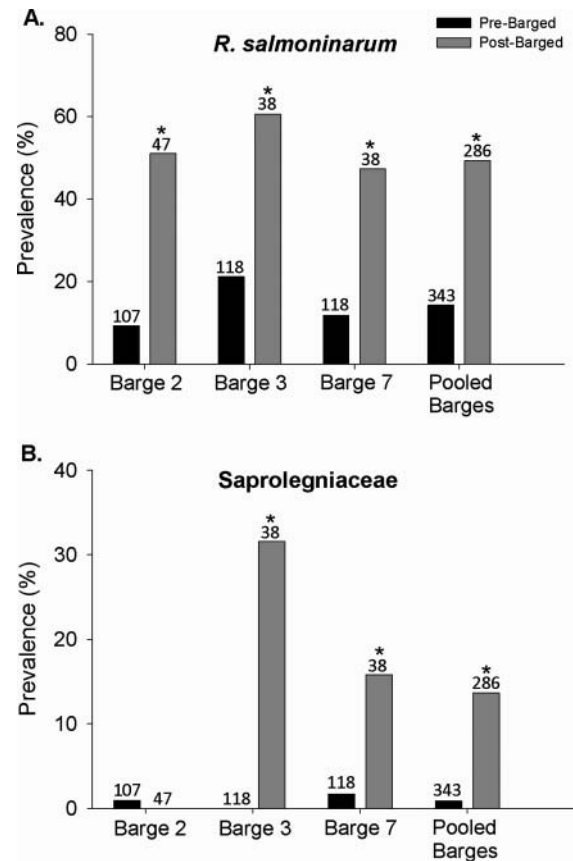


FIGURE 4. Detection of (A) *Renibacterium salmoninarum* and (B) Saprolegniaceae in hatchery spring juvenile Chinook salmon before and after barge transport. The “pooled barges” category includes the prebarged samples from barges 2, 3, and 7 and the postbarged samples from barges 1 through 8. Asterisks indicate significant differences between pre- and postbarged samples for particular barges or the pooled barges (Fisher’s exact test: $P < 0.05$). Sample sizes are indicated above the bars. Note the differences in scale of the y-axes.

Renibacterium salmoninarum prevalence in fish reared at Rapid River SFH showed the same trend as in the Dworshak NFH fish, except that the differences were only significant for samples collected at McNary and Bonneville dams (Table 5). Prevalence of *R. salmoninarum* at Lower Granite Dam was greater in the early cohort than in the late cohort ($P = 0.477$), though not significantly. There were statistically significant decreases in prevalence from the early cohort at McNary Dam to the middle ($P \leq 0.001$) and late cohorts ($P \leq 0.001$), although the middle and late cohorts were not significantly different from each other ($P = 1.0$). Additionally, the *R. salmoninarum* prevalence observed in the early and middle cohorts for the post-in-river treatment was significantly greater than that observed in the late cohort ($P \leq 0.006$), although the early and middle cohorts were not significantly different from each other ($P = 0.306$). Finally, prevalence of *R. salmoninarum* in the postbarged treatment was neither significantly different between passage cohorts nor did it follow the trend of decreasing prevalence over time.

TABLE 5. Prevalence of *R. salmoninarum* and Saprolegniaceae in the kidneys of juvenile Chinook salmon from Dworshak NFH and Rapid River SFH. The values are the number of positive juvenile salmon/the total number of juvenile salmon analyzed; positives are denoted by bold italics, with percentages in parentheses.

Hatchery	Site	Passage cohort		
		Early	Middle	Late
<i>R. salmoninarum</i>				
Dworshak NFH	Lower Granite Dam		20/120 (16.7)	8/58 (13.8)
	McNary Dam	24/60 (40.0)		10/118 (8.5)
	Post-in-river	23/35 (65.7)	19/55 (34.5)	28/145 (19.3)
	Postbarged		40/66 (60.6)	31/78 (39.7)
Rapid River SFH	Lower Granite Dam	15/105 (14.3)		6/60 (10.0)
	McNary Dam	18/49 (36.7)	6/60 (10.0)	5/59 (8.5)
	Post-in-river	6/11 (54.5)	17/49 (34.7)	20/133 (15.0)
	Postbarged	22/39 (56.4)	18/42 (42.9)	31/61 (50.8)
Saprolegniaceae				
Dworshak NFH	Lower Granite Dam		0/120	1/58 (1.7)
	McNary Dam	0/60		5/118 (4.2)
	Post-in-river	4/35 (11.4)	5/55 (9.1)	13/145 (9.0)
	Postbarged		9/66 (13.6)	15/78 (19.2)
Rapid River SFH	Lower Granite Dam	1/105 (1.0)		1/60 (1.7)
	McNary Dam	1/49 (2.0)	0/60	0/59
	Post-in-river	0/11	3/49 (6.1)	8/133 (6.0)
	Postbarged	4/39 (10.3)	7/42 (16.7)	5/61 (8.2)

Comparison of barged and in-river out-migrants.—*Renibacterium salmoninarum* prevalence was significantly greater in postbarged fish (49.3%) than in fish in the post-in-river treatment (26.4%; $P < 0.001$; Figure 3a) for fish pooled across hatcheries and the out-migration season. Similarly, Saprolegniaceae prevalence in the postbarged treatment (13.6%) was significantly greater than that observed in post-in-river out-migrants (7.7%; $P < 0.001$; Figure 3b). Contrary to the pattern seen in *R. salmoninarum* and Saprolegniaceae prevalence, the prevalence of IHNV in post-in-river fish (3.0%) was greater than that in fish from the postbarged treatment (2.4%), although the difference was not significant ($P = 0.818$).

DISCUSSION

In this first comprehensive survey of salmonid pathogens and Saprolegniaceae in out-migrating Snake River spring hatchery Chinook salmon, we found 9 of the 10 pathogen targets by PCR, as well as significant differences in the spatial and temporal distributions of those most commonly detected. *Renibacterium salmoninarum*, Saprolegniaceae, and IHNV were detected at the greatest prevalence in fish sampled. This survey also indicated a high frequency of co-infection in fish. The pathogen preva-

lence increased with distance downriver in the FCRPS among fish, irrespective of out-migration history. In contrast, pathogen prevalence decreased over time at all sampling locations as the out-migration progressed. Finally, pathogens were more common among postbarged fish than post-in-river out-migrants.

Survey Results

A greater diversity of pathogens was detected in this study than in similar published surveys. For example, Arkoosh et al. (2004) did not detect *A. salmonicida*, *F. psychrophilum*, *Y. ruckeri*, IHNV, IPNV, or VHSV in a survey of wild and hatchery coho salmon *O. kisutch* and Chinook salmon collected from the mouth of the Columbia River estuary in 1998 and 1999, although *L. anguillarum* was found in 14% of the samples pooled across years. In addition, a survey of salmonids in the much smaller Elwha River, Washington, did not detect *A. salmonicida* or *Y. ruckeri* in 660 screened fish, nor did the authors detect IHNV, VHSV, or IPNV in any of 943 fish screened (Brenkman et al. 2008). Our study is unique because of the sheer number of samples screened for pathogens (1,517 fish screened for bacterial pathogens and Saprolegniaceae and 1,472 fish screened for viruses) and the use of highly sensitive PCR assays, which may have enabled us to detect pathogens occurring in the Columbia and Snake rivers at very low levels.

Molecular techniques are faster, more sensitive, and provide higher throughput than traditional methods that are commonly used to identify fish pathogens, such as culture, serology, and histology. However, the ability of the PCR technique to detect genetic material from a pathogen that may or may not be alive or virulent raises the question of biological significance. Nevertheless, we used PCR exclusively in this study because the additional time, cost, and labor associated with traditional methods would have necessitated a trade-off in the number of fish and pathogens surveyed. Our results do not provide information on whether a fish was an asymptomatic carrier or diseased, or of the concentration of pathogens in water samples. Therefore, pathogen prevalence is not a direct measure of disease prevalence.

Our data are limited to 1 year of observation. We would expect interannual variation in the total prevalence and diversity of pathogens found in out-migrating fish owing to such factors as broodstock health, river flow and temperature, out-migration timing, and hydropower operations such as spill level, transport conditions, and transport itself. The complexity of disease dynamics in the river system emphasizes the necessity of continued monitoring of pathogens in out-migrating fish to understand the extent of seasonal and yearly variation.

Renibacterium salmoninarum.—Historical pathogen surveys indicate a high degree of interannual variability of *R. salmoninarum* prevalence in salmonids in the Columbia and Snake rivers and variation in the methodology used for detection. The PCR assay employed in this survey (modified from Powell et al. 2005) is more sensitive than the nested PCR developed by Chase and Pascho (1998; data not shown), which has been shown to be more sensitive than the enzyme-linked immunosorbent assay, or ELISA (Pascho et al. 1998). The juvenile hatchery Chinook salmon collected at Lower Granite and McNary dams during this study had considerably lower prevalences of *R. salmoninarum* (14.3% and 18.2%, respectively) than were observed in previous studies in the Columbia River that employed less sensitive methods. For example, wild and hatchery fish collected between 1988 and 1991 had *R. salmoninarum* prevalences of 86–97% at Lower Granite Dam and 92–100% at McNary Dam, as determined by ELISA (Elliott et al. 1997). Furthermore, samples collected between 1988 and 1992 of Snake River hatchery spring Chinook salmon had *R. salmoninarum* prevalences of 92% at Lower Granite Dam and 84% at McNary Dam, as tested by ELISA (Maule et al. 1996). Changes made to hatchery practices such as culling the eggs of highly infected females (Pascho et al. 1991), the segregation of eggs and juvenile salmon based on severity of the parental BKD infections, antibiotic treatment, and reduced loading densities in raceways (Maule et al. 1996; VanderKooi and Maule 1999) may be responsible for the decreased prevalence of *R. salmoninarum* detected at dams in our 2007 survey compared with surveys conducted on hatchery fish in the 1980s and 1990s.

Saprolegniaceae.—Members of the family Saprolegniaceae were the second most frequently detected PCR target in this

survey. We developed PCR primers to detect Saprolegniaceae because of the high number of fish observed with external signs of a fungal-like infection typical of saprolegniasis. However, the PCR primers used in this study cannot differentiate between pathogenic and nonpathogenic species of Saprolegniaceae. In presenting these results, we made the assumption that Saprolegniaceae species detected by PCR in the fish kidney were probably pathogenic, because pathogenic species such as *Saprolegnia parasitica* germinate and send hyphae into the kidneys and other internal organs of adult Chinook salmon (Mueller and Whisler 1994) and channel catfish *Ictalurus punctatus* (Bly et al. 1992) following experimental exposure to infective zoospores. The high prevalence of Saprolegniaceae detected in out-migrating juvenile Chinook salmon suggests that saprolegniasis is important in the Snake and Columbia rivers.

Infectious hematopoietic necrosis virus.—Infectious hematopoietic necrosis virus was the third most common target and the most common virus detected in fish collected from the Snake and Columbia rivers. The literature describes IHNV as responsible for outbreaks in salmonid fry and having significant economic impact when an outbreak occurs (e.g., Leong et al. 1995). As fish increase in age and weight they are generally less susceptible to disease and mortality due to IHNV (Bootland and Leong 1999). The relatively low prevalence of IHNV observed in this study compared with *R. salmoninarum* and Saprolegniaceae may be explained by the relatively large size (22.8–30.6 g) of our sample fish although other factors, such as yearly variation in broodstock health, may also be responsible.

Co-infections.—The majority of co-infections observed in juvenile Chinook salmon in this study were found among pathogens known to be opportunistic. For example, 62.4% of all fish positive for Saprolegniaceae were also positive for another screened pathogen. Saprolegniaceae and *A. hydrophila* are considered opportunistic and often act as secondary pathogens (Austin and Austin 1993; Bruno and Wood 1999). Ulcerative dermal necrosis (UDN) is a well-known condition that causes skin ulcers in fish, which is suspected (though never confirmed) to be caused by the secondary infection of salmonids by *Saprolegnia* spp. following an initial viral infection (Stuart and Fuller 1968).

The high number of concurrent infections observed in our study fish suggests either that pathogen susceptibility increased owing to a primary infection or that stress, injury, or both worked in combination with a primary infection to increase pathogen susceptibility. Subclinical primary infections may render a fish more susceptible to secondary infection, which can decrease fitness and result in a higher rate of mortality. In addition, St-Hilaire et al. (2001) suggest that immunosuppression from co-infections of IHNV and *R. salmoninarum* in Chinook salmon permits the proliferation of IHNV in carrier fish.

The Influence of Hatcheries

We observed greater overall prevalence of Saprolegniaceae in Dworshak NFH fish than in fish from Rapid River SFH.

Differences in the hatchery rearing environment such as disease outbreaks and resulting antibiotic treatments, or size at release, can influence disease resistance (Maule et al. 1989; Saloniuss and Iwama 1993). In addition, fish raised and released from Dworshak NFH migrated 117 rkm to reach Lower Granite Dam, taking an average of 26 d in 2007, while fish raised at Rapid River SFH migrated 280 rkm to Lower Granite Dam, which took an average of 50 d in 2007 (CSSOC and FPS 2009). Estimates made by the comparative survival study of in-river survival rates of PIT-tagged spring juvenile Chinook salmon between the hatcheries and Lower Granite Dam in 2007 were higher for fish released from Dworshak NFH (82.8%) than those released from Rapid River SFH (74.3%; CSSOC and FPS 2009). The decrease in pathogen prevalence observed between fish sampled at the hatcheries and Lower Granite Dam suggests that a proportion of the fish released from the hatcheries succumb to disease-related mortality before their arrival at Lower Granite Dam, resulting in a decreased prevalence of pathogens upon entering the FCRPS. Since fish from Rapid River SFH are in-river for twice as long as those from Dworshak NFH before reaching Lower Granite Dam, they could succumb to additional disease-related mortality during this period, consequently decreasing the pathogen prevalence of the Rapid River SFH fish throughout the FCRPS corridor.

Pathogen Prevalence Decreases Late in the Out-Migration Season

In general, the prevalence of *R. salmoninarum* was greater in fish that out-migrated early in the season than in those that out-migrated later in the season. The *R. salmoninarum* prevalence at Lower Granite Dam was not significantly different between passage cohorts, but a difference was observed when post-in-river and postbarged fish arrived at Bonneville Dam. The trend seen in the post-in-river fish may be explained by the amount of time spent in the river. Late out-migrants spend more time than early out-migrants upstream of the FCRPS. If disease-induced mortality is high in the river before fish reach the FCRPS, then we would expect fish populations that spend more time upstream to have fewer detectable pathogens as they enter the FCRPS. Alternatively, if pathogen exposure and transmission is high in the FCRPS, we may detect more pathogens in the early out-migrants because they spend approximately 4 weeks in the FCRPS, compared with 2 weeks for the late out-migrants (Muir et al. 2006). Fish from Dworshak NFH that were barged later in the season also had significantly fewer *R. salmoninarum* detections than fish barged earlier, suggesting that there would be less opportunity for pathogen transmission in later barges. Our findings support previous studies providing evidence of greater benefit to barging in the middle and late part of the out-migration season (Williams et al. 2005; Muir et al. 2006).

Out-Migration Experience May Explain Higher Pathogen Prevalence in Barged Fish

The increase in pathogen prevalence was greater in post-barged fish as they out-migrated through the FCRPS than in post-in-river fish, suggesting that differences in out-migration experience are responsible. Two mechanisms that may explain differences in pathogen prevalence are stressors and mortality rates that vary between barged and in-river out-migration histories. First, stressful conditions over the course of barging due to collection, holding, loading densities within the barge, mechanical damage, crowding, and cotransport with juvenile steelhead may have increased exposure and susceptibility to infection (NRC 1996; Budy et al. 2002; Sandford and Smith 2002). The detection of *R. salmoninarum* from the water in the barge hold and increased prevalence among fish after barging, together with the failure to detect pathogens in the river-water samples, suggests that the barge hold provided an environment for pathogen transmission. All pathogens detected in significant numbers in the postbarged treatment have the potential for waterborne transmission. *Renibacterium salmoninarum* and IHNV can be transmitted horizontally via shedding or by the ingestion of contaminated fecal material (Pilcher and Fryer 1980; Mitchum and Sherman 1981; Traxler et al. 1993; Balfry et al. 1996). Similarly, dispersal and infection by *Saprolegnia* spp. is accomplished by motile zoospores that attach to the host (Roberts 2001). Transmission of pathogens on the barges may have occurred among our experimental fish, between the run-of-the-river populations loaded onto the barges and our experimental fish, or both. In addition, the river water circulated into the barge holds during transportation may have been a source of pathogen exposure. Waterborne transmission and subsequent infection by IHNV can occur quickly, both within and between species (Mulcahy et al. 1983; Hsu et al. 1986; Wolf 1988; Traxler et al. 1993). For example, adult kokanee *O. nerka* were experimentally infected with IHNV after a 30-min low-dose immersion in IHNV (Yamamoto et al. 1989). Infectious hematopoietic necrosis virus was not detected in any barged fish before barging, but 16.7% of the sampled fish from a single barge were positive for IHNV at the end of that barging event. Although fish were not sampled upon loading of this specific barge, the evidence suggests the potential for IHNV transmission within the barged treatment. Other researchers have demonstrated, through live box experiments with brook trout *Salvelinus fontinalis*, that transmission of *R. salmoninarum* can occur under barging and raceway conditions (Elliott and Pascho 1991).

The primary stressors experienced by in-river fish are prolonged out-migration and navigation of an increasing number of dams and reservoirs within the FCRPS (NRC 1996; Budy et al. 2002). Adult return studies have shown decreasing smolt-to-adult ratios (SAR) in Snake River salmonids as the number of dams bypassed increased (Sandford and Smith 2002). However, the pathogen prevalence of in-river fish with a multiple bypass history was not significantly different from fish with a

one-bypass history in this survey (data not shown), suggesting that this phenomenon does not influence the prevalence of pathogens. Nevertheless, multiple-bypassed fish experience stress and potential mechanical injury at each dam they encounter, which could potentially lead to an increase in the severity of infections. Second, mortality during the 2- to 4-week FCRPS in-river out-migration can be up to 50% (FPS and CSSOC 2006), while mortality is generally less than 1% during the relatively short barge trip (32–44 h; Budy et al. 2002). The onset of disease and resultant mortality could have been greater in the in-river fish during their lengthy out-migration than in the barged fish, resulting in a decline of fish with detectable pathogens in the sampled post-in-river treatment.

Our results also suggest that the greater delayed mortality observed in barged fish than in in-river fish in previous studies (see Sindermann 1990; Budy et al. 2002) may be partly attributed to disease. The relatively higher pathogen prevalence observed in postbarged fish in this study may lead to higher rates of disease-related mortality upon seawater entry. The physiological and behavioral changes that ready anadromous fish for seawater entry (smoltification) can increase the severity of *R. salmoninarum* infections in juvenile salmonids (Mesa et al. 1999) and accelerate mortality in salmonids with BKD (Fryer and Sanders 1981; Banner et al. 1983). Furthermore, salmonids are particularly susceptible to saprolegniasis during smoltification (Pickering 1994). High pathogen prevalence in postbarged fish may also lead to increased predation pressure below Bonneville Dam. For example, salmon infected with *R. salmoninarum* have compromised predator avoidance capabilities and may be preferentially preyed upon by birds (Mesa et al. 1998; Schreck et al. 2006). In addition, saprolegniasis results in lethargy and loss of equilibrium, which can increase susceptibility to predation (Bruno and Wood 1999).

CONCLUSIONS

This study is the first to survey the prevalence of a diverse set of pathogens in out-migrating juvenile spring Chinook salmon in the Snake and Columbia rivers from their natal hatchery to the final dam on the Columbia River. This survey was also unique in providing an opportunity to assess spatial and temporal variability of pathogens in fish with barged and in-river out-migration histories. Our research indicates that the prevalence of pathogens in hatchery spring Chinook salmon at the conclusion of barging from Lower Granite Dam to Bonneville Dam is greater than for fish with an in-river out-migration history. Although the prevalence of pathogens increased at downstream locations as fish out-migrated in-river, the increase was greater in fish that were barged. Accordingly, transportation of out-migrating fish may increase pathogen transmission. Although the presence of a pathogen will not always result in direct mortality due to disease, the detection of pathogens associated with out-migrating fish from the Snake and Columbia rivers may affect or reflect survival. Stressors encountered during out-migration probably

diminish the ability of juvenile salmonids to resist disease, which can, in turn, potentially reduce reproductive capability, increase susceptibility to predation, reduce host competitive fitness, and cause disease-induced delayed mortality (Sindermann 1990). Furthermore, the high prevalence of certain pathogens in treatment groups with different out-migration histories provides an indication of stress and pathogen exposure within the FCRPS. Monitoring of pathogens in out-migrating fish over several years is needed to understand the extent of seasonal and yearly variation and the ultimate importance of pathogen prevalence in this system. Finally, management strategies to reduce pathogen exposure and transmission during barging may increase survival and ultimately aid in the recovery of endangered and threatened salmonids in this river system.

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