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Fish Ecology Division

Northwest Fisheries Science Center

National Marine Fisheries Service

Seattle, Washington

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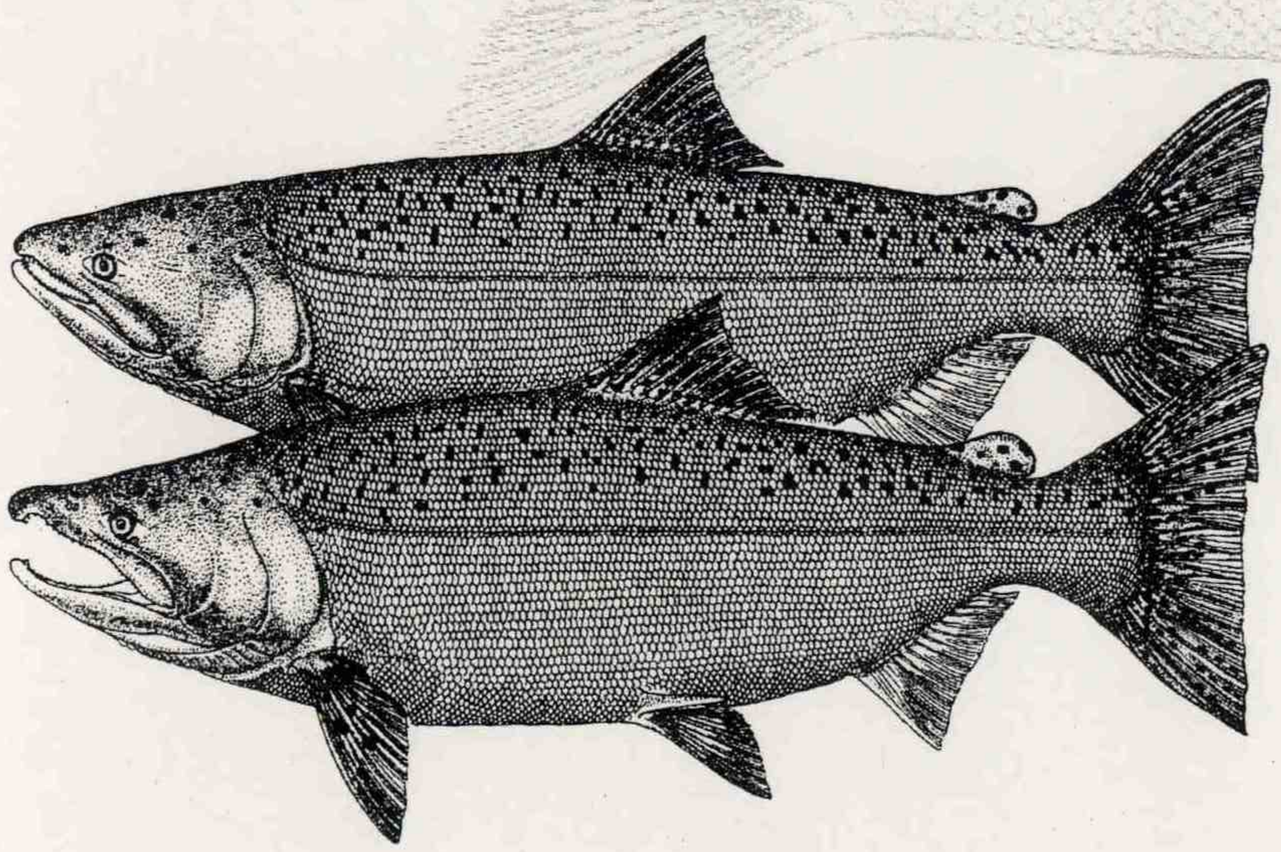

Alternative Barging Strategies to Improve Survival of Transported Juvenile Salmonids, 2008

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EXECUTIVE SUMMARY

In spring 2008, we continued a multi-year study to test the hypothesis that release of transported fish in the lower Columbia River estuary near Astoria (river kilometer 10) would produce higher adult returns than release at Skamania Landing, the established location just below Bonneville Dam (rkm 225). To evaluate this hypothesis, we released transported groups of juvenile Pacific salmon *Oncorhynchus* spp. to both sites for comparison of smolt-to-adult return rates (SARs) from each group. We speculated that moving the release site 215 km downstream could decrease smolt mortality due to predation by piscivorous fish and birds. Releases in 2008 made up the third and final consecutive year of juvenile releases for this study. Adults returning over the next several years will provide data to test this hypothesis.

In addition to evaluating a release location for transported fish, we used new, non-lethal techniques to collect fish pathogen data. We determined pathogen loads in study fish to evaluate whether pathogens in individual fish affect vulnerability to avian predators as well as SARs.

On six consecutive Sundays, from mid April to May 2008, we collected river-run yearling Chinook salmon *O. tshawytscha* and steelhead *O. mykiss* at the Lower Granite Dam juvenile fish facility. All study fish were tagged with passive integrated transponder (PIT) tags. After tagging, fish were transferred to raceways and held until the following day, when they were loaded on barges for transport. Fewer yearling Chinook salmon were collected than planned; total releases of yearling Chinook were 19,555 at Astoria (16,519 hatchery and 3,036 wild) and 28,237 at Skamania Landing (23,717 hatchery and 4,520 wild). More steelhead were collected than planned, with total releases of 31,039 at Astoria (rkm 10; 25,353 hatchery and 5,686 wild) and 40,546 at Skamania Landing (rkm 225; 32,920 hatchery and 7,626 wild).

All releases at rkm 10 were made after dark on an outgoing tide to reduce avian predation by Caspian Terns *Hydroprogne caspia* and Double-crested Cormorants *Phalacrocorax auritas* from nearby nesting colonies on East Sand Island. After the nesting season, abandoned bird colonies were scanned to detect PIT tags from fish released from this and other studies, and these data were used to estimate the minimum numbers of fish from each release group preyed upon by piscivorous birds (only minimum predation rates can be estimated, as not all tags are detected).

During each tagging day, about 300 non-lethal gill clip samples were collected for pathogen analyses (*Renibacterium salmoninarum* and *Nucleospora salmonis*), for a total

of about 1,800 samples over the season. These data allowed us to determine whether infection with *R. salmoninarum, N. salmonis,* or both was correlated with predation vulnerability. There was no conclusive evidence from studies in 2006, 2007, or 2008 that infection of fish with one or both pathogens influenced rates of predation, but infection levels of *R. salmoninarum* were low in the majority of test fish during all 3 years.

In 2008, the first year that *N. salmonis* levels in gill samples could be quantified, the majority of *N. salmonis*-infected fish were determined to have low infection levels. However, PIT tags from two of the six fish (hatchery steelhead) with the highest recorded *N. salmonis* levels (>1,000 DNA copies/reaction) were recovered from the East Sand Island Caspian Tern colony in 2008. In addition, the PIT tag from one of the two fish (hatchery steelhead) with the highest recorded *R. salmoninarum* levels (>1,000 bacteria/mg tissue) was recovered from the tern colony in 2007. These data suggested increased vulnerability to avian predation of steelhead with high infection levels of either pathogen. Because of the lower avian predation rates on juvenile Chinook salmon than on juvenile steelhead, relatively few PIT tags from pathogen-tested Chinook salmon were detected on East Sand Island (7 to 33 tags) in comparison to PIT tags from pathogen-tested steelhead (98 to 139 tags) during the three years of pathogen testing.

After three years of releases at the two sites, we have clear evidence that the new release location affected vulnerability to avian predators. Mean minimum avian predation rates in 2008 were 3.9% for yearling Chinook salmon released at rkm 225, but only 0.9% for those released at rkm 10. Minimum avian predation rates were 14.9% for steelhead released at rkm 225 but only 4.4% for their cohorts released at rkm 10. These results were nearly identical to those from releases in 2006 and 2007, and show that releasing fish farther downstream, at night, and on an outgoing tide will reduce avian predation substantially, particularly for steelhead, the species most vulnerable to avian predation. This finding is relevant for management actions related to recovery of juvenile salmonids that pass the world's largest Caspian Tern and Double-crested Cormorant colonies during their downstream migration.

We will need to wait several years for complete adult returns from all three release years to determine the efficacy of releasing transported salmonids at Astoria vs. Skamania Landing. Based on complete adult returns from 2006 releases, the release site at Astoria (rkm 10) provided a modest improvement in SARs for hatchery steelhead and hatchery and wild steelhead combined, but not for wild steelhead. Complete adult returns from 2006 indicate that the Astoria release site was detrimental for yearling Chinook salmon.

For wild and hatchery steelhead combined, 551 adults returned from releases at Skamania for a SAR of 1.32, while 509 adults returned from releases at Astoria for a SAR of 1.75. This resulted in a SARs ratio of 1.20 (95% CI, 1.01-1.41) for Skamania:Astoria. For wild and hatchery yearling Chinook salmon combined, 139 adults returned from releases at rkm 225 for a SAR of 0.57, while 53 returned from releases at rkm 10 for a SAR of 0.33: thus the Skamania-to-Astoria ratio was only 0.49 (95% CI, 0.18-1.31). These results may vary by release year. However, based on 2006 releases, transporting steelhead smolts to the estuary increased the rate of straying and lowered conversion rates between Bonneville Dam and Lower Granite Dam, likely due to greater impairment of homing ability.

CONTENTS

INTRODUCTION

At transport dams on the Snake and Columbia Rivers, salmonid smolts are guided away from turbine intakes, collected, and either returned to the river to continue migration or transported by truck or barge to a release site below Bonneville Dam. The purpose of transporting fish is to avoid mortality caused by dam passage, but the benefit provided by transportation has varied for different fish stocks and with the timing of transport during the juvenile migration season (Muir et al. 2006; Williams et al. 2005).

Typically, about 50% of Snake River smolts migrating downstream survive to below Bonneville Dam (Williams et al. 2005), while about 98% of transported smolts survive (Budy et al. 2002). Therefore, one would expect about twice as many transported as inriver migrant fish to return as adults. Nevertheless, on an annual basis, the ratio of transported to inriver migrant adult returns is usually lower than expected. This indicates that higher mortality is experienced for transported smolts after release than for inriver migrants that survived to below Bonneville Dam. The difference in smolt-to-adult return rates (SARs) between transported fish and inriver migrants that survive past Bonneville Dam is termed "*D*" to represent the differential delayed mortality of transported fish. Use of a lower-estuary release site may increase SARs and reduce *D* for transported fish.

Fish condition and health have been assessed prior to and after transport in previous studies (Pascho and Elliott 1989; Elliott and Pascho 1991, 1992, 1993, 1994; Elliott et al., 1997; Congleton et al. 2000, 2005; Kelsey et al. 2002; Schreck et al. 2005). These studies examined stress and stressors in detail, and modifications to the collection and transport system have been made to reduce stress (Williams and Matthews 1995). In spite of these improvements, transport has not provided the benefit expected, particularly for wild Chinook salmon *Oncorhynchus tshawytscha* (Williams et al. 2005). The present study contributes to an ongoing effort by the U.S. Army Corps of Engineers Anadromous Fish Evaluation Program to improve post-release survival of transported fish.

In studies of Coho salmon *O. kisutch*, Solazzi et al. (1991) found that smolts transported to a release point near Columbia River km 29 returned at a rate 1.6 times greater than those released upriver. Similarly, Gunnerod et al. (1988) found that Atlantic salmon *Salmo salar* released in salt water returned at a higher rate. Marsh et al. (1996, 1998, 2000) compared steelhead *O. mykiss* released at Columbia River km 225 with those released at rkm 29, but too few adults returned from either release point for a meaningful evaluation.

The primary objective of the alternate release-site study in 2008 was to determine whether releasing barged fish farther downstream near Astoria, Oregon, at rkm 10 would improve SARs of spring Chinook salmon and steelhead (Figure 1). This release strategy would minimize the time spent moving into and through the estuary. To provide additional insight into the vulnerability of smolts to predators, we planned to document

fish condition prior to release. Our approach was to tag transported smolts with passive integrated transponder (PIT) tags (Prentice et al. 1990), collect samples for pathogen analysis, and release fish at either rkm 225 (the present release site near Skamania Landing) or rkm 10 (the alternate site near Astoria, Oregon). Similar releases were made during 2006 and 2007 at these two locations (Ryan et al. 2007, Marsh et al. 2008). When the adults return, we will compare the SARs between release sites to determine the benefit (if any) of transporting smolts farther downstream. Adult returns for the evaluation of this objective will be complete in 2011.

Our second objective was to determine the prevalence and levels of the fish pathogens *Renibacterium salmoninarum* and *Nucleospora salmonis* within each release group. The infection profiles of *R. salmoninarum* and *N. salmonis* reported here can then be correlated with avian predation rates and SARs, although the latter correlation will have to wait several years for adult returns. Our third and final objective was to compare avian predation rates between Skamania Landing releases and Astoria releases.

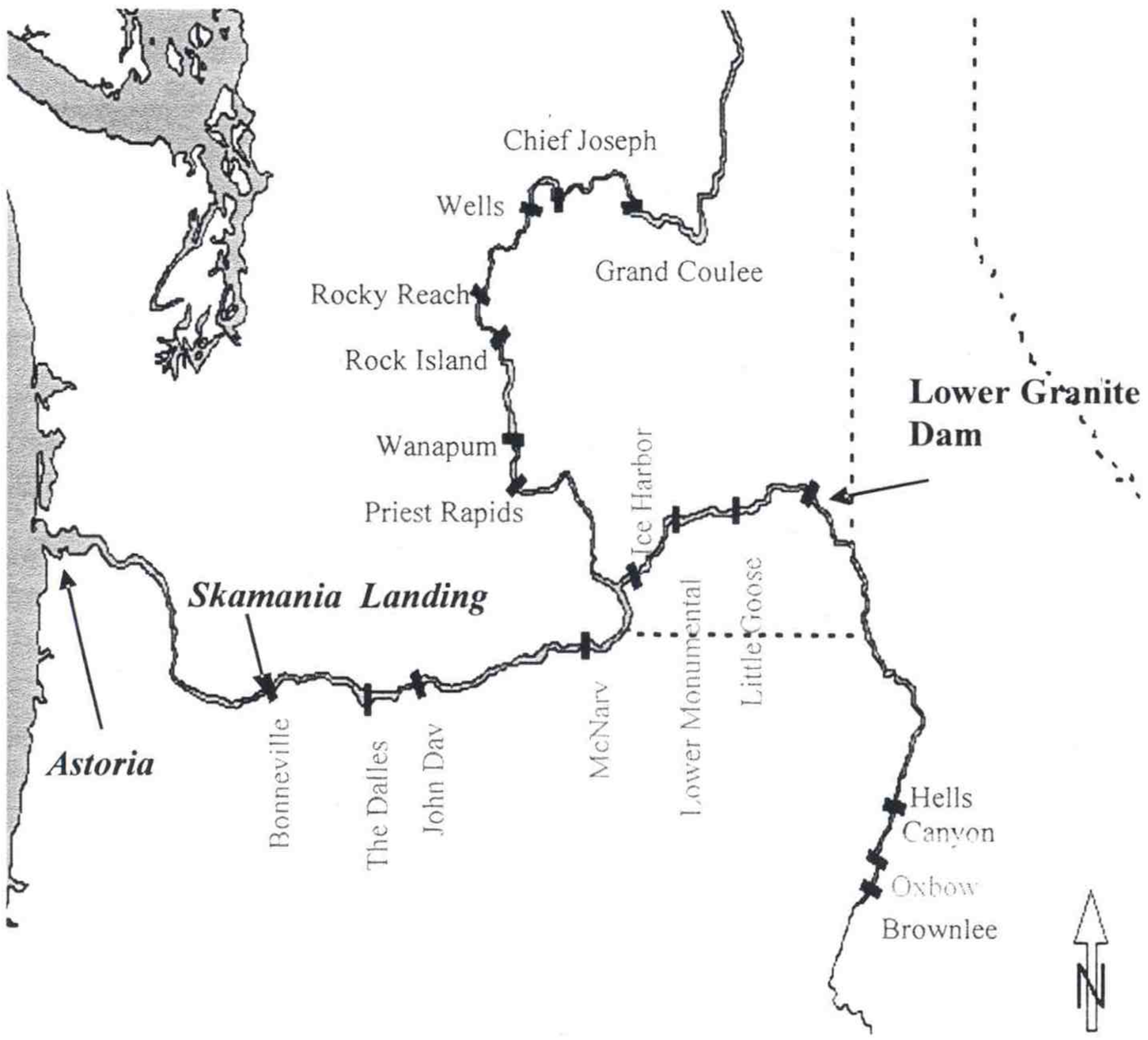

Figure 1. Study area showing collection and tagging site at Lower Granite Dam and release sites at Skamania Landing (rkm 225), and Astoria (rkm 10), 2008.

METHODS

Fish Collection and Tagging

During spring 2008, we collected and PIT-tagged two groups of steelhead and two groups of yearling Chinook salmon smolts at Lower Granite Dam. Fish were tagged at the NOAA tagging facility on six consecutive Sundays from mid April through May. Tagging followed the protocols and standards outlined in the PIT Tag Marking Procedures Manual (CBFWA 1999) for mass marking using simple PIT-tag injectors (see Marsh et al. 2001 for description of tagging methods used at this facility). After each tagging session, fish were transferred to the east bank transport raceways for 24-h recovery.

The following day, one group of each species was loaded on an 8000-series transport barge (a 2000-series barge was used for the first two releases because general transport had yet to start) for release at Skamania Landing (rkm 225), the standard transportation release site. A second group of each species was loaded on a 2000-series barge for release at Astoria (rkm 10), an alternate release site in the lower estuary.

We attempted to tag sufficient numbers of both yearling Chinook salmon and steelhead to test a SARs ratio of 1.3 for transported fish released at Astoria (T_A) to those release at Skamania Landing (T_S). A T_A/T_S ratio of 1.3 or higher would indicate SARs at least 30% higher for fish released at the alternate site. This ratio was based on an expected SAR of 1.0% at Lower Granite Dam for the Astoria releases. For both yearling Chinook salmon and steelhead, we tagged hatchery and wild fish in proportion to those entering the juvenile bypass facility. While the expected SAR required us to tag 53,000 fish of each species, the actual number tagged varied in accordance with numbers of fish arriving at the dam.

Fish Releases

Skamania Landing release groups were transported and released with normal transport fish (except for the first two releases prior to the start of general transport). We attempted to keep loading density and water-volume replacement times as equal as possible between the Skamania Landing (8000 series) and Astoria (2000 series) barges. For both barges, we complied with the loading density and replacement rates set by the U.S. Army Corps of Engineers. However, due to the unpredictable nature of fish passage, keeping loading densities equal proved to be difficult and was not always achieved.

The barge used for Astoria releases was towed with a separate vessel mirroring the path of the Skamania Landing barge until after it passed Bonneville Dam, when it continued downstream to rkm 10 (except for the first two trips in 2008 where both barges were towed by the same vessel). Astoria releases were timed to occur at night on an ebb tide to reduce predation by Caspian Terns *Hydroprogne caspia* and Double-crested Cormorants *Phalacrocorax auritas* from the nearby nesting colonies on East Sand Island (Table 1). Dissolved oxygen levels, water temperatures, and mortalities were monitored on the 2000 series barge using the same standard procedures used on the 8000 series barge.

Table 1. Release dates, times, and locations for PIT-tagged juvenile steelhead and yearling Chinook salmon smolts released at Skamania Landing and near Astoria during 2008. High tides for the Astoria releases are noted.

	Astoria releases (rkm 10)		Skamania releases (rkm 225)	
Release date	Time (PDT)	High tide at rkm 10 (PDT)	Release date	Time (PDT)
24-Apr	0445	0252	23-Apr	0710
1-May	0010	2226	30-Apr	0515
8-May	0410	0223	6-May	1957
15-May	0012	2230	13-May	2300
22-May	0315	0151	20-May	1820
28-May	2230	2041	27-May	1915

Pathogen Sampling

Fish were analyzed for the presence of two salmonid pathogens known to occur in the Snake and Columbia River basins; *R. salmoninarum*, the causative agent of bacterial kidney disease (BKD), and *N. salmonis*, an intranuclear microsporidian parasite that primarily infects lymphoblast cells and can cause a chronic, severe lymphoblastosis and a leukemic-like condition. Gill filament samples for determining the presence and levels of *R. salmoninarum* and the presence of *N. salmonis* were collected from fish in every release group during tagging. On each tagging day, the goal was to sample 75 fish each of wild and hatchery Chinook salmon and of wild and hatchery steelhead, for a total of 300 fish per replicate. The total number of fish sampled over the season was close to the goal of 1,800, but proportions of fish by species and origin varied depending on their availability at the dam on each tagging date (Table 2).

Table 2. Release numbers (mortalities removed) of PIT-tagged hatchery and wild steelhead and yearling Chinook salmon smolts that were gill-clipped, transported, and released at Skamania Landing by tag date during 2008.

	Chinook salmon		Steelhead		
Tag date	Hatchery	Wild	Hatchery	Wild	Total
19 and 20-Apr	76	73	73	77	299
26 and 27-Apr	79	66	82	73	300
4-May	67	73	87	73	300
11-May	86	65	73	76	300
18-May	74	78	75	72	299
24 and 25-May	92	54	93	61	300
Total	474	409	483	432	1,798

Sample collection methodology followed the protocol for non-lethal gill filament sampling described by Schrock et al. (1994). Briefly, a 2 × 3-mm gill sample (approximately 10 mg) was removed from each fish using surgical scissors. Samples were placed in individual pre-weighed and labeled tubes, frozen immediately on dry ice, and transported to the USGS Western Fisheries Research Center for analysis. The use of pre-weighed tubes and transport of undiluted samples on dry ice instead of dilution in ethanol before transport enabled accurate weighing of samples. The PIT-tag code associated with each gill filament sample number was recorded.

At the same PIT-tagging stations where fish were collected for pathogen analyses, water samples were taken for quantification of *R. salmoninarum* in water at the juvenile fish facility. Water samples were taken four times during each tagging day; twice before the re-circulating water in the tagging system was changed, and twice after the water was changed. Samples were preserved by addition of 0.01% thimerosal (final concentration) to each 500-mL water sample.

Bird Colony Sampling

Using PIT tags allowed us to use avian predation data from the NOAA Fisheries avian predation project (Ryan et al. 2007, Sebring et al. 2009) to estimate predation rates of the fish released in this study. The avian predation project evaluates the impacts of predation by Caspian Terns and Double-crested Cormorants on juvenile salmonids by detecting PIT tags on piscivorous water bird colonies in the Columbia River Basin (Ryan

et al. 2001, 2003). Comparing the rates of predation of PIT-tagged salmonids allowed us to determine whether fish released at Skamania Landing were more susceptible to predation by piscivorous birds than fish released at Astoria. The data also allowed us to observe any differences in predation rate that may be due to *R. salmoninarum* or *N. salmonis* infection. For each species, to compare predation rates between release locations, a geomean of the replicate T_A/T_S ratios with a 95% confidence interval was constructed (Burnham et al. 1987) where T_A and T_S were predation proportions rather than SARs as defined above. If the 95% confidence interval did not contain the value 1, then predation rates were significantly different at the $P < 0.05$ level.

Pathogen Analyses

Gill samples were weighed, processed and tested for *R. salmoninarum* by two PCR procedures; nested PCR (nPCR) and real-time quantitative PCR (qPCR). The nPCR was done according to the method of Chase and Pascho (1998). For the qPCR, the procedure of Chase et al. (2006) was followed, except that a non-fluorescent quencher was substituted for the fluorescent quencher dye (TAMRA) on the 3' end of the internal probe RS1262. The use of this modified probe, MGBRS1262, was intended to increase the sensitivity of the qPCR. Some previous work had indicated that the original qPCR method of Chase et al. (2006) had a lower sensitivity than the nPCR (McMichael et al. 2006), but only the qPCR can provide a measure of the infection levels in fish. Thus, testing a single sample by both PCR techniques was desirable to provide the most information.

For detection of *N. salmonis* in gill samples, the nested PCR method of Barlough et al. (1995) was followed, with several modifications. A commercially available PCR buffer (Qiagen, Inc.[1]) was included in the master mix, 0.5 U of TaqDNA polymerase was used, and the use of gelatin was omitted. A 2-µL aliquot of the first round product was used in the nested round of amplification. The cycling conditions for the first PCR round were changed to the following: initial denaturing at 94°C for 5 min, followed by 35 cycles, each including denaturing at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 60 s, and a final extension of 72°C for 7 min. The second PCR round (35 cycles) was the same as the first, except that the annealing step was accomplished at 60°C for 30 s. The nPCR methodology can only determine the presence or absence of *N. salmonis*. For quantification of *N. salmonis* in samples in 2008, the qPCR method of Foltz et al. (2009) was used.

[1] Use of trade names does not imply endorsement by the National Marine Fisheries Service or the USGS.

For enumeration of *R. salmoninarum* in water samples, a procedure modified from that of Elliott and McKibben (1997) was used. Water samples were shaken to mix the contents, and large debris was allowed to settle for 5 min. Triplicate sub-samples were prepared from each water sample. For each sub-sample, a 5-mL aliquot of the sample was combined with 3 mL of phosphate-buffered saline (PBS, 0.01 M phosphate, pH 7.1) with 0.5% (by volume) Triton X-100 added (PBS-Triton). After vortex mixing, each sub-sample was triturated through a 22-gauge needle and then filtered through a Nuclepore 0.2-μm pore diameter filter.

After each filter was rinsed with 1-3 mL PBS Triton, 100 μL of a 1:40 dilution (by volume) of fluorescein isothiocyanate-labeled anti-*R. salmoninarum* polyclonal antiserum (Kirkegaard and Perry Laboratories) was pipetted onto each filter. Filters were incubated in a humid chamber for 1 h at room temperature, then rinsed with 1-3 mL PBS-Triton, and counterstained with 1 mL Eriochrome Black T (Sigma; diluted 1:2000 wt:vol in PBS). Filters were air dried, and cover glasses were mounted with pH 9 glycerol-DABCO mounting medium (Johnson et al. 1982). Each filter was examined by epifluorescence microscopy at 1000× magnification with a Zeiss Axiophot microscope. A total of 150 microscope fields were examined on each of three filters per water sample, and *R. salmoninarum* cells were counted.

Several statistical methods were used for pathogen analyses among groups of steelhead and Chinook salmon (Motulsky 1995; InStat 3, Graph Pad). Contingency tables were used to compare relative proportions of uninfected and infected fish for *R. salmoninarum*, *N. salmonis*, or both pathogens. Fisher's exact test was used for analysis of 2 × 2 tables, and a chi-square test was used to analyze larger contingency tables. For fish testing positive for *R. salmoninarum* by qPCR, *R. salmoninarum* level data (both raw data and log-transformed data) were first tested for normality by the Kolmogorov-Smirnov method. Because at least one data set in each comparison failed the normality test ($P < 0.05$) even after log transformation, the nonparametric Mann-Whitney test was used for comparison of two groups, and the Kruskal-Wallis test (single-factor analysis of variance by ranks) was used to compare *R. salmoninarum* levels among three or more groups. Dunn's multiple comparison test was applied when a significant result ($P < 0.05$) was observed using the Kruskal-Wallis test.

For each species, fork lengths of fish infected with one or both pathogens were compared with fork lengths of fish in which neither pathogen was detected. Because length data were not normally distributed, the Mann-Whitney test or Kruskal-Wallis test was used for these comparisons as previously described. Correlation of *R. salmoninarum* level with fork length was evaluated using the nonparametric Spearman rank correlation test.

Comparison of Smolt-to-Adult Returns

Lower Granite Dam served as the principal recovery site for adults. Data acquired from other areas were considered ancillary. When adult returns were complete, a geomean of the replicate T_A/T_S ratios with a 95% confidence interval was calculated for yearling Chinook salmon (hatchery, wild, and combined) and for steelhead (hatchery, wild, and combined) (Burnham et al. 1987). If the 95% confidence interval did not contain the value 1, then the T_A/T_S ratio was significantly different at the $P < 0.05$ level. A similarly bounded geomean was constructed for relative conversion rates from Bonneville to Lower Granite Dam.

RESULTS

Fish Collection and Tagging

On six consecutive Sundays from mid-April through May, river-run yearling Chinook salmon and steelhead were collected and tagged with PIT tags at the Lower Granite Dam juvenile fish facility (Table 3). A total of 19,555 yearling Chinook salmon were tagged and released downstream from Astoria at rkm 10 (16,519 hatchery and 3,036 wild), while 28,237 were tagged and released at Skamania Landing (rkm 225; 23,717 hatchery and 4,520 wild). For steelhead, a total of 31,039 were released at rkm 10 (25,353 hatchery and 5,686 wild) and 40,546 released at rkm 225 (32,920 hatchery and 7,626 wild). Fewer yearling Chinook salmon, but more steelhead, were tagged than planned. Additional fish were added to the holds of both barges in an attempt to equalize densities. Due to the unpredictable nature of fish arrival and collection at the dam, equalizing densities proved to be difficult and was not always achieved (Table 4). However, final fish loading densities were far below maximum capacities on all barges.

Table 3. Release numbers of PIT-tagged wild (W) and hatchery (H) juvenile steelhead and yearling Chinook salmon by date at the Astoria and Skamania Landing release sites during 2008.

	Skamania Landing (rkm 225)				Astoria (rkm 10)				
Release	Chinook salmon		Steelhead		Chinook salmon		Steelhead		
date	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild	Total
23 and 24 Apr	2,927	474	6,212	1,365	1,560	388	4,549	921	18,396
30 Apr and 1 May	2,395	954	9,554	1,236	1,337	576	8,074	869	24,995
6 and 8 May	3,001	1,234	3,572	931	3,118	844	3,730	865	17,295
13 and 15 May	6,014	692	2,583	855	4,649	500	2,439	729	18,461
20 and 22 May	6,384	682	4,051	899	4,088	405	2,039	766	19,314
27 and 28 May	2,996	484	6,948	2,340	1,767	323	4,522	1,536	20,916
Total	23,717	4,520	32,920	7,626	16,519	3,036	25,353	5,686	119,377

Table 4. Numbers of PIT-tagged fish, and the number of untagged fish added to increase barge hold densities for the Skamania Landing (8000 series) and Astoria (2000 series) release barges, 2008. Total pounds and pounds/gallon of fish in the barge holds are also shown.

Release date	Barge	Number of tagged fish	Number non-tagged fish	Total pounds	Pounds/gallon
23 Apr	Skamania	10,978	7,435	2,557	0.18
24 Apr	Astoria	7,418	9,731	2,382	0.17
30 Apr	Skamania	14,139	10,693	3,599	0.26
1 May	Astoria	10,856	11,016	3,170	0.23
6 May	Skamania	8,738	7,466	2,568	0.10
8 May	Astoria	8,657	6,395	2,378	0.17
13 May	Skamania	10,144	77,418	10,420	0.42
15 May	Astoria	8,317	19,076	3,265	0.23
20 May	Skamania	12,016	34,418	6,107	0.24
22 May	Astoria	7,298	19,688	3,553	0.25
27 May	Skamania	12,768	23,930	5,242	0.21
28 May	Astoria	8,148	16,271	3,489	0.25

Avian Predation

Based on PIT tag recoveries on East Sand Island, it was estimated that avian predators consumed a significantly higher proportion of fish released at Skamania Landing than of those released at Astoria. For both steelhead and yearling Chinook salmon, the avian predation rates were significantly lower for fish released at Astoria during nighttime and on an ebb tide than for fish released 215 km upstream at Skamania Landing. On the East Sand Island tern and Double-crested Cormorant colonies, 14.9% of the tags from steelhead released at Skamania Landing were recovered, while 4.4% of the tags from steelhead released at Astoria were recovered (Table 5). Smaller proportions of PIT tags from Chinook salmon were recovered on the colonies, but these recoveries still showed the trend of significantly higher predation rates for fish released at Skamania Landing (Table 6). A slightly higher proportion of hatchery smolts compared to wild smolts were preyed upon for both yearling Chinook salmon and steelhead. There were no consistent trends in predation rates through the season.

Table 5. Number of juvenile steelhead released, number of PIT tags detected on the East Sand Island Caspian Tern and Double-crested Cormorant colonies, and predation rate from 2008 releases at Astoria (rkm 10) and Skamania Landing (rkm 225). Geomean ratios of predation rates for Astoria/Skamania (P_A/P_S) releases are shown with 95% confidence intervals.

Release day	Astoria (rkm 10) Juveniles released	PIT tags detected	Predation rate	Skamania Landing (rkm 225) Juveniles released	PIT tags detected	Predation rate	P_A/P_S
			Wild steelhead				
Apr 24	921	63	6.84	1,365	220	16.12	0.42
May 1	869	9	1.04	1,236	164	13.27	0.08
May 8	865	7	0.81	932	55	5.90	0.14
May15	729	5	0.69	855	75	8.77	0.08
May 24	766	1	0.13	900	25	2.78	0.05
May 31	1,536	37	2.41	2,340	202	8.63	0.28
Total	5,686	122	2.15	7,628	741	9.71	0.22
Geomean P_A/P_S = 0.13 (95% CI 0.05-0.34)							
			Hatchery steelhead				
Apr 24	4,549	553	12.16	6,212	1,272	20.48	0.59
May 1	8,074	347	4.30	9,555	1,927	20.17	0.21
May 8	3,730	52	1.39	3,572	428	11.98	0.12
May15	2,439	18	0.74	2,583	253	9.79	0.08
May 24	2,039	14	0.69	4,051	253	6.25	0.11
May 31	4,522	274	6.06	6,948	1,156	16.64	0.36
Total	25,353	1,258	4.96	32,921	5,289	16.07	0.31
Geomean P_A/P_S = 0.19 (95% CI 0.08-0.43)							
			Wild and hatchery steelhead combined				
Apr 24	5,470	616	11.26	7,577	1,492	19.69	0.57
May 1	8,943	356	3.98	10,790	2,091	19.38	0.21
May 8	4,695	59	1.26	4,503	483	10.73	0.12
May15	3,168	23	0.73	3,438	328	9.54	0.08
May 24	2,805	15	0.53	4,950	278	5.62	0.10
May 31	6,058	311	5.13	9,288	1,358	14.62	0.35
Total	31,039	1,380	4.45	40,546	6,030	14.87	0.30
Geomean P_A/P_S = 0.18 (95% CI 0.08-0.42)							

Table 6. Number of yearling Chinook salmon released, number of PIT tags detected on the East Sand Island Caspian Tern and Double-crested Cormorant colonies, and predation rate from 2008 releases at Astoria (rkm 10) and Skamania Landing (rkm 225). Geomean ratios of predation rates for Astoria/Skamania (P_A/P_S) releases are shown with 95% confidence intervals.

Release day	Astoria (rkm 10) Juveniles released	PIT tags detected	Predation rate	Skamania Landing (rkm 225) Juveniles released	PIT tags detected	Predation rate	P_A/P_S
			Wild Chinook salmon				
Apr 24	388	12	3.07	474	17	3.59	0.86
May 1	576	6	1.04	954	62	6.50	0.16
May 8	844	2	0.24	1,234	59	4.78	0.05
May15	500	0	0.00	692	17	2.46	
May 24	405	0	0.00	682	5	0.73	
May 31	323	1	0.31	484	5	1.03	0.30
Total	3,036	21	0.69	4,520	165	3.65	0.19
Geomean P_A/P_S = 0.14, 95% CI 0.02-1.14							
			Hatchery Chinook salmon				
Apr 24	1,560	78	5.00	2,927	193	6.59	0.76
May 1	1,337	29	2.17	2,395	162	6.76	0.32
May 8	3,118	14	0.45	3,001	217	7.23	0.06
May15	4,649	20	0.43	6,014	241	4.01	0.11
May 24	4,088	4	0.10	6,384	88	1.38	0.07
May 31	1,767	6	0.34	2,996	49	1.64	0.21
Total	16,519	151	0.91	23,717	950	4.01	0.23
Geomean P_A/P_S = 0.17, 95% CI 0.06-0.47							
			Wild and hatchery Chinook salmon combined				
Apr 24	1,948	90	4.62	3,401	210	6.17	0.75
May 1	1,913	35	1.83	3,349	224	6.69	0.27
May 8	3,962	16	0.40	4,235	276	6.52	0.06
May15	5,149	20	0.39	6,706	258	3.85	0.10
May 24	4,493	4	0.09	7,066	93	1.32	0.07
May 31	2,090	7	0.33	3,480	54	1.55	0.22
Total	19,555	172	0.88	28,237	1,115	3.95	0.22
Geomean P_A/P_S = 0.16, 95% CI 0.06-0.45							

Most detections of PIT tags from both fish species and both release sites were on the Caspian Tern colony. The Astoria release groups showed lower tag proportions detected on both tern and cormorant colonies than did the Skamania Landing release groups for all but one replicate (first Chinook salmon replicate for cormorants) in 2008 (Tables 7).

Table 7. Percentage of PIT tags detected by date on the East Sand Island Caspian Tern and Double-crested Cormorant colonies for both steelhead and yearling Chinook salmon released during 2008.

	Steelhead tags detected (%)			
	Caspian Tern		Double-crested Cormorant	
Date release	Astoria	Skamania	Astoria	Skamania
23 and 24 Apr	11.01	19.16	0.26	0.53
30 Apr and 1 May	3.82	18.58	0.16	0.80
6 and 8 May	0.71	7.13	0.56	3.66
13 and 15 May	0.50	7.13	0.22	2.49
20 and 22 May	0.46	5.07	0.07	0.57
27 and 28 May	5.07	13.40	0.07	1.23
Total	4.22	13.62	0.22	1.28

	Chinook salmon tags detected (%)			
	Caspian Tern		Double-crested Cormorant	
Date release	Astoria	Skamania	Astoria	Skamania
23 and 24 Apr	2.86	4.82	1.74	1.35
30 Apr and 1 May	1.31	5.08	0.52	1.61
6 and 8 May	0.20	4.56	0.20	1.96
13 and 15 May	0.25	2.83	0.14	1.01
20 and 22 May	0.07	1.00	0.02	0.31
27 and 28 May	0.33	0.95	0.00	0.60
Total	0.57	2.91	0.31	1.04

Pathogen Analyses

Overall results for prevalence of *R. salmoninarum* detected by nPCR and qPCR are shown in Table 8. The proportion of fish in which *R. salmoninarum* was detected by qPCR was significantly higher than that detected by nPCR in gill samples from wild and hatchery Chinook salmon and wild and hatchery steelhead ($P < 0.0001$ for all comparisons). By nPCR testing alone, no difference in *R. salmoninarum* prevalence was detected among wild Chinook salmon, hatchery Chinook salmon, wild steelhead, and hatchery steelhead ($P = 0.84$). By qPCR testing alone, *R. salmoninarum* prevalence was not significantly different among wild Chinook salmon, hatchery Chinook salmon, and wild steelhead ($P = 0.41$), but the prevalence was significantly higher in these fish groups than in hatchery steelhead ($P = 0.006$).

Results for *N. salmonis* prevalence detected by nPCR and qPCR are shown in Table 8. For fish groups (wild and hatchery steelhead) for which there were sufficient *N. salmonis*-positive fish for statistical analysis, there was no significant difference in prevalence detected by nPCR and qPCR ($P \geq 0.33$). The prevalence of *N. salmonis* was significantly higher in hatchery steelhead than in wild Chinook salmon or hatchery Chinook salmon by both nPCR and qPCR testing ($P < 0.0001$). The *N. salmonis* prevalence was also higher in hatchery steelhead than in wild steelhead by both PCRs ($P \leq 0.0002$), and the prevalence of this pathogen was higher in wild steelhead than in wild and hatchery Chinook salmon by both PCRs ($P \leq 0.0005$).

Table 8. Pathogen detection in gill samples from all tested fish. Detection of *Renibacterium salmoninarum* and *Nucleospora salmonis* by nested PCR (nPCR) and quantitative PCR (qPCR) in gill tissues from hatchery and wild Chinook salmon smolts and hatchery and wild steelhead smolts sampled non-lethally at the time of tagging from the five release groups of fish marked with PIT tags at Lower Granite Dam during 2008.

	Number of fish affected/number tested (%)			
	Renibacterium salmoninarum		*Nucleospora salmonis*	
	nPCR	qPCR	nPCR	qPCR
Yearling Chinook salmon				
Wild	109/409 (26.7)	228/409 (55.7)	3/409 (0.7)	2.409 (0.5)
Hatchery	128/474 (27.0)	284/473 (60.0)	8/474 (1.7)	0/474 (--)
Steelhead				
Wild	125/432 (28.9)	246/432 (56.9)	20/432 (4.6)	14/432 (3.2)
Hatchery	128/483 (26.5)	237/483 (49.1)	55/483 (11.4)	66/483 (13.7)

Among fish testing positive for *R. salmoninarum* by qPCR, *R. salmoninarum* levels were generally less than 100 bacteria/mg of gill sample; samples from only 14 fish had *R. salmoninarum* concentrations exceeding this level. These included 2 hatchery steelhead (highest concentration 601 *R. salmoninarum*/mg), 2 wild steelhead (highest concentration 114 *R. salmoninarum*/mg), 9 hatchery Chinook salmon (highest concentration 204 *R. salmoninarum*/mg), and 1 wild Chinook salmon (concentration 146,038 *R. salmoninarum*/mg). However, more than half (61 to 71%) the qPCR-positive fish of each species had *R. salmoninarum* levels above the threshold for consistent detection by the assay (5 bacteria/qPCR reaction; Table 9). *R. salmoninarum* levels in wild and hatchery Chinook salmon and wild and hatchery steelhead were not significantly different ($P = 0.29$; Table 8).

Table 9. Mean levels of *Renibacterium salmoninarum* detected by qPCR in smolts sampled non-lethally from the six release groups of fish marked with PIT tags at Lower Granite Dam during 2008.

Fish species	Number above threshold for consistent detection of *R. salmoninarum*[a]/Total number positive by qPCR (%)	Geometric mean number *R. salmoninarum*/mg gill sample[b] (±SD)
Yearling Chinook salmon		
Wild	149/228 (65)	14 (±3)
Hatchery	175/284 (62)	14 (±2)
Juvenile steelhead		
Wild	151/246 (61)	13 (±2)
Hatchery	168/237 (71)	15 (±2)

[a] Five bacteria/qPCR reaction.
[b] Number of *R. salmoninarum*/mg calculated according to the following formula:
(Number of *R. salmoninarum*/reaction × 40) / Sample weight, where 40 is the dilution factor

The newly available qPCR test for *N. salmonis* enabled quantification of levels of this parasite in gill samples for the first time during testing of the 2008 samples. No hatchery Chinook salmon were positive for *N. salmonis* by qPCR, and the two wild Chinook salmon testing qPCR-positive for the parasite had very low levels (3 DNA copies/PCR reaction; Table 10). Among fish testing positive for *N. salmonis* by qPCR, *N.* levels were greater than 100 DNA copies per PCR reaction for 3 (21%) of positive wild steelhead and 20 (30%) of positive hatchery steelhead. The highest *N. salmonis* concentration detected in wild steelhead was 131 DNA copies per reaction, and the highest concentration detected in hatchery steelhead was 5,605 DNA copies per reaction, but there was no significant difference between the two steelhead groups in the median *N. salmonis* copy numbers detected ($P = 0.27$). Half or more of the qPCR-positive wild and hatchery steelhead had *N. salmonis* levels above the threshold for consistent detection by the assay (16 DNA copies/qPCR reaction; Table 10).

Table 10. Mean levels of *Nucleospora salmonis* detected by qPCR in smolts sampled non-lethally from the six release groups of fish marked with PIT tags at Lower Granite Dam during 2008.

Fish species	Number above threshold for consistent detection of *N. salmonis**/Total number positive by qPCR (%)	Geometric mean *N. salmonis* DNA copy number/reaction (±SD)
Chinook salmon wild	0/2	3 (---)
Chinook salmon hatchery	0/0	---
Steelhead wild	7/14 (50)	19 (±4)
Steelhead hatchery	42/66 (64)	47 (±8)

* Sixteen DNA copies/qPCR reaction

Proportions of fish testing positive for *R. salmoninarum*, *N. salmonis*, or both varied by species and sample date (Table 11). For hatchery Chinook salmon and steelhead, the highest percentages of fish positive for one or both pathogens (79 and 74%, respectively) were recorded on 11 May (70% and 65% of hatchery Chinook salmon and steelhead, respectively, had passed by this date). For wild fish, the highest percentage of Chinook salmon positive for one or both pathogens (83%) was recorded on 27 April, and the highest percentage of steelhead positive for either or both pathogens (81%) was recorded on 4 May. For wild Chinook salmon, proportions of fish positive for either or both pathogens were significantly higher for samples collected on 27 April (13% of unclipped Chinook salmon had passed by this date) than for the samples collected on all other dates ($P = 0.01$). Proportions of hatchery Chinook salmon positive for either or both pathogens did not differ significantly among sample dates ($P = 0.14$). For wild steelhead, proportions of fish positive for one or both pathogens were significantly higher for samples collected on 4 May than for samples collected on all other dates ($P = 0.007$). For hatchery steelhead, the proportions of fish positive for either or both pathogens were not significantly different among sample dates ($P = 0.72$).

When data were combined for all sample dates, the proportions of hatchery and wild Chinook salmon and steelhead positive for either or both pathogens were not significantly different ($P = 0.37$). Overall, one or both pathogens were detected in 68% of the wild Chinook salmon, 72% of the hatchery Chinook salmon, 66% of the wild steelhead, and 68% of the hatchery steelhead.

Table 11. Proportions of fish testing positive for *Renibacterium salmoninarum* only, *Nucleospora salmonis* only, both, or neither of these pathogens among all fish by sample date from PIT tagged groups at Lower Granite Dam in 2008.

		Number and proportion (%) of fish			
Sample date and fish species	Number tested	Positive *R. salmoninarum* only[a]	Positive *N. salmonis* only[b]	Positive *R. salmoninarum* and *N. salmonis*	Neither pathogen detected
20 April					
Chinook W	73	38 (52)	0	1 (1)	34 (47)
Chinook H	76	46 (61)	0	1 (1)	29 (38)
Steelhead W	77	36 (47)	2 (3)	4 (5)	35 (45)
Steelhead H	73	37 (51)	9 (13)	1 (1)	25 (35)
27 April					
Chinook W	66	55 (83)	0	0	11 (17)
Chinook H	79	55 (70)	0	0	24 (30)
Steelhead W	73	39 (53)	1 (1)	1 (1)	32 (44)
Steelhead H	82	50 (61)	0	6 (7)	26 (32)
4 May					
Chinook W	73	47 (64)	1 (1)	1 (1)	24 (33)
Chinook H	67	46 (69)	2 (3)	2 (3)	17 (25)
Steelhead W	73	52 (71)	3 (4)	4 (5)	14 (19)
Steelhead H	87	51 (59)	6 (7)	5 (6)	25 (29)
11 May					
Chinook W	65	46 (71)	0	0	19 (29)
Chinook H	86	68 (79)	0	0	18 (21)
Steelhead W	76	49 (64)	0	5 (7)	22 (29)
Steelhead H	73	34 (47)	7 (10)	13 (18)	19 (26)
18 May					
Chinook W	78	53 (68)	0	1 (1)	24 (31)
Chinook H	74	56 (76)	0	1 (1)	17 (23)
Steelhead W	72	49 (47)	1 (1)	0	22 (31)
Steelhead H	75	37 (49)	2 (3)	8 (11)	28 (37)
25 May					
Chinook W	54	35 (65)	0	1 (2)	18 (33)
Chinook H	92	60 (65)	1 (1)	1 (1)	30 (33)
Steelhead W	61	37 (61)	0	3 (5)	21 (34)
Steelhead H	93	39 (42)	6 (6)	17 (18)	31 (33)
All dates					
Chinook W	409	274 (67)	1 (<1)	4 (1)	130 (32)
Chinook H	474	331 (70)	3 (1)	5 (1)	135 (28)
Steelhead W	432	262 (61)	6 (1)	17 (4)	146 (34)
Steelhead H	483	248 (51)	30 (6)	50 (10)	155 (32)

[a] Positive for *R. salmoninarum* by nPCR, qPCR, or both PCRs.
[b] Positive for *N. salmonis* by nPCR, qPCR, or both PCRs

Hatchery or wild Chinook salmon and wild steelhead that tested positive for either *R. salmoninarum* or *N. salmonis* or both pathogens by nPCR and/or qPCR did not differ significantly in length compared to fish that tested negative for both pathogens ($P \geq 0.08$). However, hatchery steelhead that tested positive for one or both pathogens were significantly longer ($P = 0.049$) than those that tested negative for both pathogens. Hatchery steelhead that tested positive for *R. salmoninarum* did not differ significantly in length ($P = 0.39$) from those that tested negative; however, hatchery steelhead that tested positive for *N. salmonis* were significantly longer ($P < 0.0001$) than those that tested negative. Mean lengths of hatchery steelhead testing positive for *N. salmonis* by either PCR were consistently greater than mean lengths of fish testing negative for *N. salmonis* on all sample dates, but significant differences in length were detected only in fish sampled on 4 May ($P = 0.04$) and 25 May ($P = 0.006$).

There was no significant correlation between length and *R salmoninarum* level detected by qPCR in gill samples for wild Chinook salmon ($P = 0.22$) or wild steelhead ($P = 0.48$). However, there was a significant negative correlation between length and *R. salmoninarum* levels for hatchery Chinook salmon ($P = 0.02$) and hatchery steelhead ($P = 0.03$). No significant correlation between length and *N. salmonis* levels was detected for wild steelhead ($P = 0.71$) or hatchery steelhead ($P = 0.25$); numbers of Chinook salmon testing qPCR-positive for *N. salmonis* were insufficient for statistical analysis.

Among PIT tags recovered from the East Sand Island Caspian Tern and Double-crested Cormorant colonies, 141 (33 Chinook salmon and 108 steelhead) were from fish that had been sampled at Lower Granite Dam for detection of *R. salmoninarum* and *N. salmonis*. The majority of PIT tags (121) were recovered from the tern colony; the remaining 20 were recovered from the cormorant colony. Of fish with PIT tags recovered on bird colonies, 90% of wild and 74% of hatchery Chinook salmon and 56% of wild and 59% of hatchery steelhead were positive for one or both pathogens (Table 12).

Overall, 63% of the fish testing positive for *R. salmoninarum* by qPCR had levels of the bacterium above the threshold for consistent detection by the assay (5 bacteria/qPCR reaction; Table 13). *R. salmoninarum* concentrations for qPCR-positive fish ranged from 3 to 56 bacteria/mg for wild Chinook salmon, from 3 to 47 bacteria/mg for hatchery Chinook salmon, from 2 to 54 bacteria/mg for wild steelhead and from 4 to 39 bacteria/mg for hatchery steelhead. *N. salmonis* concentrations for qPCR-positive hatchery steelhead ranged from 2 to 5,605 DNA copies per reaction; 4 of the 10 qPCR-positive fish had *N. salmonis* levels ≥143 DNA copies per reaction.

Table 12. Proportions of fish testing positive for *Renibacterium salmoninarum* only, for *Nucleospora salmonis* only, for both pathogens, or for neither pathogen among fish sampled at Lower Granite Dam during 2008, with PIT tags subsequently recovered on the East Sand Island piscivorous bird colonies.

		Number and proportion (%) of fish			
Fish species	Number tested	*R. salmoninarum* positive only[a]	*N. salmonis* positive only[b]	*R. salmoninarum* and *N. salmonis* positive	Neither pathogen detected
Chinook W	10	9 (90)	0	0	1 (10)
Chinook H	23	17 (74)	0	0	6 (26)
Steelhead W	39	22 (56)	0	0	17 (44)
Steelhead H	69	28 (41)	7 (10)	6 (9)	28 (41)

[a] Positive for *R. salmoninarum* by nPCR, qPCR, or both PCRs.
[b] Positive for *R. salmoninarum* by nPCR, qPCR, or both PCRs.

Table 13. Mean levels of *Renibacterium salmoninarum* and *Nucleospora salmonis* detected by qPCR in smolts sampled at Lower Granite Dam during 2008, with PIT tags subsequently recovered on the East Sand Island piscivorous bird colonies. No PIT tags recovered from the bird colonies were from hatchery or wild Chinook salmon or wild steelhead that had tested positive for *N. salmonis* by qPCR at Lower Granite Dam.

Fish species	Number of fish above threshold for consistent detection of *R. salmoninarum*[a] by qPCR /total positive (%)	Geometric mean number *R. salmoninarum*/mg gill sample[b] (±SD)
Chinook (W)	6/8 (75)	15 (±3)
Chinook (H)	9/15 (60)	10 (±2)
Steelhead (W)	11/17 (65)	11 (±2)
Steelhead (H)	18/30 (60)	13 (±2)

	Number of fish above threshold for consistent detection of *N. salmonis*[a] by qPCR/total positive (%)[c]	Geometric mean *N. salmonis* DNA copy number/reaction (±SD)
Steelhead (H)	4/10 (40%)	47 (±17)

[a] Five bacteria/qPCR reaction.
[b] Number of *R. salmoninarum*/mg calculated according to the following formula: (Number of *R. salmoninarum*/reaction × 40)/Sample weight, where 40 is the dilution factor.
[c] Sixteen DNA copies/qPCR reaction

R. salmoninarum concentrations in water samples taken from the Lower Granite Dam tagging station ranged from no bacteria detected to 4 bacteria/mL (Table 14). For all sample dates, daily mean *R. salmoninarum* concentrations in water samples were ≤ 1 bacteria/mL.

Table 14. Mean *Renibacterium salmoninarum* concentrations in water samples taken from the PIT-tagging station from which fish were also sampled for pathogen testing at Lower Granite Dam, 2008. After the first two water samples were taken each day, water in the recirculating system was changed.

Sample date and time (PDT)	Mean *R. salmoninarum*/mL (range)
20 April	
1000	1 (0-2)
1145	1 (0-2)
1240	2 (0-4)
1430	1 (0-2)
27 April	
0925	0
1135	1 (0-2)
1240	1 (0-2)
1415	1 (0-4)
4 May	
0900	0
1101	1 (0-2)
1240	2 (0-4)
1415	0
11 May	
0900	1 (0-2)
1125	0
1230	0
1405	1 (0-2)
18 May	
0900	2 (0-4)
1145	0
1230	1 (0-4)
1430	1 (0-2)
11 May	
0915	0
1130	1 (0-2)
1225	1 (0-2)
1355	0

For qPCR-positive gill snip samples, daily mean *R. salmoninarum* levels ranged from 8 to 24 bacteria/mg tissue, and daily mean *N. salmonis* levels ranged from 20 to 99 DNA copies/qPCR reaction (Table 15). The highest daily mean *R. salmoninarum* concentration was recorded on 27 April, and the highest daily mean *N. salmonis* concentration was recorded on the same date. The lowest daily mean gill tissue concentrations for both pathogens were recorded on 20 April.

Table 15. Mean levels of *Renibacterium salmoninarum* and *N. salmonis* detected by qPCR in daily samples of smolts (qPCR-positive smolts of both species combined) sampled non-lethally during PIT tagging at Lower Granite Dam in 2007. For the *R. salmoninarum* qPCR results, means not sharing a common letter are significantly different ($P < 0.0.05$). There were no significant differences between daily mean *N. salmonis* levels ($P > 0.05$).

Sample date	Geometric daily mean *R. salmoninarum* per mg gill sample (±SD)	Geometric daily mean *N. salmonis* DNA copies per qPCR reaction (±SD)
20 April	8 (±2) x	20 (±4)
27 April	24 (±3) z	99 (±5)
4 May	20 (±2) z	45 (±13)
11 May	13 (±2) y	23 (±6)
18 May	10 (±2) x	22 (±3)
25 May	13 (±2) y	66 (±10)

Comparison of Smolt-to-Adult Returns

Adult returns are now complete for study fish released in 2006. Based on adult returns of juvenile steelhead released in 2006, use of an alternate release site in the lower estuary for transported smolts appeared to provide a modest improvement in SARs for hatchery, but not wild fish (Table 16). From wild and hatchery steelhead combined, 509 adults returned from the Astoria releases for a SAR of 1.75, while 551 adults returned from the Skamania Landing releases for a SAR of 1.32. Geomean T_A/T_S from these SARs were 0.95 (95% CI, 0.51-1.77) for wild steelhead, 1.22 (1.04-1.44) for hatchery steelhead, and 1.20 (1.01-1.41) for wild and hatchery fish combined.

Table 16. Numbers of juvenile steelhead released in 2006, adult returns from these releases, and smolt-to-adult return ratios (SARs) at Astoria (rkm 10) and Skamania Landing (rkm 225). Ratios of SARs for transported fish released at Astoria vs. Skamania (T_A/T_S) and geomean with 95% CIs are also shown.

	Fish released in 2006						
	Astoria (rkm 10)			Skamania Landing (rkm 225)			SARs ratio
Release day	Juveniles released	Adults	SAR	Juveniles released	Adults	SAR	T_A/T_S
Wild steelhead							
Apr 24	455	15	3.30	526	14	2.66	1.24
May 1	439	1	0.23	807	5	0.62	0.37
May 8	553	9	1.63	912	9	0.99	1.65
May15	290	4	1.38	609	5	0.82	1.68
May 24	1,227	6	0.49	1,977	15	0.76	0.64
May 31	479	8	1.67	775	14	1.81	0.92
Total	3,443	43	1.25	5,606	62	1.11	1.13
Geomean Astoria/Skamania = 0.95 (95% CI, 0.51-1.77)							
Hatchery steelhead							
Apr 24	6,694	169	2.52	5,655	97	1.72	1.47
May 1	3,239	41	1.27	4,915	65	1.32	0.96
May 8	3,893	90	2.31	5,362	92	1.72	1.35
May15	4,292	75	1.75	6,500	99	1.52	1.15
May 24	5,503	61	1.11	9,703	92	0.95	1.17
May 31	2,091	30	1.43	4,064	44	1.08	1.33
Total	25,712	466	1.81	36,199	489	1.35	1.34
Geomean Astoria/Skamania = 1.22 (95% CI 1.04-1.44)							
Wild and hatchery steelhead combined							
Apr 24	7,149	184	2.57	6,181	111	1.80	1.43
May 1	3,678	42	1.14	5,722	70	1.22	0.93
May 8	4,446	99	2.23	6,274	101	1.61	1.38
May15	4,582	79	1.72	7,109	104	1.46	1.18
May 24	6,730	67	1.00	11,680	107	0.92	1.09
May 31	2,570	38	1.48	4,839	58	1.20	1.23
Total	29,155	509	1.75	41,805	551	1.32	1.32
Geomean Astoria/Skamania = 1.20 (95% CI 1.01-1.41)							

For yearling Chinook salmon, use of the lower estuary release site did not improve SARs for either hatchery or wild fish (Table 17). For wild and hatchery yearling Chinook salmon combined, 53 adults returned from the Astoria releases for a SAR of 0.33, while 139 adults returned from the Skamania Landing releases for a SAR of 0.57. These returns resulted in a geomean T_A/T_S of 0.40 (95% CI, 0.02-10.64) for wild Chinook salmon, 0.55 (0.19-1.59) for hatchery Chinook salmon, and 0.49 (0.18-1.31) for wild and hatchery fish combined. Based on returns from 2006 releases, the effect of transporting smolts to the estuary did not appear to vary seasonally.

Table 17. The number of juveniles released, returning adults, and smolt-to-adult returns (SAR) from 2006 releases of yearling Chinook salmon at Astoria (rkm 10) and Skamania Landing (rkm 225). The ratio of Astoria/Skamania SARs and geomean with 95% confidence intervals are also shown.

	Fish released in 2006						
	Astoria (rkm 10)			Skamania Landing (rkm 225)			SARs ratio
Release day	Juveniles released	Adults	SAR	Juveniles released	Adults	SAR	T_A/T_S
Wild Chinook salmon							
Apr 24	902	0	0.00	958	2	0.21	0.14*
May 1	747	1	0.13	1,332	8	0.60	
May 8	262	4	1.53	495	1	0.20	1.76*
May15	184	1	0.54	289	4	1.38	
May 24	329	1	0.30	561	6	1.07	0.26*
May 31	32	0	0.00	103	1	0.97	
Total	2,456	7	0.29	3,738	22	0.59	0.48
Geomean Astoria/Skamania = 0.40 (95% CI 0.02-10.64)							
Hatchery Chinook salmon							
Apr 24	2,852	1	0.04	2,192	7	0.32	0.11
May 1	3,568	5	0.14	5,929	32	0.54	0.26
May 8	3,312	16	0.48	5,568	37	0.66	0.73
May15	3,309	18	0.54	5,456	33	0.60	0.90
May 24	703	5	0.71	1,448	7	0.48	1.49*
May 31	15	0	0.00	46	0	0.00	
Total	13,759	45	0.33	20,639	116	0.56	0.58
Geomean Astoria/Skamania = 0.55 (95% CI 0.19-1.59)							
Wild and hatchery Chinook salmon combined							
Apr 24	3,754	1	0.03	3,150	9	0.29	0.09
May 1	4,315	6	0.14	7,261	40	0.55	0.25
May 8	3,574	20	0.56	6,063	38	0.63	0.89
May15	3,493	19	0.54	5,745	37	0.64	0.84
May 24	1,032	6	0.58	2,009	13	0.65	0.86*
May 31	47	0	0.00	149	1	0.67	
Total	16,215	52	0.32	24,377	138	0.57	0.57
Geomean Astoria/Skamania = 0.49 (95% CI 0.18-1.31)							

* Due to low numbers of returning adults, SARs for some weekly releases were combined for analysis.

Preliminary adult returns from 2007 and 2008 releases are shown in Table 18. Adult returns will be complete in 2010 and 2011 from releases made in 2007 and 2008, respectively.

Table 18. Number of adults and smolt-to-adult return rates (SARs) from 2007 and 2008 releases of yearling Chinook salmon and steelhead at Skamania Landing (rkm 225) and Astoria (rkm 10) based on returns through October 6, 2009.

Release year and location	Yearling Chinook salmon		Steelhead	
	Adults	SAR	Adults	SAR
2007				
Skamania	136	0.78	468	1.50
Astoria	84	0.74	282	1.24
2008				
Skamania	92	0.33	312	0.77
Astoria	72	0.37	281	0.90

Comparison of Adult Conversion and Straying Rates

Conversion rates (the percent of adults detected at Bonneville Dam and later detected at Lower Granite Dam) were significantly lower for smolts transported to Astoria for release in 2006 than those released at the traditional release site at Skamania Landing (Table 19). For steelhead, 48% of fish released at Astoria were detected at both Bonneville and Lower Granite Dams, while 60% of those released at Skamania Landing were detected at both dams. The geomean ratio of their conversion rates (Astoria/Skamania) was 0.78 (95% CI, 0.68-0.90). Conversion rates were lower in the Bonneville Dam to McNary Dam reach than in the McNary Dam to Lower Granite Dam reach, likely due to zone 6 fisheries. There was no apparent seasonal trend in conversion rates for steelhead. Conversion rates were similar between hatchery and wild steelhead and slightly lower for 1-ocean than 2-ocean steelhead.

Fifty-three steelhead from 2006 releases were detected straying into tributary streams; 45 into the John Day River, 4 into the Walla Walla River, 2 into the Yakima River, and 2 into the Umatilla River, with 10 of the 53 eventually crossing Lower Granite Dam. The majority of steelhead that strayed (64%) were from Astoria releases. An additional 21 steelhead were detected at upper Columbia River Dams with 3 of the 21 eventually crossing Lower Granite Dam.

Table 19. Conversion rates for steelhead (hatchery and wild combined) released from barges at Astoria (rkm 10) and Skamania Landing (rkm 225) in 2006. The ratio of conversion rates from Bonneville to Lower Granite Dam is also shown.

Release group	Release location	Steelhead conversion rates			Astoria/Skamania ratio for Bonneville to Lower Granite
		Bonneville to McNary Dam	McNary to Lower Granite Dam	Bonneville to Lower Granite Dam	
1	Astoria	0.6050	0.8035	0.5000	0.80
	Skamania	0.6872	0.8720	0.6257	
	Total	0.6322	0.8277	0.5416	
2	Astoria	0.6744	0.6613	0.4767	0.77
	Skamania	0.7636	0.8023	0.6182	
	Total	0.7245	0.7432	0.5561	
3	Astoria	0.6906	0.7578	0.5359	0.91
	Skamania	0.7209	0.8065	0.5872	
	Total	0.7054	0.7817	0.5609	
4	Astoria	0.7029	0.7778	0.5507	0.89
	Skamania	0.7301	0.8443	0.6196	
	Total	0.7176	0.8145	0.5880	
5	Astoria	0.5398	0.6735	0.3750	0.70
	Skamania	0.7164	0.7431	0.5323	
	Total	0.6340	0.7149	0.4589	
6	Astoria	0.5326	0.7451	0.4130	0.65
	Skamania	0.7889	0.7945	0.6333	
	Total	0.6593	0.7742	0.5220	
Total	Astoria	0.6213	0.7541	0.4821	0.81
	Skamania	0.7268	0.8101	0.5967	
	Total	0.6708	0.7823	0.5359	

Geomean Astoria/Skamania = 0.78, 95% CI 0.68-0.90

For yearling Chinook salmon, 60% of the 2006 Astoria released fish were detected at both Bonneville and Lower Granite Dams, while 73% of those released at Skamania Landing were detected at both dams (Table 20). The geomean ratio of their conversion rates (Astoria/Skamania) was 0.76 (95% CI 0.47-1.23). Conversion rates were lower in the Bonneville Dam to McNary Dam reach than in the McNary Dam to Lower Granite Dam reach, likely due to zone 6 fisheries. Conversion rates showed an improving trend during the migration season. Conversion rates were similar between hatchery and wild yearling Chinook salmon and slightly lower for jacks than for older age classes. Only one yearling Chinook salmon from 2006 releases was detected straying into the upper Columbia River.

Table 20. Conversion rates for yearling Chinook salmon (hatchery and wild combined) released from barges at Astoria (rkm 10) and Skamania Landing (rkm 225) in 2006. The ratio of conversion rates (A/S) from Bonneville Dam (BON) to Lower Granite Dam (LGR) is also shown.

		Yearling Chinook salmon conversion rates			Astoria/Skamania ratio for Bonneville to Lower Granite
Release group	Release location	Bonneville to McNary Dam	McNary to Lower Granite Dam	Bonneville to Lower Granite Dam	
1	Astoria	0.5000	1.0000	0.5000	
	Skamania	0.7857	0.8182	0.6429	0.78
	Total	0.7500	0.8333	0.6250	
2	Astoria	0.5556	0.6000	0.3333	
	Skamania	0.9020	0.8696	0.7843	0.43
	Total	0.8116	0.8214	0.6667	
3	Astoria	0.6389	0.8696	0.5556	
	Skamania	0.8298	0.9500	0.8085	0.69
	Total	0.7470	0.9206	0.6988	
4	Astoria	0.6389	1.2609	0.8056	
	Skamania	0.7885	0.9024	0.7115	1.13
	Total	0.7273	1.0313	0.7500	
5	Astoria	0.7000	0.8571	0.6000	
	Skamania	0.5600	0.8571	0.5200	1.15
	Total	0.6000	0.8571	0.5429	
6	Astoria	1.0000	--	--	
	Skamania	1.0000	1.0000	1.0000	--
	Total	1.0000	0.5000	0.5000	
Total	Astoria	0.6311	0.9538	0.6019	
	Skamania	0.8000	0.8954	0.7263	0.83
	Total	0.7406	0.9128	0.6826	

Geomean Astoria/Skamania = 0.76, 95% CI 0.47-1.23

DISCUSSION

One goal of this study was to evaluate whether an alternate release site downstream from the Astoria Bridge could increase survival to ocean entry for transported salmonids. We hypothesized that fish released at this site would survive to the ocean at higher rates by avoiding some avian predators in the Columbia River estuary. Steelhead are particularly vulnerable to predation by piscivorous birds; Collis et al. (2001) reported that over 15% of the PIT tags from steelhead detected at Bonneville Dam in 1998 were later found on estuarine bird colonies. In contrast, they found only 2% of the PIT tags from yearling Chinook detected at the dam that year.

In 1998 the greatest number of PIT tags was recovered on Rice Island, which hosted the largest Caspian Tern colony in North America (Collis et al. 2002). Ryan et al. (2002, 2003) and Glabek et al. (2003) reported similar results on East Sand Island after the USACE began efforts to relocate the tern colony from Rice to East Sand Island. To optimize survival, our study fish were released at night on an ebb tide. Based on data from evaluations of estuary travel time (Ledgerwood et al. 2001), we expected that most study fish would reach the ocean during one tidal cycle. Thus the majority of fish would pass the colonies in hours of darkness, when foraging by avian predators was not likely. Our results supported this hypothesis: of the total number of PIT-tags found on both large avian colonies in the estuary, a significantly higher proportion were from salmonids released at Skamania than from those released at Astoria.

Use of the alternate release site near Astoria could improve survival for other reasons, in addition to facilitating the avoidance of avian predators. Ward et al. (1995) sampled multiple locations in the lower Snake and middle and lower Columbia Rivers to develop an index of predation by northern pikeminnow *Ptychocheilus oregonensis*. They found the highest rates of predation in the Columbia River below Bonneville Dam, where the Skamania Landing release site is located. By transporting smolts farther downstream to Astoria, this source of potential mortality can be avoided.

Release in the lower estuary would also allow migrating smolts to avoid the area near the Willamette River confluence, where high levels of toxic chemicals have been found (Spromberg et al. 2008). Barged fish would still pass through this area (and be exposed to the same water as migrating fish), but they would likely be exposed to any toxic chemicals for a shorter duration. Finally, during years with low flow/high water temperature, steelhead migrants often residualize in reservoirs late in the migration. Few of these residuals survive to migrate the following spring (Williams et al. 2005).

Releasing them near the mouth of the river in strong current during an ebb tide might encourage these fish to migrate rather than overwinter in reservoirs, thus improving overall SARs.

In the end, the more important question is not which release site allows juvenile salmonids to survive at greater rates to ocean entry, but rather which release site produces the greatest SAR. It is conceivable that survival to ocean entry could be higher for fish released at Astoria, but that even with higher short-term survival, these fish could return at the same or lower rates than fish released at Skamania Landing. This could occur if fish released at Astoria were not physiologically prepared to enter seawater. Conversely, the group released at Astoria could be in better condition due to avoiding migration through the lower Columbia River, and could produce higher SARs. Ultimately, the success of either release site will be determined by examining differences in SARs among release groups, and understanding the effect of differential straying on fish with different release sites.

Another goal of the study was to monitor the prevalence and levels of the fish pathogens *R. salmoninarum* and *N. salmonis* by non-lethal testing of subsamples of fish PIT-tagged at Lower Granite Dam. Both *R. salmoninarum* (see Alcorn et al. 2005 for summary) and *N. salmonis* (Wongtavatchai et al. 1995) can cause chronic infections and have immunosuppressive properties. Although infections with either pathogen can be directly fatal, they can also allow for opportunistic infections with other pathogens.

The PCR testing of gill snip samples taken from fish at Lower Granite Dam indicated significant year-to-year variation in *R. salmoninarum* prevalence. The detected *R. salmoninarum* prevalence was significantly higher in each species in 2008 than in 2007 ($P < 0.0001$). Conversely, the detected *R. salmoninarum* prevalence was significantly higher in 2006 than in 2008 in wild and hatchery yearling Chinook salmon ($P = 0.007$, $P = 0.01$, respectively) and wild steelhead ($P = 0.006$), but not in hatchery steelhead ($P = 0.24$). Data from qPCR testing also indicated significantly higher *R. salmoninarum* levels in gill samples from each species in 2008 compared with 2007 ($P < 0.001$), but no significant difference was detected in *R. salmoninarum* levels in gill samples from each species in 2008 compared with 2006 ($P > 0.05$). Whereas 61 to 71% of qPCR-positive fish in 2008 and 52 to 55% of qPCR-positive fish in 2006 had *R. salmoninarum* levels at or above the threshold for consistent detection by assay (5 bacteria/qPCR reaction), only 17 to 38% of qPCR-positive fish in 2007 had *R. salmoninarum* levels at or above this threshold. Correlation of *R. salmoninarum* infection profiles with SARS will require completion of the adult return data.

A significant negative correlation ($P \leq 0.03$) between fish length and *R. salmoninarum* levels was observed for both yearling hatchery Chinook salmon and hatchery steelhead in the 2008 Lower Granite Dam sample. A significant negative correlation ($P = 0.01$) between fish length and *R. salmoninarum* levels was also detected for yearling wild Chinook salmon in the 2006 sample. No significant correlations between fish length and *R. salmoninarum* levels was observed for any species in the 2007 sample, but levels of this pathogen detected by qPCR were significantly lower than in the other years. It is unknown whether the growth of the fish was affected directly by *R salmoninarum* infection.

Year-to-year variation in prevalence and severity of *R. salmoninarum* infections, similar to that observed in the three years of this study, has been previously reported among salmonid smolts (Pascho and Elliott 1989; Elliott and Pascho 1991, 1993; Elliott et al. 1997; Vanderkooi and Maule 1999). Although PCR results of the present research cannot be directly compared to ELISA results of past studies, limited evidence suggests that the majority of fish tested by PCR during all years of the present study would have shown negative or low *R. salmoninarum* antigen levels by ELISA testing (Chase et al. 2006). Thus they would not have been considered clinically diseased (e.g., showing grossly visible kidney lesions had they been sacrificed) at the time of sampling.

As in 2006 (Ryan et al. 2007) and 2007 (Marsh et al. 2008), the *N. salmonis* prevalence was significantly higher in hatchery steelhead than in wild steelhead or yearling wild or hatchery Chinook salmon ($P \leq 0.0002$). *N. salmonis* prevalence in yearling wild or hatchery Chinook salmon and wild steelhead was $\leq 6\%$ for all three years. In contrast, the *N. salmonis* prevalence in hatchery steelhead by nPCR (the only test available for testing the 2006 and 2007 samples) ranged from 11% in 2008 to 23% in 2006 and 30% in 2007. By nPCR testing, the *N. salmonis* prevalence in hatchery steelhead was significantly higher in 2006 and 2007 than in 2008 ($P < 0.0001$). Testing by qPCR in 2008 also showed a low *N. salmonis* prevalence (14%) in hatchery steelhead relative to the previous years. The 2008 qPCR testing indicated that hatchery steelhead were the only group in which *N. salmonis* concentrations exceeding 1,000 DNA copies per qPCR reaction were detected in some fish. Correlation of *N. salmonis* infection profiles with SARS for the three release years must await completion of the adult return data.

The explanation(s) for the significantly greater length of *N. salmonis*-infected fish in comparison to non-infected fish in 2008 is unknown. *N. salmonis*-infected fish were also found to be significantly longer than non-infected fish in 2007. *N. salmonis*-infected fish were also consistently longer than non-infected on each sample date in 2008.

However, there was no significant correlation between *N. salmonis* levels and fish length ($P > 0.05$). Because the prevalence of *N. salmonis* infections differs greatly among hatcheries in Idaho (Kathy Clemens, Idaho Fish Health Center, U.S. Fish and Wildlife Service, Orofino Idaho, personal communication), perhaps the length differences reflected differences in size of fish released from the various hatcheries.

In contrast to water samples taken from tagging stations in previous years, the *R. salmoninarum* concentrations in 2008 water samples were extremely low (≤4 bacteria/mL) and showed little variation among sample dates or among sample times on a given date. In 2006, *R. salmoninarum* concentrations exceeding 100 bacteria/mL were recorded, and in 2007, concentrations exceeding 600,000 bacteria/mL were recorded. The data from previous years suggested that elevated bacterial concentrations likely resulted when fish with severe *R. salmoninarum* infections passed through the tagging station and shed bacteria into the water within an hour before the water sample was taken. A single wild Chinook salmon with a gill *R. salmoninarum* concentration exceeding 1,000 bacteria/mL was recorded in 2008; that fish passed through the tagging station on 27 April, but no elevated *R. salmoninarum* levels were detected in water samples on that date.

The presence of *R. salmoninarum*, *N. salmonis*, or both pathogens was not shown to influence the susceptibility of steelhead and Chinook salmon to predation by piscivorous birds, although the low numbers of PIT tags recovered on East Sand Island from Chinook salmon hampered analyses for this species. A lack of preferential predation on *R. salmoninarum*-infected fish was not surprising, considering the relatively low levels of *R. salmoninarum* in the majority of fish tested during the three study years.

Nevertheless, in 2007, a PIT tag was recovered from the East Sand Island Caspian Tern colony that came from one of the two fish (both hatchery steelhead) in the sample at Lower Granite found to have an *R. salmoninarum* level exceeding 1,000 bacteria/mg of gill tissue. In addition, PIT tags from two of the six hatchery steelhead in which *N. salmonis* concentrations exceeded 1,000 DNA copies per reaction in the Lower Granite sample were recovered from the tern colony in 2008. One of these fish had the highest *N. salmonis* concentration detected by qPCR in 2008 (5,605 DNA copies per reaction). The *N. salmonis* levels could not be measured in samples prior to 2008.

Steelhead are more vulnerable than yearling Chinook salmon to predation by birds from the East Sand Island colonies, and Snake River steelhead also appear to be more heavily infected with *N. salmonis* than Snake River yearling Chinook salmon. Therefore, more testing by qPCR may be warranted to determine the possible influence

of *N. salmonis* infection levels on vulnerability of steelhead to bird predation. Previous work has indicated higher vulnerability to predation by piscivorous fish among juvenile Chinook salmon with moderate to high *R. salmoninarum* infection levels as determined by ELISA (Mesa et al. 1998), but the influence of similar levels of this pathogen or *N. salmonis* on vulnerability to avian predation has not been determined.

Previous studies with coho and Atlantic salmon found that release of transported smolts to the estuary or ocean resulted in higher adult return rates than release to freshwater (Solazzi et al. 1991; Gunnerod et al. 1988). Studies to evaluate the release of transported steelhead in the Columbia River estuary (Tongue Point) vs. Skamania Landing were conducted from 1992 to 1994 (smolt release years). For the 1994 release year, the ratio of Tongue Point to Skamania Landing adult returns was 3.0, while for the other two release years it was near 1.0. However, these results were inconclusive because too few adults returned from all three release years (Marsh et al. 1996, 1998, 2000).

Returns of PIT-tagged adults over the next several years will be required to determine whether adult returns are improved by releasing transported yearling Chinook salmon and steelhead smolts to a site lower in the Columbia River estuary (rkm 10) compared to the traditional release site at Skamania Landing (rkm 225). Based on adult returns from 2006 releases, transporting smolts to the downstream release site provided a modest benefit to steelhead, but was detrimental for yearling Chinook salmon. However, these results may vary by release year. Further, based on 2006 releases, transporting steelhead smolts (and yearling Chinook salmon smolts for the first 3 replicates) to the estuary increased the rate of straying and lowered conversion rates between Bonneville Dam and Lower Granite Dam, likely due to greater impairment of homing ability.

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