# Alternative Barging Strategies to Improve Survival of Transported Juvenile Salmonids, 2006

Report of research by

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## EXECUTIVE SUMMARY

During spring 2006, we conducted a study to test the hypothesis that releasing transported juvenile Pacific salmonids *Oncorhynchus* spp. to the lower Columbia River estuary at river kilometer (rkm) 10 would produced higher smolt-to-adult return rates (SARs) than releasing them just below Bonneville Dam at rkm 225. We speculated that releasing transported fish an additional 215 km downstream from the location presently used could decrease smolt mortality due to predation by piscivorous fish and birds. 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, starting in April 2006 and running through May, run-of-the-river yearling Chinook salmon *O. tshawytscha* and steelhead *O. mykiss* were collected and tagged with passive integrated transponder (PIT) tags at the Lower Granite Dam juvenile fish facility. Following tagging, fish were transferred to raceways and held until the following day, when they were loaded on barges for transport. A total of 13,729 hatchery and 2,435 wild yearling Chinook salmon were tagged and released downstream from Astoria at rkm 10, while 20,488 hatchery and 3,707 wild yearling Chinook salmon were tagged and released at Skamania Landing (rkm 225). In total, we released 25,726 hatchery and 3,4045 wild steelhead at rkm 10 and 36,210 hatchery and 5,612 wild steelhead at rkm 225.

During each tagging day, 300 non-lethal gill clip samples were collected for pathogen analyses (*Renibacterium salmoninarum* and *Nucleospora salmonis*), for a total of 1,800 samples over the season. All Astoria releases were made after dark on an outgoing tide to reduce avian predation by Caspian terns *Hydroprogne caspia* and double-crested cormorants *Phalacrocorax auritas* from the nearby nesting colonies on East Sand Island.

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 number of fish from each release group preyed upon by piscivorous birds. These data also allowed us to determine whether infection with *R. salmoninarum, N. salmonis,* or both pathogens was correlated with predation vulnerability. There was no evidence from the 2006 study that infection of fish with one or both pathogens influenced rates of predation, but *R. salmoninarum*

infection levels in the majority of tested fish were low. Current methodologies for assaying *N. salmonis* can only provide numbers of fish infected, not infection levels.

Both of these pathogens are associated with chronic, slowly progressing infections that may not always cause outright mortality, but may make fish vulnerable to secondary infections. Therefore, the pathogens may have more effect on smolt-to-adult returns (SARs) than on short-term mortality, including mortality by avian predation. Overall, *R. salmoninarum* was detected by one or both PCR assays in 77% of wild Chinook salmon, 72% of hatchery Chinook salmon, 73% of wild steelhead, and 70% of hatchery steelhead sampled. *N. salmonis* was detected by polymerase chain reaction (PCR) in 4% of wild Chinook salmon, 1% of hatchery Chinook salmon, 6% of wild steelhead, and 23% of hatchery steelhead tested.

We will need to wait several years for adult returns to determine the efficacy of releasing transported salmonids at rkm 10 instead of the traditional release site at rkm 225. However, we do know that the new release location affected vulnerability to avian predators; mean avian predation rates were 3.0% for yearling Chinook salmon released from Skamania Landing at rkm 225, but only 0.4% for those released near Astoria at rkm 10. Avian predation rates were 13.8% for steelhead released at Skamania Landing, but only 1.7% for their cohort released at Astoria. These are minimum estimates of the impact of avian predation, as not all tags consumed by birds are deposited or found on colonies. Our results show that releasing fish farther downstream, at night, and on an outgoing tide will reduce avian predation by up to seven-fold on average. 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.

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# INTRODUCTION

At collector dams on the Snake and Columbia Rivers, migrating salmonid smolts are guided away from turbine intakes and collected for transport 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 within the migration season (Muir et al. 2006; Williams et al. 2005).

Typically, about 50% of Snake River migrant smolts survive downstream migration 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 adults as inriver migrant fish to return as adults. Nevertheless, on an annual basis, the ratio of transported to in-river 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 migration. The difference in survival between inriver migrants and transported fish has been termed differential delayed mortality or "*D*." The purpose of this study is to determine if transporting juvenile fish farther downstream will increase smolt-to-adult return (SARs) and reduce *D* of 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). Although stress and stressors have been examined in detail in these studies, and modification to the collection and transportation system have been made to reduce stress (Williams and Matthews 1995), transportation has not provided the benefit expected, particularly for wild Chinook salmon *Oncorhynchus tshawytscha* (Williams et al. 2005). This research continues an ongoing effort by the U.S. Army Corps of Engineers Anadromous Fish Evaluation Program (AFEP) to evaluate modifications to the existing transportation program to improve post-release survival of transported fish.

Studies conducted with Coho salmon *O. kisutch* found that smolts transported to a release point near Tongue Point in the Columbia River returned at 1.6 times greater rate than those released upriver (Solazzi et al. 1991). 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 the Skamania Landing release site with a release site at Tongue Point (rkm 29) in the Columbia River estuary, but too few adult steelhead *O. mykiss* returned from either release point for a meaningful evaluation.

The primary objective of the 2006 alternate barge release site study was to determine whether releasing barged fish farther downstream near Astoria at rkm 10

(approximately 10 km downstream from the Astoria Bridge) would improve the SAR rate of spring Chinook salmon and steelhead (Figure 1). The strategy was to minimize the time spent moving into and through the estuary, while documenting fish condition to provide insight into the vulnerability of smolts to predators. 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 the current barge release site downstream from Bonneville Dam near Skamania Landing (rkm 224) and at Astoria. Since complete adult returns are needed to calculate SARs, this objective will require several years to complete.

Our second objective was to determine *Renibacterium salmoninarum* prevalence and severity, along with the presence of *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 eventually with SARs. Our third and final objective was to compare avian predation rates between Skamania Landing and Astoria release groups.

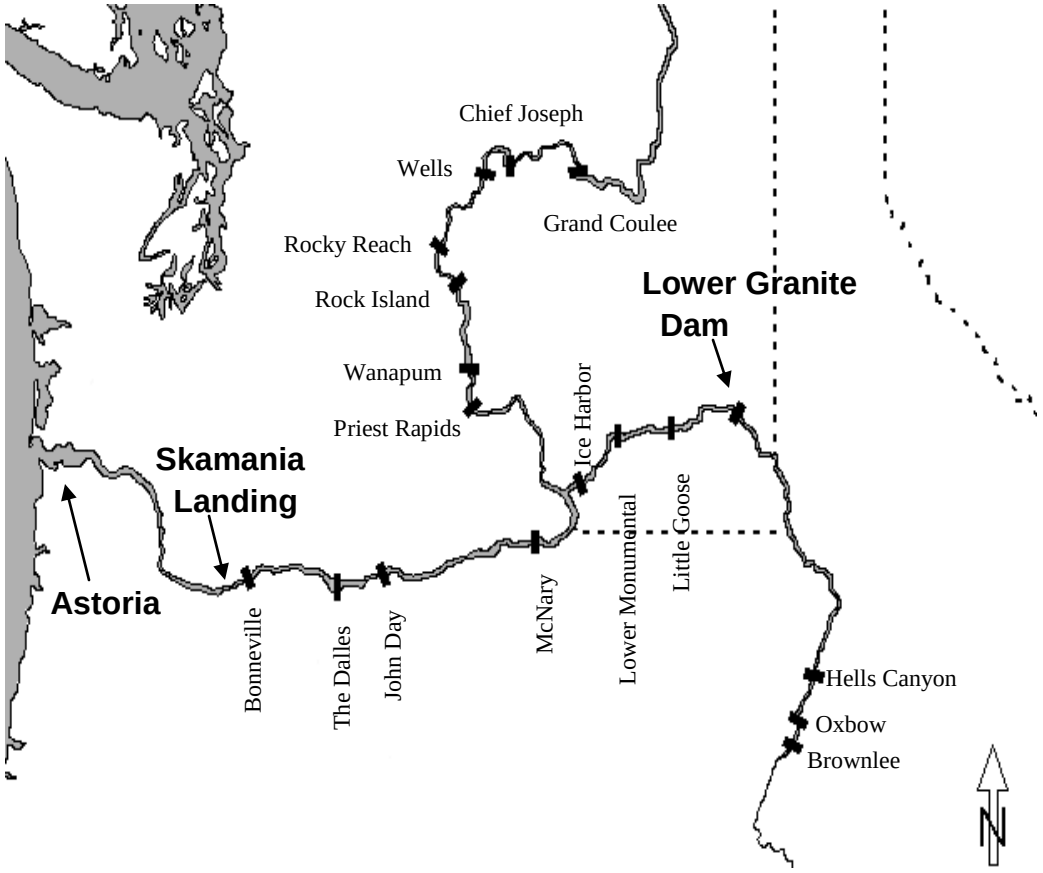


Figure 1. Study area showing Lower Granite Dam, where fish were collected and PIT tagged, the Skamania Landing barge release site (rkm 224), and the Astoria barge release site (rkm 10) during 2006.

## METHODS

### Fish Acquisition and Tagging

During spring 2006, 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 April to 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 transportation barge for release at rkm 224 (Skamania Landing), where transported fish are typically released. A second group of each species was loaded on a 2000-series barge and released at rkm 10 (Astoria release) in the lower estuary.

We attempted to tag sufficient numbers of both yearling Chinook salmon and steelhead to test a ratio of 1.3 (i.e., an SAR at least 30% higher) for adults returning from transported groups releases at Astoria ($T_A$) vs. those released Skamania Landing ($T_S$). This ratio ($T_A/T_S$) was based on an expected SAR of 1.0% at Lower Granite Dam for the Astoria releases (Table 1). 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 ratio 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.

Table 1. Required sample sizes based on expected SAR ($\alpha = 0.05$, $\beta = 0.20$) and $T_A/T_S$.

| $T_A/T_S$ ratio | Expected Astoria SAR | Fish PIT tagged at Lower Granite Dam (n) | |
|---|---|---|---|
| | | Astoria (rkm 10) | Skamania Landing (rkm 225) |
| 1.2 | 1.00 | 48,000 | 57,000 |
| 1.2 | 0.75 | 64,000 | 76,000 |
| 1.2 | 0.50 | 95,000 | 114,000 |
| 1.3 | 1.00 | 23,000 | 30,000 |
| 1.3 | 0.75 | 31,000 | 40,000 |
| 1.3 | 0.50 | 46,000 | 58,000 |

Lower Granite Dam will serve as the principal recovery site for adults. Data acquired from other areas will be considered ancillary. To analyze results, statistical tests will be applied when adult returns for the study are complete. Confidence intervals for the $T_A/T_S$ ratio will be calculated using the ratio (survival) estimate (Burnham et al. 1987) and its associated empirical variance. The study will produce an overall, statistically bound $T_A/T_S$ estimate for fish returning to Lower Granite Dam.

## Fish Releases

The Skamania Landing release groups were transported and released with normal transportation fish. We attempted to keep loading density and water volume replacement times as close as possible between the Skamania Landing (8000 series) and Astoria (2000 series) barges, and did not exceed loading density and replacement rates set by the U.S. Army Corps of Engineers. However, due to the unpredictable nature of fish arrival and collection at the dam, keeping loading densities equal proved to be difficult.

The barge used for Astoria releases was towed with a separate vessel, which mirrored the path of the Skamania Landing barge until after it passed Bonneville Dam and continued downstream to rkm 10. Astoria releases were timed to occur at night on an ebb tide to minimize predation by Caspian terns *Hydroprogne caspia* and double-crested cormorants *Phalacrocorax auritas* from the nearby nesting colonies on East Sand Island in the Columbia River estuary (Table 2). 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 2. Release dates, times, and locations for PIT-tagged juvenile steelhead and yearling Chinook salmon smolts released at Skamania Landing and near Astoria during 2006. High tides for the Astoria releases are noted.

| | Astoria releases (rkm 10) | | Skamania releases (rkm 224) | |
|---|---|---|---|---|
| Release date | Time | High tide at rkm 10 | Release date | Time |
| 26 Apr | 3:15 | 00:18 | 26 Apr | 0:35 |
| 3 May | 21:15 | 19:12 | 2 May | 21:15 |
| 11 May | 1:45 | 00:09 | 9 May | 19:25 |
| 17 May | 21:15 | 17:54 | 16 May | 19:55 |
| 26 May | 2:30 | 00:18 | 25 May | 19:05 |
| 2 June | 21:00 | 19:22 | 1 June | 19:10 |

## 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. The goal was to sample 75 fish each of wild and hatchery Chinook salmon and of wild and hatchery steelhead on each tagging date, 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 3).

Table 3. Release numbers (mortalities removed) of PIT-tagged hatchery (H) and wild (W) steelhead and yearling Chinook salmon smolts that were gill-clipped, transported, and released at Skamania Landing by release date during 2006.

| | Chinook salmon | | Steelhead | | |
|---|---|---|---|---|---|
| Release date | H | W | H | W | Total |
| 26 Apr | 74 | 69 | 75 | 75 | 293 |
| 2 May | 75 | 75 | 75 | 75 | 300 |
| 9 May | 75 | 73 | 75 | 74 | 297 |
| 16 May | 74 | 74 | 75 | 75 | 298 |
| 25 May | 73 | 67 | 75 | 74 | 289 |
| 1 Jun | 8 | 19 | 141 | 131 | 299 |
| Total | 379 | 377 | 516 | 504 | 1,776 |

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 recirculating water in the tagging system was changed, and twice after the water was changed. The 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) 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. We used paired *t*-tests ($P < 0.05$) to compare predation rates between release locations.

## 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 (Elliott and Pascho 2004; 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, 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 both PCR rounds were changed to the following: 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 65°C for 60 sec, and 72°C for 60 sec, with a final extension of 72°C for 7 min. The current assay methodology can only determine the presence or absence of *N. salmonis*. A new methodology is being developed that may allow quantitative assaying in the near future.

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. The 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 either *R. salmoninarum*, *N. salmonis*, or both pathogens. Fisher's exact test was used for analysis of $2 \times 2$ 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

1 Use of trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

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. Similar procedures were used for comparison of *R. salmoninarum* concentrations detected in water samples on different days. Correlation of *R. salmoninarum* level with fork length were evaluated using the nonparametric Spearman rank correlation test, and this test was also used to evaluate correlations of *R. salmoninarum* level with concentration of the bacterium in water samples.

## RESULTS

### Tagging

On six consecutive Sundays from 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 4). A total of 13,729 hatchery and 2,435 wild yearling Chinook salmon were tagged, loaded on a transport barge, and released at Astoria, while 20,488 hatchery and 3,707 wild yearling Chinook salmon were tagged, transported, and released at Skamania Landing. In total, 25,726 hatchery and 3,445 wild steelhead were released at Astoria, and 36,210 hatchery and 5,612 wild steelhead were released at Skamania Landing. 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 (Table 5). However, final fish loading densities were far below maximum capacities on all barges.

Table 4. Release numbers of PIT-tagged wild and hatchery juvenile steelhead and yearling Chinook salmon by date at the Astoria and Skamania Landing release sites during 2006.

| | Skamania Landing | | | | Astoria | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Release | Chinook salmon | | Steelhead | | Chinook salmon | | Steelhead | | |
| date | Hatchery | Wild | Hatchery | Wild | Hatchery | Wild | Hatchery | Wild | **Total** |
| 26 Apr | 2,199 | 950 | 5,657 | 527 | 2,837 | 897 | 6,695 | 456 | **20,218** |
| 2-3 May | 5,769 | 1,310 | 4,917 | 808 | 3,556 | 731 | 3,242 | 439 | **20,772** |
| 9 and 11 May | 5,569 | 495 | 5,364 | 914 | 3,310 | 262 | 3,899 | 554 | **20,367** |
| 16-17 May | 5,456 | 287 | 6,504 | 609 | 3,309 | 184 | 4,295 | 290 | **20,934** |
| 25-26 May | 1,449 | 562 | 9,704 | 1,978 | 702 | 329 | 5,504 | 1,227 | **21,455** |
| 1-2 June | 46 | 103 | 4,064 | 776 | 15 | 32 | 2,091 | 479 | **7,606** |
| **Total** | **20,488** | **3,707** | **36,210** | **5,612** | **13,729** | **2,435** | **25,726** | **3,445** | **111,352** |

Table 5. 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, 2006. The total number pounds of fish in the barge holds are also shown.

| Release date | Barge | Number tagged | Number not tagged | Total pounds |
|---|---|---|---|---|
| 26 April | Skamania Landing | 9,333 | 7,092 | 1,727 |
| 26 April | Astoria | 10,885 | 14,129 | 2,539 |
| 2 May | Skamania Landing | 12,804 | 1,431 | 1,585 |
| 3 May | Astoria | 7,968 | 12,139 | 2,068 |
| 9 May | Skamania Landing | 12,342 | 35,252 | 4,719 |
| 11 May | Astoria | 8,025 | 16,318 | 2,413 |
| 16 May | Skamania Landing | 12,856 | 10,357 | 3,196 |
| 17 May | Astoria | 8,078 | 17,512 | 3,480 |
| 25 May | Skamania Landing | 13,693 | 8,886 | 2,600 |
| 26 May | Astoria | 7,762 | 4,000 | 1,192 |
| 1 June | Skamania Landing | 4,989 | 14,427 | 1,517 |
| 2 June | Astoria | 2,617 | 13,867 | 1,241 |

## Avian Predation

Based on PIT tag recoveries on East Sand Island, we 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 rate was 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 colony, 11.8% of the tags from steelhead released at Skamania Landing were recovered, while 1.6% of the tags from steelhead released at Astoria were recovered. An additional 2.0% of tags from steelhead released at Skamania Landing and 0.1% of tags from steelhead released at Astoria were detected on the colonies of double-crested cormorants. Losses to both colonies combined were of 13.8% for Skamania Landing and 1.7% for Astoria release locations. 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).

Table 6. Percentage of PIT tags detected on the East Sand Island Caspian tern and double-crested cormorant colonies for both steelhead and yearling Chinook salmon smolts from 2006 releases at Skamania Landing and near Astoria. A paired *t*-test was used to compare release locations.

| | Tags detected (%) | | | |
|---|---|---|---|---|
| | Steelhead | | Chinook salmon | |
| Bird colony | Astoria | Skamania | Astoria | Skamania |
| Caspian tern | 1.61 | 11.8 | 0.32 | 1.54 |
| Paired *t*-test | $t = 5.89$, $P = 0.002$ | | $t = 7.24$ , $P = 0.001$ | |
| Double-crested cormorant | 0.13 | 1.98 | 0.09 | 1.44 |
| Paired *t*-test | $t = 4.21$ , $P = 0.008$ | | $t = 4.40$ , $P = 0.007$ | |

## Date-Specific Predation Results

Tagged fish were released on six separate occasions between April and June at both the Skamania Landing and Astoria release sites (Table 4). The Astoria release groups showed lower tag proportions detected on both tern and cormorant colonies than did the Skamania Landing release groups for all replicates (Table 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 2006.

| | Tags detected (%) | | | |
|---|---|---|---|---|
| | Caspian tern | | Double-crested cormorant | |
| Release date | Astoria | Skamania | Astoria | Skamania |
| | Steelhead | | | |
| 26 April | 1.45 | 12.39 | 0.01 | 1.63 |
| 2-3 May | 0.35 | 5.17 | 0.38 | 3.39 |
| 9 and 11 May | 0.54 | 10.07 | 0.11 | 2.84 |
| 16-17 May | 2.55 | 9.35 | 0.17 | 1.25 |
| 25-26 May | 1.71 | 13.57 | 0.12 | 2.16 |
| 1-2 June | 3.81 | 20.70 | 0.08 | 0.31 |
| | Chinook salmon | | | |
| 26 April | 0.64 | 2.45 | 0.11 | 1.11 |
| 2-3 May | 0.14 | 1.40 | 0.12 | 1.68 |
| 9 and 11 May | 0.08 | 1.75 | 0.00 | 1.42 |
| 16-17 May | 0.52 | 1.15 | 0.09 | 1.51 |
| 25-26 May | 0.10 | 1.09 | 0.19 | 1.04 |
| 1-2 June | 0.00 | 1.34 | 0.00 | 0.00 |

## Pathogen Analyses

The proportion of fish found infected with *R. salmoninarum* by qPCR was significantly higher than that found by nPCR in gill samples from wild and hatchery Chinook salmon and wild and hatchery steelhead ($P$ <0.0001; Table 8). However, the prevalence of *R. salmoninarum* did not differ among species between nPCR ($P$ = 0.76) and qPCR testing ($P$ = 0.81). Prevalence of *N. salmonis* was significantly higher in hatchery steelhead than in wild steelhead, wild Chinook salmon, or hatchery Chinook salmon (Table 8; $P$ <0.0001).

Table 8. Pathogen detection in gill samples from all tested fish. Detection of *Renibacterium salmoninarum* by nested PCR (nPCR) and quantitative PCR (qPCR) and detection of *Nucleospora salmonis* by nPCR 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 six release groups of fish marked with PIT tags at Lower Granite Dam during 2006.

| | Number of positive fish/Number sampled (%) | | |
|---|---|---|---|
| | *Renibacterium salmoninarum* | | *Nucleospora salmonis* |
| Fish species | nPCR | qPCR | nPCR |
| Wild Chinook salmon | 138/394 (35%) | 250/394 (63%) | 14/394 (4%) |
| Hatchery Chinook salmon | 124/383 (32%) | 237/383 (62%) | 2/383 (1%) |
| Wild steelhead | 181/506 (36%) | 319/498 (64%) | 29/498 (6%) |
| Hatchery steelhead | 176/510 (35%) | 313/510 (61%) | 116/510 (23%) |

Among fish testing positive for *R. salmoninarum* by qPCR, *R. salmoninarum* levels were generally less than 100 bacteria per mg of gill sample; samples from only 12 fish had *R. salmoninarum* concentrations exceeding this level. These fish included three hatchery Chinook salmon (highest concentration 636 *R. salmoninarum* per mg), three wild Chinook salmon (highest concentration 172 *R. salmoninarum* per mg), three hatchery steelhead (highest concentration 203 *R. salmoninarum* per mg), and three wild steelhead (highest concentration 513 *R. salmoninarum* per mg). Nevertheless, slightly more than half of the qPCR-positive fish of each species had *R. salmoninarum* levels above the threshold for consistent detection by the assay (5 bacteria per qPCR reaction; Table 9).

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 2006. Means not sharing a common letter are significantly different ($P < 0.05$).

| Fish species | Number above threshold for consistent *R. salmoninarum* detection[a]/Total number positive by qPCR (%) | Geometric mean number *R. salmoninarum* per mg gill sample[b] (±SD) |
|---|---|---|
| Wild Chinook salmon | 132/250 (53) | 11 (±3) y |
| Hatchery Chinook salmon | 124/237 (52) | 12 (±2) y |
| Wild steelhead | 177/319 (55) | 14 (±2) z |
| Hatchery steelhead | 173/313 (55) | 14 (±2) z |

[a] Five bacteria per qPCR reaction.

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

Mean *R. salmoninarum* levels in wild Chinook salmon were not significantly different from those in hatchery Chinook salmon ($P > 0.05$), and mean *R. salmoninarum* levels in wild steelhead were not significantly different from those in hatchery steelhead ($P > 0.05$). However, *R. salmoninarum* levels in wild steelhead were significantly higher than levels in both wild ($P < 0.01$) and hatchery Chinook salmon ($P < 0.01$). *R. salmoninarum* levels in hatchery steelhead were also significantly higher than levels in both wild Chinook salmon ($P < 0.05$) and hatchery Chinook salmon ($P < 0.01$).

The proportions of fish testing positive for *R. salmoninarum*, *N. salmonis*, or both pathogens varied by species and sample date (Table 10). For wild Chinook salmon, the proportions of fish positive for either or both pathogens did not differ significantly among sample dates ($P = 0.24$). However, for hatchery Chinook salmon, the proportions of fish positive for either or both pathogens were significantly higher ($P < 0.0001$) for samples collected on 7, 14, and 22 May than for samples collected on 23 or 30 April.

For wild steelhead, the proportions of fish positive for one or both pathogens were significantly higher ($P < 0.0001$) for samples collected on 7, 14, and 30 May than for samples collected on 23 and 30 April or 22 May, and were also significantly higher ($P = 0.006$) for samples collected on 23 and 30 April than for the samples collected on 22 May. For hatchery steelhead, the proportions of fish positive for either or both pathogens were significantly higher ($P < 0.0001$) for samples collected on 7 and 30 May than for those collected on 23 and 30 April and 14 May.

Table 10. Proportions of fish testing positive for *Renibacterium salmoninarum* only, for *Nucleospora salmonis* only, for both pathogens, or for neither pathogen among all fish sampled on each date from PIT-tagged groups at Lower Granite Dam in 2006.

| Sample date and fish species | Number tested | Number positive *R. salmoninarum* only* (%) | Number positive *N. salmonis* only (%) | Number positive *R. salmoninarum* and *N. salmonis* (%) | Number with neither pathogen detected (%) |
|---|---|---|---|---|---|
| 23 April | | | | | |
| Wild Chinook | 75 | 43 (57%) | 2 (3%) | 8 (11%) | 22 (29%) |
| Hatchery Chinook | 75 | 39 (52%) | 1 (1%) | 0 | 35 (47%) |
| Wild steelhead | 75 | 41 (55%) | 3 (4%) | 2 (3%) | 29 (39%) |
| Hatchery steelhead | 75 | 39 (52%) | 6 (8%) | 5 (7%) | 25 (33%) |
| 30 April | | | | | |
| Wild Chinook | 75 | 62 (83%) | 0 | 0 | 13 (17%) |
| Hatchery Chinook | 75 | 43 (57%) | 1 (1%) | 0 | 31 (41%) |
| Wild steelhead | 75 | 51 (68%) | 1 (1%) | 3 (4%) | 20 (27%) |
| Hatchery steelhead | 68 | 37 (54%) | 8 (12%) | 10 (13%) | 13 (19%) |
| 7 May | | | | | |
| Wild Chinook | 75 | 54 (72%) | 0 | 2 (3%) | 19 (25%) |
| Hatchery Chinook | 75 | 66 (88%) | 0 | 0 | 9 (12%) |
| Wild steelhead | 67 | 54 (81%) | 1 (1%) | 3 (4%) | 9 (13%) |
| Hatchery steelhead | 75 | 52 (69%) | 4 (5%) | 13 (17%) | 6 (8%) |
| 14 May | | | | | |
| Wild Chinook | 75 | 61 (81%) | 0 | 1 (1%) | 13 (17%) |
| Hatchery Chinook | 75 | 63 (84%) | 0 | 0 | 12 (16%) |
| Wild steelhead | 75 | 59 (79%) | 0 | 2 (3%) | 14 (19%) |
| Hatchery steelhead | 75 | 33 (44%) | 10 (13%) | 12 (16%) | 20 (27%) |
| 22 May | | | | | |
| Wild Chinook | 75 | 53 (71%) | 0 | 1 (1%) | 21 (28%) |
| Hatchery Chinook | 75 | 56 (75%) | 0 | 0 | 19 (25%) |
| Wild steelhead | 75 | 32 (43%) | 3 (4%) | 1 (1%) | 39 (52%) |
| Hatchery steelhead | 75 | 29 (39%) | 11 (15%) | 11 (15%) | 24 (32%) |
| 30 May | | | | | |
| Wild Chinook | 19 | 17 (89%) | 0 | 0 | 2 (11%) |
| Hatchery Chinook | 8 | 8 (100%) | 0 | 0 | 0 |
| Wild steelhead | 131 | 107 (82%) | 1 (<1%) | 9 (7%) | 14 (11%) |
| Hatchery steelhead | 142 | 98 (69%) | 6 (4%) | 20 (14%) | 18 (13%) |
| All dates | | | | | |
| Wild Chinook | 394 | 290 (74%) | 2 (<1%) | 12 (3%) | 90 (23%) |
| Hatchery Chinook | 383 | 275 (72%) | 2 (<1%) | 0 | 106 (28%) |
| Wild steelhead | 498 | 344 (69%) | 9 (2%) | 20 (4%) | 125 (25%) |
| Hatchery steelhead | 510 | 288 (56%) | 45 (9%) | 71 (14%) | 106 (21%) |

* Positive for *R. salmoninarum* by nPCR, qPCR, or both PCRs.

When data were combined for all sample dates, however, the proportions of fish positive for one or both pathogens did not differ significantly among species ($P = 0.095$). Overall, one or both pathogens were detected in 77% of the wild Chinook salmon, 72% of the hatchery Chinook salmon, 75% of the wild steelhead, and 79% of the hatchery steelhead.

Fish that were infected with either *R. salmoninarum* or *N. salmonis* or both pathogens did not differ significantly in length ($P \geq 0.26$) from fish not infected with either pathogen. However, among wild Chinook salmon positive for *R. salmoninarum* by qPCR testing, there was a significant negative correlation ($P = 0.01$) between length and *R. salmoninarum* infection level. There was no significant correlation between length and *R. salmoninarum* infection level for hatchery Chinook salmon ($P = 0.07$), wild steelhead ($P = 0.14$), or hatchery steelhead ($P = 0.17$).

Among the PIT tags recovered from the East Sand Island tern and cormorant colonies, 159 (20 Chinook salmon and 139 steelhead) were from fish that had been sampled at Lower Granite Dam for detection of *R. salmoninarum* and *N. salmonis*. Pathogen prevalence and levels in these fish were similar to those of all fish tested for pathogens. Of fish with PIT tags recovered on bird colonies, 67% of wild and 60% of hatchery Chinook salmon and 67% of wild and 77% of hatchery steelhead were positive for one or both pathogens (Table 11).

Table 11. 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 2006, with PIT tags subsequently recovered on the East Sand Island piscivorous bird colonies.

| Fish species | Number tested | *R. salmoninarum* positive only* n (%) | *N. salmonis* positive only n (%) | *R. salmoninarum* and *N. salmonis* positive n (%) | Neither pathogen detected n (%) |
|---|---|---|---|---|---|
| Wild Chinook | 15 | 10 (67%) | 0 | 0 | 5 (33%) |
| Hatchery Chinook | 5 | 3 (60%) | 0 | 0 | 2 (40%) |
| Wild steelhead | 52 | 33 (63%) | 0 | 2 (4%) | 17 (33%) |
| Hatchery steelhead | 87 | 53 (61%) | 6 (7%) | 8 (9%) | 20 (23%) |

***** Positive for *R. salmoninarum* by nPCR, qPCR, or both PCRs.

Overall, 52% 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 per qPCR reaction; Table 12). *R. salmoninarum* concentrations for qPCR-positive fish ranged from <1 to 148 bacteria/mg for wild Chinook salmon, from 5 to 37 bacteria/mg for wild steelhead, and from 3 to 59 bacteria/mg for hatchery steelhead. The single qPCR-positive hatchery Chinook salmon had an *R. salmoninarum* concentration of 13 bacteria/mg.

Table 12. Mean levels of *Renibacterium salmoninarum* detected by qPCR in smolts sampled at Lower Granite Dam during 2006, with PIT tags subsequently recovered on the East Sand Island piscivorous bird colonies.

| Fish species | Number above threshold for consistent *R. salmoninarum* detection[a] by qPCR/Total positive (%) | Geometric mean number *R. salmoninarum* per mg gill sample[b] (±SD) |
|---|---|---|
| Wild Chinook salmon | 4/9 (44%) | 9 (±8) |
| Hatchery Chinook salmon | 1/1 (100%) | ---[c] |
| Wild steelhead | 16/29 (55%) | 14 (±2) |
| Hatchery steelhead | 29/58 (50%) | 13 (±2) |

[a] Five bacteria per qPCR reaction.

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

[c] Single qPCR-positive fish had 13 *R. salmoninarum* per mg gill sample.

The highest mean *R. salmoninarum* concentrations detected in water samples taken from the Lower Granite Dam tagging station were usually obtained from the last sample taken each day (Table 13). Mean *R. salmoninarum* concentrations in a given sample ranged from 8 to 133 bacteria per mL. There was no significant correlation between daily mean *R. salmoninarum* levels detected in gill samples of qPCR-positive fish, and daily mean *R. salmoninarum* concentrations detected in water samples ($P = 0.56$). The lowest and highest mean *R. salmoninarum* levels in gill samples were detected on 14 and 30 May, respectively, and the lowest and highest *R. salmoninarum* concentrations in water samples were detected on 22 May and 30 April, respectively (Table 14).

Table 13. 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, 2006. After the first two water samples were taken each day, the water in the recirculating system was changed.

| Sample date and time | Geometric mean *R. salmoninarum*/mL (±SD) |
|---|---|
| 23 April | |
| 9:45 a.m. | 8 (±1) |
| 11:30 a.m. | 35 (±1) |
| 12:30 p.m. | 20 (±2) |
| 4:00 p.m. | 133 (±1) |
| 30 April | |
| 10:20 a.m. | 29 (±2) |
| 11:00 a.m. | 30 (±1) |
| 1:00 p.m. | 44 (±1) |
| 4:00 p.m. | 54 (±2) |
| 7 May | |
| 10:15 a.m. | 23 (±1) |
| 12:20 p.m. | 10 (±2) |
| 1:30 p.m. | 16 (±1) |
| 3:10 p.m. | 25 (±1) |
| 14 May | |
| 9:45 a.m. | 16 (±1) |
| 11:30 a.m. | 16 (±2) |
| 1:40 p.m. | 16 (±2) |
| 3:30 p.m. | 37 (±2) |
| 22 May | |
| 11:30 a.m. | 11 (±2) |
| 11:45 a.m. | 14 (±2) |
| 1:00 p.m. | 11 (±2) |
| 4:00 p.m. | 14 (±2) |
| 30 May | |
| 10:00 a.m. | 12 (±1) |
| 11:00 a.m. | 21 (±1) |
| 12:30 p.m. | 11 (±2) |
| 2:05 p.m. | 28 (±1) |

Table 14. Mean levels of *Renibacterium salmoninarum* detected by qPCR in daily samples of smolts (both species combined) sampled non-lethally during PIT-tagging at Lower Granite Dam in 2006. Also shown are mean daily concentrations of *R. salmoninarum* detected by MF-FAT in water samples taken at the tagging station from which fish were sampled. Within a column, means not sharing a common letter are significantly different ($P$ <0.05).

| Sample date | Geometric mean *R. salmoninarum* per mg gill sample (±SD) | Geometric mean *R. salmoninarum* per mL water sample (±SD) |
|---|---|---|
| 23 April | 10 (±2) x | 29 (±3) t |
| 30 April | 14 (±2) y | 38 (±1) tu |
| 7 May | 16 (±2) yz | 17 (±2) tv |
| 14 May | 9 (±3) x | 20 (±2) tuvw |
| 22 May | 11 (±2) x | 13 (±2) tvw |
| 30 May | 18 (±2) z | 17 (±2) tvw |

## DISCUSSION

One goal of this study was to evaluate whether releasing transported salmonids downstream from the Astoria Bridge could increase survival to ocean entry. We hypothesized that using this release site would increase survival to ocean entry by allowing fish to avoid 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 major site of tag recovery was Rice Island, which was then home to 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 in subsequent years, as the tern colony was relocated from Rice Island to East Sand Island. To optimize survival, smolts were released at night on an ebb tide, during which time most would be expected to pass the bird colonies and reach the ocean during one tidal cycle (Ledgerwood et al. 2001). Our results supported this hypothesis, showing that a significantly higher proportion of salmon released at Skamania were found on both large avian colonies in the estuary compared to their cohorts released at Astoria.

In addition to facilitating the avoidance of avian predators, the release of fish near Astoria could improve survival in other ways. The release site downstream from Bonneville Dam near Skamania Landing has the highest rate of predation by northern pikeminnow *Ptychocheilus oregonensis* in the Columbia River (Ward et al. 1995). By transporting smolts farther downstream to Astoria, this source of potential mortality can be avoided. Release in the lower estuary also allows smolts to avoid migrating through the Willamette River confluence area, where high levels of toxic chemicals have been found (Spromberg et al. *in press*). Barged fish would still pass through this area (and be exposed to the same water as migrating fish), but their duration of exposure to any toxic chemicals would be shorter. 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 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 SARs. 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 less 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.

Modification of the qPCR procedure for *R. salmoninarum* improved the sensitivity of the assay compared to that used in the 2005 study (McMichael et al. 2006). In that study, for which only hatchery steelhead were sampled, 14% of the 1,002 fish were positive for *R. salmoninarum* by qPCR, whereas 61% of the 510 hatchery steelhead tested in 2006 were positive by qPCR. In comparison, similar proportions of hatchery steelhead were positive for *R. salmoninarum* by nPCR in 2005 (33%) and 2006 (35%). Furthermore, in 2005, only 2% of the qPCR-positive hatchery steelhead had *R. salmoninarum* levels at or above the threshold for consistent detection of *R. salmoninarum* (5 bacteria per PCR reaction), while 55% of the qPCR-positive hatchery steelhead in 2006 had *R. salmoninarum* levels at or above this threshold. The biological significance of the increased detection sensitivity of the modified qPCR remains to be determined.

The present study using PCR for *R. salmoninarum* detection found no significant differences in prevalence of the bacterium between steelhead and Chinook salmon, but showed higher infection levels in steelhead. Previous comparisons of Chinook salmon and steelhead at Lower Granite Dam used the enzyme-linked immunosorbent assay (ELISA) to detect *R. salmoninarum* (Pascho and Elliott 1989; Elliott and Pascho 1991, 1993). In those studies, both levels and prevalence of *R. salmoninarum* in Chinook salmon were found to be higher than or equal to those in steelhead. In addition, these earlier studies consistently found significantly higher *R. salmoninarum* levels by ELISA in wild steelhead than in hatchery steelhead, a result that was not observed in the present PCR study. (Wild and hatchery stocks of yearling Chinook salmon could not be distinguished during 1988-1990, with the exception of a few tagged fish, so these were not separated in analyses.)

Because the ELISA detects soluble antigen produced by *R. salmoninarum* and PCR detects bacterial DNA, the results of the studies are not directly comparable. *R. salmoninarum* antigen can persist in fish in the absence of live bacteria (Pascho et al. 1997); the correlation between bacterial viability and detection by PCR has not been determined. Some studies suggest that changes in hatchery practices may have contributed to reductions in *R. salmoninarum* levels (as determined by ELISA testing) in

certain hatchery populations of Chinook salmon in the Snake and Columbia River basins (Maule et al. 1996; Vanderkooi and Maule 1999; Munson and Johnson 2005), although it is unknown whether these changes have resulted in increased long-term survival of fish.

Year-to-year variation in prevalence and severity of *R. salmoninarum* infections also occurs among salmonid smolts (Pascho and Elliott 1989; Elliott and Pascho 1991, 1993; Elliott et al. 1997; Vanderkooi and Maule 1999) and may have influenced the results of our study. 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 in the present study would have shown negative or low *R. salmoninarum* antigen levels by ELISA testing (Chase et al. 2006). Therefore, these fish would not have been considered clinically diseased (e.g., showing grossly visible kidney lesions had they been sacrificed) at the time of sampling.

The influence of low *R. salmoninarum* infection levels on long-term survival of fish is not well understood. However, in a previous study with yearling hatchery Chinook salmon, significantly higher survival was observed during downriver migration and a 3-month seawater holding period among groups of smolts with low average *R. salmoninarum* antigen levels (by ELISA testing of kidney tissue) than among groups with medium to high average *R. salmoninarum* antigen levels (Pascho et al. 1993; Elliott et al. 1995). Similar studies have not yet been conducted with groups of fish from which tissues have been tested by PCR. Laboratory research is currently underway to better define the relation between concentrations of *R. salmoninarum* DNA detected by PCR in kidney and gill samples and the infection status of fish. In addition, field studies such as this one are intended to provide further information on possible correlations between *R. salmoninarum* levels in salmonid populations and fish survival.

A surprising result was the high prevalence of *N. salmonis* in hatchery steelhead compared with wild steelhead and hatchery and wild Chinook salmon. The prevalence of *N. salmonis* detected in hatchery steelhead in 2006 (23%) was similar to the 25% prevalence detected in hatchery steelhead in 2005, when this was the only group of fish sampled (McMichael et al. 2006). Although *N. salmonis* mortality has been primarily reported in juvenile and adult Chinook salmon in freshwater and seawater (see Gresoviac et al. 2000 for summary), the pathogen also causes mortality in certain stocks of steelhead reared in hatcheries in Idaho (Kathy Clemens, Idaho Fish Health Center, U.S. Fish and Wildlife Service, Orofino Idaho, personal communication).

Regular monitoring of *N. salmonis* is not done for many hatchery steelhead populations because of the difficulty of detection prior to the development of PCR assays. Therefore, the possible contribution of the pathogen to delayed mortality of steelhead smolts has not been determined. Similar to *R. salmoninarum* infections, *N. salmonis*

infections can result in reduced immune function, allowing for other opportunistic infections, but can also be directly fatal (Hedrick et al. 1990).

The presence of one or both pathogens did not appear to influence the susceptibility of steelhead and Chinook salmon to predation by piscivorous birds, although the low number of PIT tags recovered on East Sand Island from infected Chinook salmon (20 fish total, including 15 wild fish and 5 hatchery fish) precluded meaningful analyses for this species. A lack of preferential predation on infected fish was not surprising, considering the relatively low *R. salmoninarum* levels in the majority of fish tested. The *N. salmonis* levels were not determined.

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.

Changes in *R. salmoninarum* concentrations in the water of the tagging trough from which fish were sampled for pathogen analyses did not correspond to changes in mean infection levels in fish on different sample days. This may suggest that *R. salmoninarum* levels in gill samples were not significantly affected by surface contamination with *R. salmoninarum* in the water. The consistent detection of the highest *R. salmoninarum* concentrations at the end of the day suggested that bacteria may continue to concentrate in the trough, despite the midday water change. On most days, however, the increase in bacteria in the last sample was minimal, and overall concentrations were similar to those observed in other recirculating systems in conventional tagging trailers (D. G. Elliott, USGS, unpublished data). The MF-FAT cannot distinguish live from dead bacteria, so the viability of *R. salmoninarum* in the troughs was unknown.

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). The return 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.

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