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Survey of Pathogens in Juvenile Salmon *Oncorhynchus* Spp. Migrating through Pacific Northwest Estuaries

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Abstract.—Although the adverse impact of pathogens on salmon populations in the Pacific Northwest is often discussed and recognized, little is currently known regarding the incidence and corresponding significance of delayed disease-induced mortalities. In the study reported herein, we surveyed the presence and prevalence of selected micro- and macroparasites in out-migrant juvenile coho salmon *Oncorhynchus kisutch* and Chinook salmon *O. tshawytscha* from 12 coastal estuaries in the Pacific Northwest over a 6-year period (1996–2001). The major finding of this study was the widespread occurrence of pathogens in wild salmon from Pacific Northwest estuaries. The six most prevalent pathogens infecting both juvenile Chinook and coho salmon were *Renibacterium salmoninarum*, *Nanophyetus salmincola*, an erythrocytic cytoplasmic virus (erythrocytic inclusion body syndrome or erythrocytic necrosis virus), and three gram-negative bacteria (*Listonella anguillarum*, *Yersinia ruckeri*, and *Aeromonas salmonicida*). The most prevalent pathogen in both Chinook and coho salmon was *N. salmincola*, followed by the pathogens *R. salmoninarum* and the erythrocytic cytoplasmic virus. Statistically significant differences in the prevalence of *R. salmoninarum* and *N. salmincola* were observed between Chinook and coho salmon. Based on the prevalence of pathogens observed in this study, disease appears to be a potentially significant factor governing the population

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numbers of salmon in the Pacific Northwest. Development of a detailed understanding of the principal components influencing the ecology of infectious disease will aid in the development of management and control strategies to mitigate disease in and hence further the recovery of salmon stocks listed under the Endangered Species Act.

Wild Pacific salmonids have disappeared from approximately 40% of their historical spawning ranges. Many of the remaining stocks that were once abundant have now declined precipitously (Ruckelshaus et al. 2002; Lackey 2003). Their declines have led the United States to list many evolutionarily significant units of salmonids as either endangered or threatened. Besides physical detriments related to dam passage, factors affecting population fitness include predation, gill nets, and disease (NMFS 2000). Importantly, for virtually every life history stage, disease has been identified as a potentially critical factor.

Previous studies have shown that pathogens can affect the abundance of various wild fish species. For example, the pathogen *Ichthyophonus hoferi* substantially reduced the population of Atlantic herring *Clupea harengus* in the North Sea (Patterson 1996). *Ichthyophthirius multifiliis* was responsible for significant mortalities in wild spawning sockeye salmon *Oncorhynchus nerka* in British Columbia, Canada (Traxler et al. 1998). Substantial losses of Pacific herring *C. pallasii* were associated with viral hemorrhagic septicemia virus (VHSV; Sanders et al. 1996). Finally, high mortalities in brown trout *Salmo trutta* were attributed to the pathogen *Lepeophtheirus salmonis*, commonly known as sea lice (Tully et al. 1993). Although the adverse impact of pathogens on salmon populations in the Pacific Northwest is often discussed and recognized, little is currently known regarding the incidence and corresponding significance of delayed disease-induced mortalities.

Disease is influenced by the concurrent interaction of the host and pathogen with the environment. Hedrick (1998) and Wobeser (1994) refer to this complex interaction of factors as the web of causation. Accordingly, an important first step in understanding the ecology of infectious disease in salmon populations in the Pacific Northwest is to characterize the outcome of these complex interactions. In the study reported herein, we surveyed the presence and prevalence of selected micro- and macroparasites in out-migrant juvenile coho salmon *O. kisutch* and Chinook salmon *O. tshawytscha* from 12 coastal estuaries in the Pacific Northwest over a 6-year period. We specifically focused on juveniles because of the importance of the early life stage on recruitment (Holtby et al. 1990; Beamish and Mahnken 2001; Zabel and Williams 2002). Pathogens may affect recruitment either by reducing survival directly or by causing reduced growth, impaired locomotion, delayed metamorphosis, or increased mortality from predation (Sissenwine 1984; Sindermann 1990). Also, juvenile salmon undergoing parr–smolt transformations appear to be at a greater risk of infection than other life stages (Schreck 1996).

Methods

Collection of juvenile salmon.—Juvenile coho salmon and subyearling Chinook salmon were collected from a number of Washington and Oregon estuaries from 1996 to 2001 (Table 1). The geographic location of each estuary is provided in Figure 1. Appropriate sampling permits were obtained from the National Marine Fisheries Service (NMFS), the Oregon Department of Fish and Wildlife, and the Washington Department of Fish and Wildlife prior to sampling. Due to the pattern of salmon movement in the estuaries, we generally sampled during early-morning outgoing tides. In addition, to ensure the preponderant collection of wild fish, we attempted to collect fish prior to hatchery releases or other program (e.g., the Salmon and Trout Enhancement Program) releases. Although a few fin-clipped hatchery fish were collected, we did not include these fish in analyses.

At each site, 60–120 (depending on the year) subyearling Chinook salmon and juvenile coho salmon were collected, assigned a distinctive identification number, and examined externally for parasites, lesions, and other abnormalities. Sample size was based on the assumption that we would detect the pathogen with 95% confidence if 5% of the population were infected (Thoesen 1994). Upon completion of external examination, the salmon were placed in insulated, aerated tanks and transported to the nearest laboratory for immediate necropsy. Laboratories used for necropsy included the Hatfield Marine Science Center (Newport, Oregon), the University of Oregon's Oregon Institute of Marine Biology (Charleston, Oregon), the U.S. Fish and Wildlife Service's Olympia Fish Health Center (Olympia, Washington), Point Adams Field Station (Hammond, Oregon), and the Northwest Fisheries Science Center (Seattle, Washington).

TABLE 1.—Sites and months sampled in Washington and Oregon for juvenile Chinook salmon (CH) and coho salmon (CO); NS = not sampled.

Sampling site	Year					
	1996[a]	1997[b]	1998[c]	1999[d]	2000[d]	2001[d]
Washington						
Skokomish Estuary	NS	NS	CH (May)	CH (Jun)	NS	NS
Duwamish Estuary	NS	NS	CH (May)	CH (May)	NS	NS
Nisqually Estuary	NS	NS	CH (May)	CH (May)	NS	NS
Grays Harbor	NS	NS	CH (Aug) CO (May)	CH (Jul)	NS	NS
Willapa Bay	NS	NS	CH (Aug) CO (May)	CH (Jul)	NS	NS
Columbia River	NS	NS	CH (Jun)	CH (Jun)	NS	NS
Oregon						
Salmon River	CH (Aug)	NS	NS	CH (Jul)	CH (Jun)	CH (Jul)
Yaquina Bay	NS	NS	CH (Aug) CO (May)	CH (Jul)	CH (Jun)	CH (Jun) CO (May)
Alsea Bay	CH (Aug)	CH (Jun)	CH (Aug) CO (May)	CH (Jul)	CH (Jun)	CH (Jun) CO (May)
Coos Bay	CH (Sep)	CH (Aug)	CH (Jul) CO (Jun)	CH (Aug)	NS	NS
Coquille River	CH (Sep)	NS	NS	NS	NS	NS
Elk River	CH (Sep)	CH (Sep)	CH (Sep)	NS	CH (Aug)	CH (Aug)

[a] One hundred twenty Chinook salmon were collected per site; 60 fish were used for virology and bacterial assays and 60 for the detection of parasites. An enzyme-linked immunosorbent assay (ELISA) was used to detect *Renibacterium salmoninarum*.

[b] ELISA was used to detect *R. salmoninarum* in fish (Elk River $N = 60$; Coos Bay $N = 51$; Alsea River $N = 62$). Fish with damaged digestive tracts were used to determine the prevalence and intensity of *Nanophyetus salmincola* and the presence of *Myxobolus cerebralis*, *Ceratomyxa shasta*, erythrocytic inclusion body syndrome/erythrocytic necrosis virus (EIBS/ENV), and *Bothriocephalus acheilognathi* (Elk River $N = 60$; Coos Bay $N = 20$; Alsea River $N = 70$).

[c] Kidney and spleen tissue from 3–5 fish were pooled, resulting in 12–20 pooled samples per site that were examined for *R. salmoninarum* (by ELISA), *Listonella anguillarum*, *Aeromonas salmonicida*, *Flavobacterium psychrophilum*, *Yersinia ruckeri*, infectious pancreatic necrosis virus, infectious hematopoietic necrosis virus, viral hemorrhagic septicemia virus, and *Oncorhynchus masou* virus. Individual fish were sampled for EIBS/ENV, *N. salmincola*, *C. shasta*, and *M. cerebralis*.

[d] Fish ($N = 60$ from each estuary) were examined for all targeted bacteria (except *F. psychrophilum*) as well as for EIBS/ENV and *N. salmincola*. Polymerase chain reaction was used to detect *R. salmoninarum*.

Analyses of pathogen prevalence.—The targeted pathogens of interest in this study are summarized in Table 2. Diagnostic procedures were similar to techniques described in the *National Wild Fish Health Survey* (U.S. Fish and Wildlife Service 2001), *Blue Book*: *Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens* (Thoesen 1994), and *Fish Disease: Diagnosis and Treatment* (Noga 1996). A brief description of each procedure is provided below.

Bacterial culture-based assays.—The anterior kidney section and spleen were homogenized in a 1.5-mL microcentrifuge tube by use of a disposable, 1-mm sterile plastic inoculation loop (VWR International, West Chester, Pennsylvania). The loop was streaked onto a petri plate containing either trypticase soy agar (Sigma, St. Louis, Missouri) or cytophaga agar. The plates were placed in a 25°C incubator and inspected for bacterial growth at 24 and 48 h. Bacterial isolates were screened based on a series of presumptive assays, including Gram stain, novobiocin (Becton Dickinson and Co., Cockeysville, Maryland), vibriostatic agent O/129 (2,4-diamino-6,7-di-isopropylpteridine; Sigma, St. Louis, Missouri), catalase test (Thoesen 1994), cytochrome oxidase test (BioMerieux Vitek, Inc., Hazelwood, Missouri), and secondary growth on *Listonella anguillarum* (formerly *Vibrio anguillarum*) media (Alsina et al. 1994) or thiosulfate citrate bile sucrose (Kobayashi et al. 1963). Putative isolates were confirmed with rapid diagnostic agglutination assays. *Listonella anguillarum* was confirmed by use of a Mono-Va agglutination test kit (Bionor, A/S, Skien, Norway). *Flavobacterium psychrophilum*, *Aeromonas salmonicida*, and *Yersinia ruckeri* serotypes 01 and 02 were confirmed by agglutination assay based on commercial antibodies (Microtek International, Ltd., Saanichton, British Columbia).

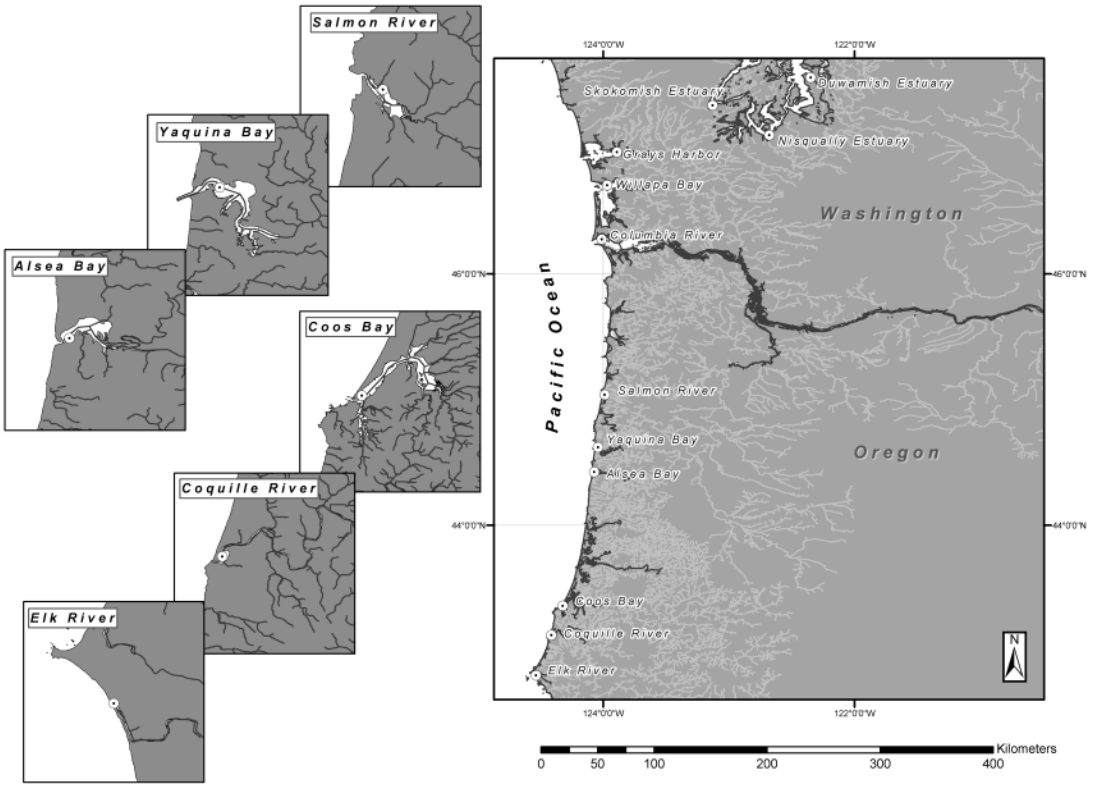

FIGURE 1.—Map showing the geographic locations of Washington and Oregon estuaries sampled to determine pathogen prevalence in juvenile Chinook and coho salmon.

Bacterial molecular-based assays.—To detect *Renibacterium salmoninarum*, we used either polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA). The anterior third of the kidney was removed aseptically from up to 30 fish per site. In the PCR-based assay, bacterial DNA was extracted and amplified by use of the nested PCR protocol described by Chase and Pascho (1998). Twenty-five microliters of each amplified DNA product, including positive and neg-

TABLE 2.—Pathogens targeted for detection in juvenile Chinook salmon and coho salmon collected from Washington and Oregon estuaries.

Target pathogen	Type of parasite
Renibacterium salmoninarum	Gram-positive bacteria
Aeromonas salmonicida	Gram-negative bacteria
Listonella anguillarum	Gram-negative bacteria
Flavobacterium psychrophilum	Gram-negative bacteria
Yersinia ruckeri	Gram-negative bacteria
Infectious hematopoietic necrosis virus	Rhabdovirus
Infectious pancreatic necrosis virus	Birnavirus
Viral hemorrhagic septicemia virus	Rhabdovirus
Oncorhynchus masou virus	Herpesvirus
Erythrocytic inclusion body syndrome/Erythrocytic necrosis virus	Togavirus/iridovirus
Nanophyetus salmincola	Digenean trematode
Sanguinicola spp.	Digenean trematode
Cryptobia salmositica	Internal protozoan (flagellate)
Ceratomyxa shasta	Internal protozoan (myxosporean)
Myxobolus cerebralis	Internal protozoan (myxosporean)
Bothriocephalus acheilognathi	Cestode

ative *R. salmoninarum* controls, and 10 μL of a PCR molecular weight marker (Promega Corp., Madison, Wisconsin) were visualized by electrophoresis in a 2% agarose gel with ethidium bromide under ultraviolet illumination. In the ELISA (Pascho and Mulcahy 1987; Pascho et al. 1987), the kidney sample was considered positive for *R. salmoninarum* if the optical density (OD) was greater than two standard deviations above the mean OD obtained with negative control tissue. The ELISA was used for samples collected between 1996 and 1998. We switched to the PCR assay in 1999 due to the small amount of kidney tissue that was available for analyses.

Viral diagnostics.—The base of the tail was severed with a scalpel blade, blood from the caudal vein was collected in a heparinized capillary tube, and a blood smear prepared on a glass slide was examined under oil immersion. Fifty fields were examined at 1,000× magnification. The sample was considered positive for erythrocytic inclusion body syndrome (EIBS) and/or erythrocytic necrosis virus (ENV) if more than two cells showed inclusion bodies in the cytoplasm. Infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), *Oncorhynchus masou* virus (OMV), and viral hemorrhagic septicemia virus (VHSV) were identified with the aid of cell culture as detailed by Thoesen (1994).

Parasitology.—The posterior half of each kidney was removed and placed in a labeled Whirlpak bag. The tissue was flattened between two glass slides and examined under a dissection microscope at 100× for the prevalence and intensity of *Nanophyetus salmincola* metacercariae. In addition, anterior intestines of salmon were screened microscopically for Asian tapeworms *Bothriocephalus acheilognathi* (Schmidt 1986). The posterior section of the intestine was prepared as a wet mount and examined for multicellular myxosporean trophozoites of *Ceratomyxa shasta* (Bartholomew et al. 1989). The pepsin–trypsin digest method with observation of typical spores was used on individual cranial cartilage to determine the presence of *Myxobolus cerebralis*, the causative agent of whirling disease (Markiw and Wolfe 1974). Wet mounts of gill tissue were examined for ova or miracidia of *Sanguinicola* spp. and the kinetoplastid flagellates of *Cryptobia salmositica*.

Statistical analyses.—Chi-square analyses were used to determine whether pathogen prevalence differed between coho and Chinook salmon ($P \leq 0.05$). The chi-square tests were performed in the StatView computer program (SAS Institute, Inc., 1998).

Results

All of the fish examined in our surveys appeared to be clinically healthy, as they did not exhibit external signs of clinical disease. However, the pathogens of interest were present at varying levels of prevalence in fish from all the sampled Oregon and Washington estuaries. The pathogens infecting both juvenile Chinook salmon (Figure 2A) and coho salmon (Figure 2B) were *N. salmincola*, *R. salmoninarum*, EIBS/ENV, and three gram-negative bacteria, *A. salmonicida*, *Y. ruckeri*, and *L. anguillarum*. The prevalence of the macroparasite *N. salmincola* was found to be greater than any of the other target pathogens in both Chinook (33–100%) and coho salmon (73–98%). Macroparasites that were surveyed in selected years but that were not detected included *C. salmositica* and *Sanguinicola* spp. in 1996, *F. psychrophilum* and *B. acheilognathi* in 1998, *C. shasta* in 1996–1998, and *M. cerebralis* in 1997–1998.

The prevalence of bacterial pathogens in both Chinook and coho salmon was much lower than the prevalence of *N. salmincola*, although prevalence levels were still deemed significant. The most prevalent microparasite in Chinook salmon and coho salmon was *R. salmoninarum*. The prevalence of *R. salmoninarum* in Chinook salmon ranged from 3% to 93% in fish examined by PCR (1999–2001) and from 0% to 71% in fish examined by ELISA (1996–1998) (Table 3). Coho salmon were examined for the presence of *R. salmoninarum* by ELISA in 1998 and by PCR in 2001. Prevalences ranged from 0% to 14% in 1998 and from 0% to 28% in 2001 (Table 4). The majority of fish infected with *R. salmoninarum* had low quantitative numbers based on low ELISA OD readings. We were not able to determine the intensity (e.g., semiquantitative number) of the pathogen in later years because the PCR technique we employed was not quantitative. The prevalence of *L. anguillarum* ranged from 0% to 31% in Chinook salmon (Table 3) and from 0% to 15% in coho salmon (Table 4). The ranges of prevalence for *A. salmonicida* and *Y. ruckeri* were much lower. The prevalence of *A. salmonicida* in Chinook salmon ranged from 0% to 5%; prevalence in coho salmon was 0–2%. The prevalence of *Y. ruckeri* ranged from 0% to 8% in Chinook salmon and from 0% to 2% in coho salmon.

The only virus detected throughout the study was one that produced cytoplasmic inclusion bod-

TABLE 3.—Percent pathogen prevalence in Chinook salmon collected from Washington and Oregon estuaries. See footnotes in Table 1 for sample sizes. Site codes are as follows: Duwamish (DUW), Nisqually (NIS), Skokomish (SKO), Grays Harbor (GRA), Willapa Bay (WIL), Columbia River (COL), Salmon River (SAL), Yaquina Bay (YAQ), Alsea Bay (ALS), Coos Bay (COO), Coquille River (COQ), and Elk River (ELK).

Pathogen and year	DUW	NIS	SKO	GRA	WIL	COL	SAL	YAQ	ALS	COO	COQ	ELK
Listonella anguillarum												
1996									0	0	0	0
1997												
1998	25	0	0	0	0	0		0	0	0		0
1999	18	17	9	12	25	18	5	7	10	13		
2000							17	19	20			31
2001							12	15	19			22
Aeromonas salmonicida												
1996							0		0	0	0	0
1997												
1998	0	0	0	0	0	0		0	0	0		0
1999	0	0	0	0	0	0	0	0	3	0		
2000							0	2	2			5
2001							5	0	0			2
Renibacterium salmoninarum												
1996							6		7	16	68	24
1997									44	71		33
1998	0	0	0	22	67	0		0	0	0		0
1999	13	13	17	7	10	93	41	10	23	10		
2000							33	21	30			21
2001							3	29	14			17
Yersinia ruckeri												
1996							0		0	0	0	0
1997												
1998	8	0	0	0	0	0		0	0	0		0
1999	0	0	0	0	0	0	2	0	0	0		
2000							2	0	4			3
2001							5	2	2			0
Nanophyetus salmincola												
1996							100		100	100	96	70
1997									100	95		80
1998	90	63	58	84	73	77		97	97	95		55
1999	60	47	80	83	77	33	93	100	100	86		
2000							66	100	100			69
2001							100	77	79			63
Erythrocytic inclusion body syndrome/erythrocytic necrosis virus												
1996							8		23	11	13	0
1997									11	5		0
1998	31	19	12	7	22	29			15	12		17
1999	16	9	13	20	10	5	5	0	2	2		
2000							5	2	2			10
2001							0	10	2			7

ies in peripheral red blood cells. The prevalence of the erythrocytic cytoplasmic virus ranged from 0% to 31% in Chinook salmon and from 8% to 28% in coho salmon during the study (Tables 3, 4). In 1996 and 1998, we also surveyed for but did not detect IHNV, IPNV, OMV, and VHSV.

Although coho and Chinook salmon were infected by similar parasites, we observed some species differences in parasite prevalence. First, we found that Chinook salmon generally had a greater prevalence of the pathogen *R. salmoninarum* (0–93%) than did coho salmon (0–28%). Chi-square *P*-values addressing the likelihood that this observed difference in prevalence occurred by chance alone were equal to or less than 0.0001 in three (Grays Harbor, Willapa Bay, and Yaquina Bay) of the four sites with nonzero values of prevalence where both species were sampled. Contrary to this trend, salmon collected in 2001 from one sample site, Alsea Bay, had a statistically greater

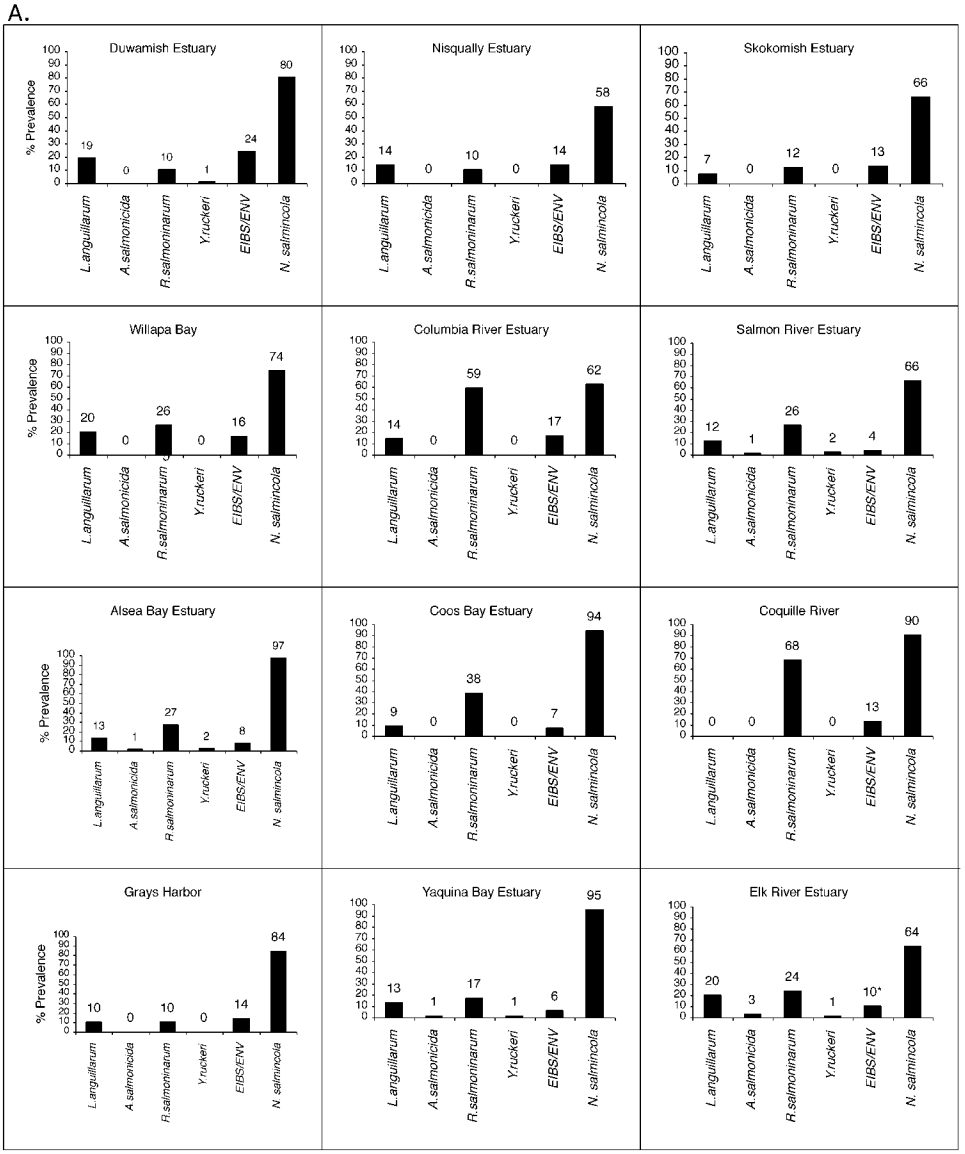

FIGURE 2.—The average prevalence of selected pathogens in **(A)** chinook salmon and **(B)** coho salmon sampled in Washington and Oregon estuaries from 1996 to 2001. The pathogens include *Listonella anguillarum*, *Aeromonas salmonicida*, *Renibacterium salmoninarum*, *Yersinia ruckeri*, erythrocytic inclusion body syndrome/erythrocytic necrosis virus (EIBS/ENV), and *Nanophyetus salmincola*. The number at the top of each bar represents the percent prevalence of the corresponding pathogen.

($P < 0.0001$) prevalence of *R. salmoninarum* in coho salmon (28%) than in Chinook salmon (14%). Second, Chinook salmon generally had a greater prevalence of *L. anguillarum* (0–31%) than did coho salmon (0–15%), although this difference was not statistically significant. Third, we found that coho salmon had a significantly greater ($P < 0.0001$) prevalence of *N. salmincola* (73–98%) than did Chinook salmon (33–100%) in five out of the seven sites sampled for both species; this trend was reversed at the remaining two sites.

Discussion

The major finding of this study was the widespread occurrence of pathogens in wild salmon from Pacific Northwest estuaries. The six most

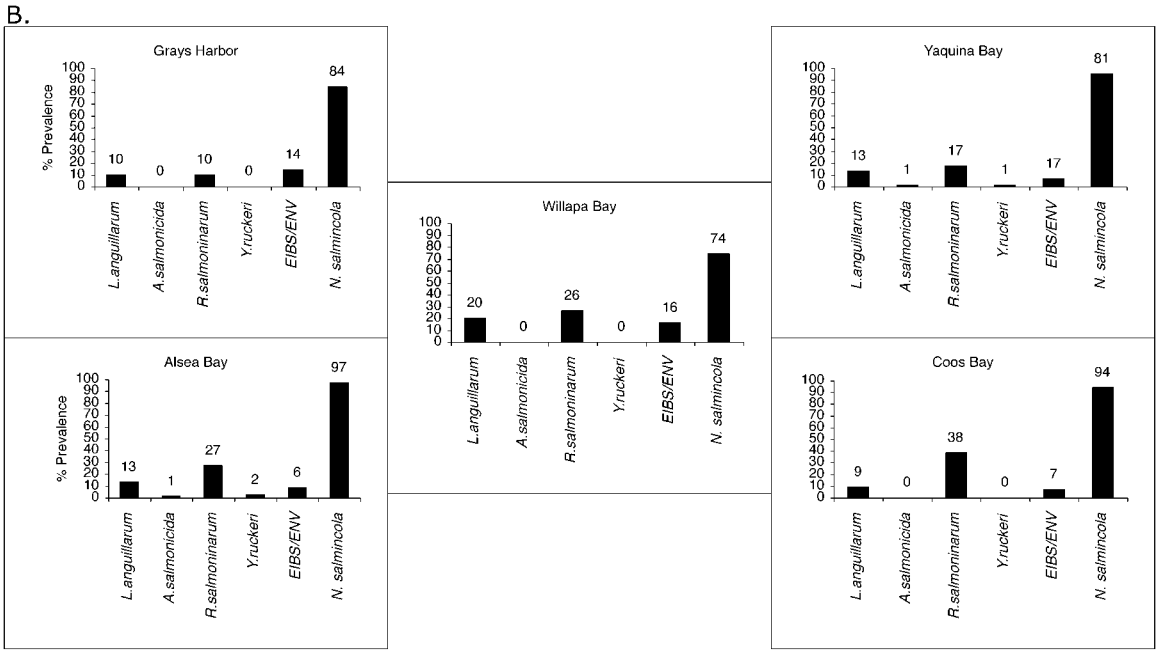

FIGURE 2.—Continued.

TABLE 4.—Percent pathogen prevalence in coho salmon collected from Washington and Oregon estuaries. See legend in Table 1 for sample size. Site codes are as follows: Grays Harbor (GRA), Willapa Bay (WIL), Yaquina Bay (YAQ), Alsea Bay (ALS), and Coos Bay (COO).

Pathogen and year	GRA	WIL	YAQ	ALS	COO
Listonella anguillarum					
1998	0	0	0	0	0
2001			15	7	
Aeromonas salmonicida					
1998	0	0	0	0	0
2001			2	0	
Renibacterium salmonicida					
1998	6	14	0	0	0
2001			0	28	
Yersinia ruckeri					
1998	0	0	0	0	0
2001			2	1	
Nanophyetus salmincola					
1998	96	98	73	88	93
2001			97	88	
Erythrocytic inclusion body syndrome/ erythrocytic necrosis virus					
1998	18	12	28	15	8
2001			7	1	

prevalent pathogens infecting both juvenile Chinook salmon and coho salmon from coastal rivers during the study period were *R. salmoninarum*, *N. salmincola*, an erythrocytic cytoplasmic virus (EIBS/ENV), and three gram-negative bacteria (*L. anguillarum*, *Y. ruckeri*, and *A. salmonicida*). The most prevalent pathogen in both Chinook salmon and coho salmon was *N. salmincola*, followed by the pathogens *R. salmoninarum* and the erythrocytic cytoplasmic virus.

Accordingly, disease is probably a significant factor in the modulation of population dynamics, particularly in listed stocks, which by definition have low numbers of fish. As an example, *N. salmincola* is a digenean trematode that uses salmonids as a secondary intermediate host. The metacercarial cyst stage of the parasite can cause exopthalmia, erratic swimming, blood vessel blockage, kidney damage, and mortality in salmon fry (Baldwin et al. 1967). Weiseth et al. (1974) estimated prevalence of *N. salmincola* in adult ocean-caught salmon off the Oregon coast at 31% in Chinook salmon ($N = 542$) and 53% in coho salmon ($N = 2{,}049$). In the present study, we observed even higher prevalence of this parasite in juveniles of both coho (73–98%) and Chinook salmon (33–100%) in Oregon and Washington estuaries, suggesting disease-induced mortality associated with this parasite during ocean residence.

Alternatively, *R. salmoninarum*, the causative agent of bacterial kidney disease, which is a chronic and often lethal disease in salmon (Evelyn et al. 1973; Fryer and Lannan 1993), appears to persist when fish move out of the estuary into the ocean. Two studies have examined the prevalence of *R. salmoninarum* in ocean-caught salmon. Banner et al. (1986) sampled juvenile Chinook salmon from the Pacific Ocean just off the coast of Oregon and Washington and found an 11% incidence of infection based on ELISA. Kent et al. (1998) found *R. salmoninarum* incidences of 58% in Chinook salmon, 42% in coho salmon, and 6% in sockeye salmon. The values reported in these other studies are similar to values obtained in our study. Banner et al. (1986) suggested that since the disease is chronic and salt water does not appear to eliminate pathogen prevalence, the pathogen likely persists throughout the life of the fish. Therefore, the possibility exists that fish infected with this pathogen will return to spawn and infect new generations through vertical transmission (Evelyn et al. 1986).

Specific health outcomes associated with the endemic levels of infection observed in this study are difficult to anticipate given the significance of coupled environmental interactions in modulation between carrier, chronic, and acute states. *Listonella anguillarum*, the bacterial agent of vibriosis, is a marine pathogen that can cause mortality at significant rates. The severity of the disease, ranging from acute to chronic, is highly dependent upon environmental stress (Noga 1996); up to a 90% incidence of mortality was documented in a saltwater environment (Cisar and Fryer 1969). Although low mortality is associated with ENV, the causative agent of viral erythrocytic necrosis, stress has been documented to activate latent infections (Smail 1982). In addition, latent infections of ENV can synergistically increase susceptibility to other diseases. For example, chum salmon *O. keta* infected with ENV have a greater mortality from vibriosis and less tolerance to oxygen depletion (Evelyn and Traxler 1978; MacMillan et al. 1980). Direct mortality due to the EIBS virus is low, but the ability of the infected fish to survive is compromised due to anemia and increased susceptibility to secondary infections caused by either bacterial or fungal pathogens (Arakawa et al. 1989). The disease appears to be greatly influenced by temperature; more outbreaks occur during the cold winter months (Piacentini et al. 1989). Finally, both *A. salmonicida*, the causative agent of furunculosis, and *Y. ruckeri*, the causative agent of enteric red mouth, can occur in acute, chronic, and carrier forms. The severity of both diseases increases as the quality of the environment decreases (Rucker 1966; Busch and Lingg 1975).

Differences in the prevalence of *N. salmincola* and *L. anguillarum* in Chinook salmon (33–100% and 0–31%, respectively) and coho salmon (73–98% and 0–15%, respectively) may be attributed to a genetic predisposition, as with *R. salmoninarum* (Kaattari et al. 1988; Starliper et al. 1997), but may also reflect preferential habitat use (freshwater and estuarine). Chinook salmon spend more time in the estuary as juveniles than the other salmon species (Healey 1982) and, as such, have a longer time and higher likelihood of exposure to *L. anguillarum*, a marine pathogen. Winter et al. (1980) speculated that expression of acute diseases like vibriosis depends more on the host's environment at the time of exposure than on genotype. Conversely, juvenile coho salmon typically stay in small streams until their second year of life, thus increasing the time and likelihood of exposure to *N. salmincola* (a freshwater pathogen) prior to their rapid migration to sea (Lichatowich 1999). Differences in the prevalence of these two pathogens in salmon with different early life histories highlight the potential importance of spatial and temporal variations in pathogen prevalence and habitat use in understanding the ecology of infectious disease.

Based on the prevalence of pathogens observed in this study, disease appears to be a potentially significant factor governing the population numbers of salmon in the Pacific Northwest. Development of a detailed understanding of the principal components of the web of causation influencing the ecology of infectious disease will aid in the development of management and control strategies to mitigate disease in, and hence recovery of, listed salmon stocks. This goal is admittedly complicated given that disease associated with pathogens not only directly affects host survival through disease-induced mortality, but also indirectly reduces reproductive potential, increases susceptibility to predation, and reduces host competitive fitness (Sindermann 1990). Immediate areas of focus in developing a more detailed understanding of the ecology of infectious disease include (1) coupled environmental interactions that increase host susceptibility (Arkoosh et al. 1991, 1994, 1998, 2001), (2) spatial and temporal variations in pathogen reservoirs in the environment, and (3) specific habitat use in both the estuarine and riverine environment.

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