Establishing Baseline Information on the Geographic Distribution of Fish Pathogens Endemic in Pacific Salmonids Prior to Dam Removal and Subsequent Recolonization by Anadromous Fish in the Elwha River, Washington

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Abstract

After the removal of two dams, wild, natural, and hatchery produced fish are expected to recolonize historic habitats in the Elwha River. Fish populations previously isolated by the dams will interact, and potentially transmit pathogens. Geomorphic changes caused by dam removal could disrupt the balance between host and pathogen, resulting in pathogen transmission and amplification, potentially leading to disease. We reviewed historic stocking records and conducted an initial survey to better understand the distribution of salmonid pathogens in the Elwha River before dam removal. Review of hatchery plantings revealed that seven salmonid species were released throughout the Elwha River Basin since 1914. Approximately 61 million Chinook salmon (*Oncorhynchus tshawytscha*) and 40 million chum salmon (*O. keta*), coho salmon (*O. kisutch*), and steelhead trout (*O. mykiss*) were released below Elwha Dam from various stock origins. Additionally, 19 million salmonids were planted above Elwha Dam beginning in 1930. From 2003 to 2006, five salmonid species from the lower, middle, and upper Elwha River and tributaries were tested for bacteria (n=684), viruses (n=943), and were screened for *Myxobolus cerebralis* (n=740). *Renibacterium salmoninarum* was the only target pathogen found, and was detected in five salmonid species in each segment of the river. In Elwha hatcheries, erythrocytic inclusion body syndrome, *R. salmoninarum*, and *Flavobacterium psychrophilum* were most commonly detected. Information from baseline surveys in the Elwha River highlight the benefit of including fish pathogen distribution as an important factor in risk assessment for future dam removals.

Introduction

There are over 75,000 dams present in rivers of the United States (Graf 1999), and over 500 dams have been removed in the last two decades (Stanley and Doyle 2003). Salmonid populations can be negatively impacted by hydroelectric dams. These impacts include, but are not limited to, decreased spawning and juvenile rearing habitats, altered river flows, injury to fish that pass through the turbines, and elevated water temperatures in regulated sections of the river (Gregory et al. 2002). Even with such impacts, many authors have warned against removal of dams without consideration of the full range of ecological consequences (Bednarek 2001, Babbitt 2002, Hart et al. 2002). One study examined the risk of allowing recolonization of anadromous fish in Skaha Lake, British Columbia, Canada, and concluded that perhaps the greatest risk of recolonization would arise from introduction of a pathogen to which the resident fish had not been previously exposed (Hammel et al. 2000).

The removal of two dams in the Elwha River will re-establish seven anadromous salmonid species that are expected to recolonize historic habitats throughout the watershed (Pess et al. 2008). The recolonization of the watershed is expected to increase both salmonid carrying capacity and competitive interactions between anadromous and potamodromous forms (e.g., fish that migrate entirely within freshwater) that have been physically isolated over the last 95 years (Brenkman et al. 2008, Pess et al. 2008).

Despite many potential benefits of dam removal, the interaction between potamodromous

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fish upstream of the dams and recolonizing anadromous salmonids poses risks from a fish health perspective. Fish populations in the Elwha River can potentially be exposed to a greater number of a given pathogen or to a pathogen to which the population has not been previously exposed. This exposure may result in transmission and amplification of pathogens which can lead to disease. In addition to changes associated with salmonid recolonization, changes in river hydrology and geomorphology may shift the equilibrium between host and pathogen. Many factors affect this balance in addition to environmental conditions, such as stresses on the host and virulence of the pathogens. If the balance shifts in favor of the pathogen, then disease can occur, and possibly become a limiting factor at the population level (Hudson et al. 1998). If a goal of dam removal is restoration of self-sustaining populations of salmonids, then a comprehensive monitoring program should include assessments of important factors that could potentially limit population size. Although it is unlikely that fish diseases would be the only factor in a dam removal decision or that might prevent successful restoration, it could be an important consideration when restoration of migratory or federally threatened fish populations is a goal.

A rare opportunity exists to establish baseline information on the presence of fish pathogens above and below the Elwha River dams prior to their removal. These baseline data will allow for the comparison of pathogen distribution prior to and after co-mingling among wild, natural (descendents of hatchery fish that are naturally reproducing), and hatchery-origin Pacific salmonids. With the knowledge that stocking of hatchery fish has occurred above the dams, we summarized information on fish planted in the watershed. The repeated exposure to anadromous fish and their pathogens from the lower Elwha may influence pathogen distribution above the dams. The purpose of this paper is to: 1) summarize the historic spatial and temporal hatchery plantings of anadromous and non-anadromous salmonids; and 2) report the results of a survey for target fish pathogens in native and non-native salmonids in the upper, middle, and lower Elwha River Basin.

Study Area

The salmonid community in the Elwha River Basin is comprised of wild, natural, hatchery, and non-native fish. In the lower river, hatchery programs currently supplement the limited populations of coho salmon (*Oncorhynchus kisutch*), federally listed Chinook salmon (*O. tshawytscha*), and federally listed steelhead trout (*O. mykiss*). Pink salmon (O. *gorbuscha*), chum salmon (*O. keta)*, summer steelhead (*O. mykiss*), federally listed bull trout (*Salvelinus confluentus)*, and cutthroat trout (*O. clarkii)* also inhabit the lower river. Sockeye salmon (*O. nerka)* are considered extirpated in the basin. In the middle river between the dams, populations of federally listed bull trout, rainbow trout, and non-native brook trout (*S. fontinalis)* exist and kokanee salmon (*O. nerka)* inhabit Lake Sutherland. In the river upstream of the dams, there are potamodromous forms of bull trout, rainbow trout, and cutthroat trout (Brenkman et al. 2008). Brook trout are known to inhabit some of the high lakes in the basin.

There are a total of 49 named tributaries of the Elwha River and two reservoirs, Lake Aldwell and Lake Mills (Figure 1). Upstream of Elwha Dam, Lake Sutherland drains into Indian Creek and ultimately into the Elwha River. Below the dams, there are two hatcheries on the Elwha River administered by Washington Department of Fish and Wildlife (WDFW) and Lower Elwha Klallam Tribe (LEKT) that have operated since 1976 and 1978, respectively (Figure 1).

Methods

Historic Hatchery Plantings

To estimate the relative magnitude of hatchery releases in areas above, between, and below the Elwha River dams over the last century, we summarized readily available information on plantings of salmonid species from Schoeneman and Junge Jr. (1954), Johnson (1994), Johnson (1997), James River (1988a), and unpublished records from Olympic National Park (ONP), LEKT, and WDFW. Our findings are not a precise summary of historical hatchery releases because the records were incomplete, disparate, or difficult to verify. The health status of the planted fish was similarly limited.

Fish Pathogen Distribution Survey

We summarized results of pathogen surveys of Chinook salmon, coho salmon, and steelhead trout from State and Tribal hatcheries in the lower river

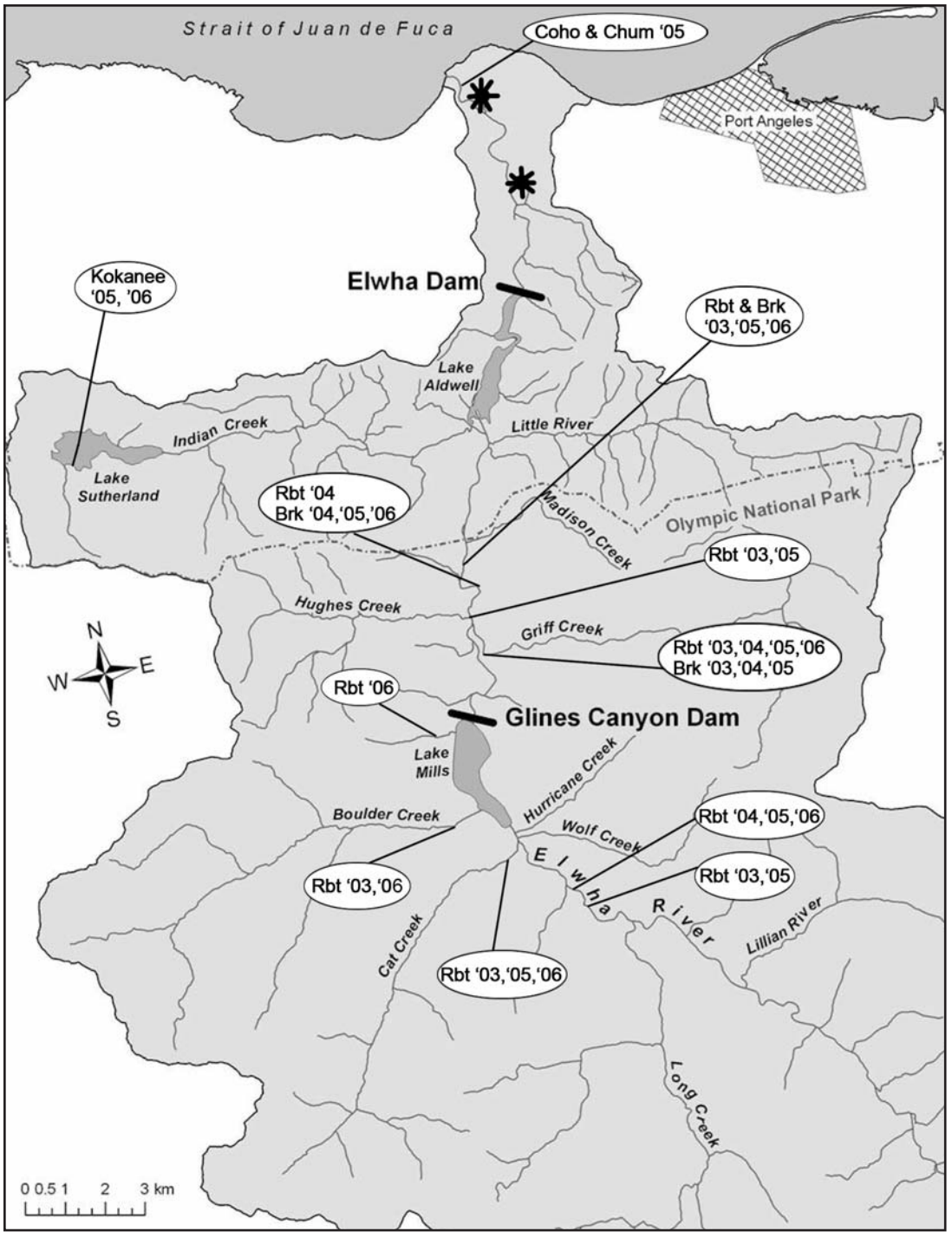

Figure 1. Map that depicts collection sites for rainbow trout (Rbt), brook trout (Brk), kokanee salmon, coho salmon, and chum salmon in the Elwha River Basin. Location of hatcheries is depicted by stars.

from 1988 to present. We also sampled salmonids and tested for the presence of bacteria, viruses, and for *Myxobolus cerebralis* in the Elwha River and numerous tributaries between and above the dams from July to August, 2003 to 2006 (Figure 1). The primary emphasis of this study was to collect fish from areas located downstream of the lower dam, between the two dams, and immediately upstream from Glines Canyon Dam (Figure 1).

Rainbow trout were collected from 10 sites in the middle and upper sections of the river and non-native brook trout were collected from three sites in the middle river by backpack electrofishing (Figure 1). In 2005, coho salmon and chum salmon were collected from the river below the Elwha Dam from a rotary screw trap (Figure 1). Bull trout were not collected due to their designation as a federally listed species under the Endangered Species Act. Three electrofishing crew members proceeded in an upstream direction electrofishing in creeks and the main stem river. Although block nets can increase efficiency under certain conditions, they were not used due to logistical constraints. For sample sites in the main stem Elwha River, electrofishing was limited to wadeable channel margins and side channel areas. Upon capture, juvenile and adult rainbow trout and brook trout were held in aerated five gallon buckets prior to necropsy. At each sample site, fish were euthanized by an overdose (> 250 ppm) of tricaine methanesulfonate, Tricaine-S (Western Chemical, Ferndale, WA). Depending on fish length, fish were sampled for viruses, bacteria, and/or parasites (Table 1).

Laboratory analysis was conducted to determine presence of infectious pancreatic necrosis virus (IPNV), infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), *Aeromonas salmonicida* (the causative agent of furunculosis), *Yersinia ruckeri* (the causative agent of red mouth disease), *Renibacterium salmoninarum* (the causative agent of bacterial kidney disease [BKD]), and *Myxobolus cerebralis* (the causative agent of whirling disease). At each site, samples from up to five fish of the same species were pooled. For more detailed information regarding the laboratory procedures, please refer to the National Wild Fish Health Survey (NWFHS) Laboratory Procedures Manual (Puzach 2006). The polymerase chain reaction (PCR) and real-time quantitative polymerase chain reaction (qPCR) protocols used in this study were based on those reported in Chase et al. (2006). For both the PCR and qPCR, the positive control was kidney tissue from a clinically diseased Spring Chinook salmon.

Virology

Depending on fish size, either whole body, viscera, or kidney and spleen were collected and processed (Table 1). Processed samples were plated on *epithelioma papulosum cyprini* (EPC) and Chinook salmon embryo (CHSE-214) cells incubated at 15° C and monitored for at least 18 days.

Bacteriology

Depending on fish size, samples for easily culturable bacteria or an enzyme linked immunosorbent assay (ELISA) sample for *R. salmoninarum* or both were taken. Samples for culturable bacteria were plated on brain heart infusion agar. The samples were either taken with an inoculation loop directly from the kidney, or from a homogenate of kidney and spleen. If bacteria were isolated, they were further characterized to determine whether they were target pathogens. Because *R. salmoninarum* is not easily cultured, an ELISA was used as a screening method. The ELISA assays were considered positive if the signal was two standard deviations above a negative control. Of

TABLE 1. Tissues used for testing virology, bacteriology, and parasitology in salmonids as dependent on fork length (mm).

Fish Length (mm)	Virology	Bacteriology	Parasitology
<40 mm	Whole Body	Not Tested	Not Tested
>40 mm, but <100 mm	Viscera	Kidney With or Without Spleen[1]	Head
>100mm	Kidney & Spleen	Kidney & Spleen[1] Kidney	Head

[1]Kidney tissue was used for ELISA. When bacterial culture was utilized kidney and spleen or kidney only were used.

the positive ELISA results for each species and site, three samples from each group were chosen to be confirmed by a PCR. In 2006, for all species except kokanee salmon, samples from the same species in the same location were combined for up to five fish per pool. A homogenate of kidney and spleen was used instead of kidney only.

Parasitology

The only parasite that was routinely screened for was *M. cerebralis*. The samples were tested by the pepsin trypsin digest method.

Results

Summary of Historic Hatchery Plantings

We summarized spatial and temporal plantings of salmonids throughout the Elwha River Basin over the last century. At least seven different salmonid species were planted into the Elwha River Basin and hatchery releases occurred from areas below Elwha Dam upstream to river kilometer (rkm) 66. Hatchery releases occurred in the main stem river, several tributaries, Lake Sutherland, Lake Aldwell, and Lake Mills. The following species were regularly planted into the Elwha River Basin: Chinook salmon, chum salmon, coho salmon, steelhead and rainbow trout, sockeye salmon, kokanee salmon, cutthroat trout, and brook trout.

The first supplementation of anadromous hatchery fish occurred in 1914 at a facility located at the base of the Elwha Dam (James River 1988a). From 1914 to 1923, a total of 22.8 million eggs were taken from Pacific salmon and steelhead trout in the Elwha River (James River 1988a), although the location and numbers of released fish from those eggs was not determined. We did not locate detailed records of hatchery releases into the lower river from 1924 to the late 1950's, and it is unclear how many fish of each species were released. However, hatchery releases did occur during those years (Johnson 1997).

We determined from hatchery records that a total of approximately 61 million Chinook salmon were released into the Elwha River below Elwha Dam from 1953 to 2006. The current annual egg-take goal for Chinook salmon is 2.5 million fish. The stock origins of Chinook salmon released into the Elwha River (reared at the Dungeness hatchery) from 1953 to 1971 included fish from the Elwha River, Spring Creek, Dungeness River, Sol Duc River, Green River, Hood Canal, Big Soos Creek, Isaaquah Creek, Finch Creek, and Minter Creek (LEKT unpublished data). An additional approximately 26 million coho salmon, 10 million chum salmon, 3.5 million winter steelhead trout, and 0.5 million summer steelhead trout were released into the lower Elwha River. The stock origin for coho salmon that were released into the Elwha River included fish from Satsop, Dungeness, and Elwha Rivers (LEKT unpublished data).

Anadromous salmonids were also released upstream of Glines Canyon Dam in ONP as early as 1952 during a study that was designed to evaluate survivorship of Chinook salmon and coho salmon (Schoeneman and Junge Jr. 1954). The last records of anadromous salmonids released upstream of Glines Canyon Dam occurred in 1989 when Chinook salmon were released from rkm 31 to rkm 66. Review of historic records revealed that at least 500,000 Chinook salmon, 350,000 coho salmon, and 165,000 winter steelhead were released upstream of Glines Canyon Dam (Schoeneman and Junge Jr. 1954, James River 1988a, LEKT and ONP unpublished data). The release sizes of those fish were subyearling, yearling, and adult salmonids.

In addition to the releases of anadromous salmonids downstream of Elwha Dam, ONP and WDFW regularly planted non-anadromous rainbow trout, brook trout, kokanee salmon, and cutthroat trout in areas upstream of Elwha Dam (Johnson 1994, 1997). These salmonids were raised at numerous hatchery facilities that included: Lake Crescent Hatchery, Lakewood Hatchery, Mossyrock Hatchery, Quilcene National Fish Hatchery, Shelton Hatchery, University of Washington Hatchery, Lake Whatcom Hatchery, Steilacoom Hatchery, and Bogachiel Hatchery (WDFW unpublished data). Fish were transported into the backcountry by backpack, horses, and fixed-wing aircraft where they became established in even the most remote locations (Olson and Meyer 1994). It was difficult to verify stock origins for most species, but some rainbow trout strains were most likely from McCloud River, California (Phelps et al. 2001).

At least 19 million hatchery raised fish were planted into the Elwha River Basin upstream of the dams beginning in 1930 (WDFW and ONP, unpublished data). Specifically, hatchery plantings of cutthroat trout, rainbow trout, brook trout, and kokanee and sockeye salmon occurred in the

main stem river and in high elevation lakes, Lake Aldwell, Boulder Creek, Griff Creek, McCartney Creek, Prescott Creek, Stoney Creek, Wolf Creek, Lillian River, and Little River. Current hatchery releases above Elwha Dam are restricted to Lake Sutherland.

The following provides a relative gage to address the magnitude of fish plantings among fish species above Elwha Dam: 9.7 million kokanee/sockeye salmon, 7.1 million rainbow trout, 1.5 million cutthroat trout, and 0.6 million brook trout. Lake Sutherland was the most heavily planted waterway, and received approximately 11.6 million fish from 1930 to 1994 (WDFW unpublished data).

Summary of Fish Pathogen Detections in Hatcheries in the Lower Elwha River

Regular monitoring of the health of the fish raised at the WDFW and LEKT hatcheries has occurred since 1976 and 1978, respectively. Both WDFW and LEKT operate hatcheries in accordance with The Salmonid Disease Control Policy of the Fisheries Co-Managers of Washington State, which currently requires monitoring of returning adults at spawning as well as routine monitoring of their offspring while they are raised at the hatcheries (http://www.nwifc.org/enhance/documents/FinalDiseasePolicy-July2006_Ver3.pdf).

A total of 13 species of parasites, six species of bacteria, and three viruses were recorded in Pacific salmonids in Elwha hatcheries since 1988 (Table 2). The most routinely observed diseases and their etiological agents in recent history are erythrocytic inclusion body syndrome (EIBS) which is caused by a toga-like virus (Arakawa et al. 1989), BKD, and bacterial coldwater disease which is caused by *Flavobacterium psychrophilum*. The other fish pathogens listed in Table 2 have been detected over time, but less frequently. In most cases, no disease was associated with the detection. Importantly, testing for IHNV, IPNV, and VHSV failed to detect any positive samples (Table 2).

TABLE 2. Summary of presence or non-detection of fish pathogens in lower Elwha River Tribal and State hatchery facilities from 1988 to present (in alphabetical order by group).

Parasites	*Ambiphyra* sp.
	Crytptobia sp.
	Dermocystidium sp.
	Epistylis sp.
	Gill amoeba
	Gyrodactylus sp.
	Hexamita sp.
	Ichthyobodo sp. (Costia)
	Ichthyophthirius multiphilis (Ich)
	Loma sp. (Pleistophora)
	Saprolegnia sp.
	Tetracapsuloides bryosalmonae
	Trichodina sp.
Bacteria	*Aeromonas hydrophila*
	Aeromonas salmonicida
	Flavobacterium psychrophilum
	Fusiform bacteria
	Renibacterium salmoninarum
	Yersinia ruckeri
Viruses or Viral Syndromes	Erythrocytic inclusion body syndrome (EIBS)
	Paramyxovirus
	Reovirus

Summary of Fish Pathogen Screening

From 2003 to 2006, salmonids were collected at 11 sites among the lower, middle and upper Elwha River (Figure 1). A total 660 fish were cultured for *A. salmonicida* and *Y. ruckeri*, 943 were cultured for IHNV, VHSV, and IPNV, and 744 were screened for *M. cerebralis* (Table 3). None of these pathogens were detected. We also tested 566 fish by ELISA for *R. salmoninarum* from 2003 to 2006. No clinical signs of BKD were observed in any of the fish sampled. A subset of fish from all species of salmonids that were tested in each section of river (upper, middle and lower river) screened positive by ELISA and were confirmed using PCR or qPCR (Table 4). As per the protocols of the National Wild Fish Health Survey, in 2003 and 2004, PCR confirmations for *R. salmoninarum* were performed on three positive ELISA samples from each population testing positive by ELISA. None of the PCR assays were positive. In 2006, all species were pooled for *R. salmoninarum* ELISA and PCR/qPCR testing except the kokanee salmon. The kokanee salmon kidney samples were run individually as in previous years. However, instead of choosing three ELISA positive samples, 12 fish were arbitrarily selected for testing by PCR and qPCR.

Discussion

We summarized historic hatchery planting and fish health records and results from field surveys

TABLE 3. Numbers of salmonids tested for bacteria, viruses, or *Myxobolus cerebralis* in the Elwha River from 2003 to 2006. Dashed lines indicate no sampling occurred and location refers to below, between, and above the dams.

		Bacteriology					Virology					*M. cerebralis*				
Location	Species	03	04	05	06	Total	03	04	05	06	Total	03	04	05	06	Total
Lower	Coho	-	-	5	-	5	-	-	10	-	10	-	-	5	-	5
	Chum	-	-	-	-	-	-	-	23	-	23	-	-	-	-	-
Middle	Brook	4	60	60	75	199	7	115	60	135	317	7	60	61	66	194
	Kokanee	-	-	60	75	135	-	-	67	95	162	-	-	60	-	60
	Rainbow	11	-	20	25	56	34	-	20	25	79	32	4	25	33	94
Upper	Brook	-	1	-	-	1	-	1	-	-	1	-	1	1	-	2
	Rainbow	87	40	99	38	264	93	115	99	44	351	104	55	11	115	385

TABLE 4. The number of fish tested by ELISA for the presence of *R. salmoninarum* in salmonids collected from the lower, middle, and upper Elwha River from 2003-2006.

		Individual Samples # Positive/total			**Pooled Samples**[1] # Positive/total pools	Total Fish
Location	Species	2003	2004	2005	2006	
Lower	Coho salmon	0	0	3/5[2]	0	0
Middle	Brook trout	3/3	41/62	40/40[2]	13/13	61
	Kokanee salmon	0	0	59/60	74/74[2]	74
	Rainbow trout	23/24	16/43	21/36[2]	9/9	45
Upper	Rainbow trout	40/41	5/13	37/47[2]	19/19[2]	92

[1] Up to 5 fish per pool

[2] Confirmed by PCR and/or qPCR

designed to detect bacteria, viruses, and parasites in salmonids in the lower, middle, and upper Elwha River Basin. Our goal was to increase the understanding of the pathogen distribution in the Elwha River prior to the elimination of the Glines Canyon and Elwha Dams, and not necessarily to document minor changes in pathogen populations over time. We plan to continue to monitor the pathogens and health of fish using the best available technology as the dynamic changes in habitat conditions and salmonid recolonization occur simultaneously.

The stocking of millions of hatchery raised salmonids occurred throughout the Elwha River, its tributaries, and reservoirs over the last 95 years. Hatchery releases of Chinook salmon, coho salmon, and steelhead trout continue today in the lower river. The last recorded hatchery plantings of anadromous salmonids above Glines Canyon Dam occurred in 1989.

From 2003 to 2006, *R. salmoninarum* was the one pathogen that was consistently identified at low levels in all sections of the river and in all species. The detection of *R. salmoninarum* was not surprising since it is known to occur in most parts of the world where salmonids exist in both hatchery and wild populations (Fryer and Lannan 1993, Pascho et al. 2002). There was no clinical disease associated with these detections in the Elwha River Basin. No other target pathogens were detected in the other testing performed.

Two decades of routine monitoring at hatcheries provided a record of parasites, bacteria, and viruses that were detected (Table 2). In most cases, the infections were subclinical and no disease was observed. Historically, *Dermocystidium sp*. infections may have resulted in high mortality in Elwha River hatcheries, but it has not been a significant cause of mortality in the past 10 years (Martin Chen, Washington Department of Fish and Wildlife, personal communication). The pathogens that most commonly cause disease at these hatcheries include the toga-like virus that causes EIBS, *R. salmoninarum,* and *Flavobacterium psychrophilum*. There have been no detections of IHNV, IPNV, and VHSV, which are routinely monitored as part of compliance with The Salmonid Disease Control Policy of the Fisheries Co-Managers of

Washington State. Additionally, yearly surveys of kokanee salmon in Lake Sutherland revealed a myxozoan parasite that may be previously undescribed (Simon Jones, Department of Fisheries and Oceans, Canada, personal communication). Because historical fish health records are limited, the fish health data collected in hatcheries may be the best guide as to what pathogens occur in the watershed.

Although we cannot predict exactly what effect pathogens will have on the restoration of salmonids in the Elwha River, changes in species composition in the upper river may be accompanied by changes in pathogen distribution. Some fish species are more susceptible to particular pathogens than others (Starliper et al. 1997, Hedrick et al. 2001). As the species composition changes, we might expect that the pathogen profile would reflect those changes. Chinook salmon are thought to be more susceptible to *R. salmoninarum* than some other salmonid species (Bullock and Wolf 1986). An increase in their presence in the upper watershed may be followed by a species-driven increase in the prevalence of this pathogen. We know from our baseline study that *R. salmoninarum* already exists in the middle and upper watershed. If a change occurs, it would be in the severity of infection and/or prevalence in the populations. We may be able to detect these changes if they are large enough.

Another possible change after dam removal is the introduction of a pathogen into a naive population, which is one that has not been previously exposed to the pathogen. The members of a naive population can be more susceptible to the pathogen and exposure may result in a more severe, if not lethal infection (Mims 1988). To date IHNV, which has been detected in other coastal Washington watersheds (Emmenegger and Kurath 2002), has not been detected in hatchery or wild fish in the Elwha River Basin. However, IHVN could be introduced by anadromous salmonids that recolonize the Elwha River. Recently, steelhead trout in coastal Washington watersheds were found to be infected with a strain of IHNV that is typically detected in the Columbia River Basin (Gael Kurath, USGS Western Fisheries Research Center, Seattle, WA, personal communication). The introduction of IHNV to the salmonid populations exposed by the removal of the Elwha dams has the potential to lead to disease outbreak. Kokanee salmon are known to be susceptible to the genotypes of IHNV that occur already in coastal watersheds of Washington and British Columbia (Garver et al. 2006). If a strain of IHNV that is virulent for sockeye salmon is introduced by returning anadromous salmonids, the naive kokanee salmon population in Lake Sutherland could be impacted.

The risk of exposure to pathogens is not confined to the potamodromous fish being exposed to pathogens by recolonizing fish. If pathogens have co-evolved with the fish in the upper watershed and infect the naive recolonizing fish, severe infections could occur. We cannot predict what impacts, if any, the myxozoan parasite seen in the Lake Sutherland kokanee salmon may have on naive populations of fish. Additionally, resumption of anadromy by rainbow trout and bull trout populations above the dams may increase interaction within and among species (Brenkman et al. 2008).

In addition to the interaction among populations of fish, dam removal will also result in changes in flow, substrate distribution, and water temperature patterns. Such changes could affect the distribution of certain intermediate hosts (Krueger et al. 2006, Stocking and Bartholomew 2007). The shift in geographic distribution of intermediate hosts may have an indirect impact on pathogen distributions. In addition, some fish pathogens are known to survive in the sediment or water for extended periods (Toranzo and Hetrick 1982, Austin and Rayment 1985, El-Matbouli and Hoffmann 1991, Hedrick and El-Matbouli 2002). It is unknown to what extent increased sediment levels will have on fish health in the Elwha River.

Changes in flow patterns after dam removal will likely change the water temperature regime in the river. Since the upper Elwha River is cooler than the lower and middle river (Connolly and Brenkman 2008), after dam removal water temperatures below Glines Canyon Dam are expected to decrease. In other watersheds, increased water temperatures have resulted in amplification of pathogens and increased disease. For example, elevated water temperatures are believed to contribute to the exacerbation of ichthyophoniasis in Yukon River Chinook salmon (Kocan et al. 2004). Cooler water temperatures following dam removal could benefit salmonid health, but other factors, such as temporarily elevated turbidity levels after dam removal and subsequent gill abrasion may favor pathogens. The balance of these factors will determine whether the outcome results in disease.

Although changes in pathogen distribution due to dam removal may occur, one should be aware of the limitations of a survey of wild fish in a large river system. Unavoidable obstacles to a robust study design include sampling bias and limitations of assays. In wild fish populations, sampling bias occurs because we are unable to randomly collect fish. If a wild fish is weakened by disease, it may be less able to avoid predation and not survive to be included in our sampling. Or, it may change its behavior and be more likely to elude capture by electrofishing techniques. Thus, it can be difficult to determine an accurate pathogen prevalence level, or even detect a pathogen in wild fish. In contrast, hatchery fish health records typically bias sampling towards diseased fish. Fish Health Specialists are more likely to target slower moving, or clinically diseased fish in order to make a diagnosis. Fish Health Specialists at hatcheries can also screen for a wider range of pathogens than those targeted by the NWFHS. For example, EIBS found in the Elwha hatcheries is not a target pathogen for the NWFHS. In addition, infections can be transitory. The wild fish were only sampled once per year at roughly the same time of year, while fish at hatcheries are monitored year round. A study design for random sampling is more achievable in the hatchery. These factors make a basic, seemingly straightforward question such as, "What is the prevalence of a certain pathogen in a hatchery relative to that of free ranging or wild fish?" extremely difficult to answer.

Additionally, although the best technology available is being employed, there are limitations to the assays. This is most apparent in methods used for detecting the presence of *R. salmoninarum* and interpreting the results. Using ELISA, we measured the amount of a specific protein produced by *R. salmoninarum*. This protein can persist for months after a fish was exposed to *R. salmoninarum*, whether or not the fish is actively infected with live bacteria (Pascho et al. 1997). Thus a positive ELISA result may indicate previous or current exposure, but does not necessarily indicate active infection or disease. With PCR or qPCR, a positive result indicates presence of the DNA of the organism in the fish kidney but is not necessarily proof of active infection or "disease."

Another challenge of the monitoring fish pathogen distribution over several years is the evolution in the techniques used to detect pathogens. When we began this study, we followed the National Wild Fish Health Survey protocol of screening a population by ELISA and confirming three positive samples with PCR. In 2003 and 2004, although our positive and negative controls ran as expected, we were unable to detect *R. salmoninarum* by PCR. In 2005, with the addition of refined extraction techniques and qPCR, our testing has become more sensitive, yielding a larger number of positives. With three years of results to evaluate we had concerns, as mentioned above, that the results of the PCR and qPCR may not correlate well with the ELISA. We decided that all fish should be tested by PCR/qPCR, in addition to ELISA. In order to achieve this increased level of testing, samples of the same species and location were combined up to five fish per pool. As new technologies emerge, we hope to incorporate them and/or improve upon our existing methods.

These baseline surveys and subsequent monitoring of pathogens in the Elwha River serve to highlight the need for inclusion of fish pathogen distributions as an important factor in a risk assessment framework. A review of historic hatchery plantings is a significant part of understanding potential pathogen distribution in the Elwha River Basin, and may ultimately allow for more informed management decisions that address the risk of exposure to pathogens. It is unknown whether a change in the balance of host and pathogens as a result of this large scale change in environment will have an effect at the population level, however, monitoring pre-and post dam removal may provide useful information for future dam removal projects.

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