Final Report Submitted To:
ARMY CORPS OF ENGINEERS, WALLA WALLA DISTRICT
Study Code: BPS-00-10, SPE-06-2

Survival and travel times of in-river and transported yearling Chinook salmon in the lower Columbia River and estuary with investigation into causes of differential mortality

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July, 2009

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I. ABSTRACT

In this study, differential mortality of transported and in-river yearling Chinook salmon in the lower Columbia River and estuary (LRE) was estimated using Juvenile Salmon Acoustic Telemetry System (JSATS) acoustic tags and concomitant detection arrays. The extent of differential mortality was assessed in terms of the Barge to In-River Survival Ratio ($\widehat{BI}$ ratio) in the LRE, with values greater than 1 indicative of a higher survival of barged fish relative to in-river fish, and values less than 1 indicative of a higher survival of in-river fish relative to barged fish. The value of the $\widehat{BI}$ ratio over the course of the entire outmigration season was 0.84 ($\widehat{SE}$ = 0.03) for fish transiting RKM 202 to 8.3. Values of the $\widehat{BI}$ ratio were 0.77 ($\widehat{SE}$ = 0.05), 0.81 ($\widehat{SE}$ = 0.05), and 0.94 ($\widehat{SE}$ = 0.05) for fish transiting RKM 202 to 8.3 during the early, middle, and late periods of the outmigration season, respectively. The $\widehat{BI}$ ratios of 0.84 and 0.77 were statistically different than a ratio of 1. The values of the $\widehat{BI}$ ratios suggest differential mortality in the LRE, with a higher incidence of mortality in barged fish than in-river fish. The lowest survival for both barged and in-river fish in the LRE occurred between RKM 35.6 and 8.3, a location representing both the furthest point of saltwater intrusion into the estuary and the nesting location of avian predators. Mean travel times of in-river fish between RKM 202 and 8.3 were consistently around 2.3 ($\widehat{SE}$ = 0.01) days for the entire outmigration season, whereas for barged fish the values decreased from 7.9 ($\widehat{SE}$ = 0.19) to 3.1 ($\widehat{SE}$ = 0.03) days over the course of the outmigration season. The longer transit times of barged fish may have increased the risk of predation: for example, bird predation estimates derived from PIT tags recovered at East Sand Island were considerably higher in barged study fish (7%) than in-river study fish (4%).

In this study, the health of barged and in-river outmigrant yearling Chinook salmon was assessed by characterizing the extent and putative causes of mortality of fish held in net pens located at Tongue Point (fresh water site) and Sand Island (saline-influenced site) in the LRE. The magnitude of cumulative net pen mortality of all groups of fish held at both net pen locations was strongly impacted by net pen location. All groups of fish held at both net pen locations experienced significantly greater mortality during holding at Tongue Point relative to Sand Island, thus suggesting that both barged and in-river fish arrive at Bonneville Dam in a compromised condition that decreases their probability of survival during extended freshwater transit time. Additionally, fish barged early in the outmigration season had a higher incidence of mortality in the net pens than fish barged later in the season. Overall, mycotic infection and metabolic disease were the main causes of mortality in barged fish held in the freshwater net pen site (Tongue Point); ceratomyxosis was the main cause of mortality in net pen fish with an in-river outmigration history. Given that the transit time in the LRE of barged fish is greater than in-river fish (data based on actively migrating JSATS-tagged barged and in-river fish in the LRE), one might hypothesize that fish health is contributing to the differences in differential mortality observed in actively migrating JSATS-tagged barged and in-river fish in the LRE. However, this study did not attempt to make specific statistically significant linkages between measures of fish health and survival of actively migrating barged and in-river fish in the LRE because of logistical difficulties in study implementation associated with unusually high river flows in the 2008 outmigration season.

II. EXECUTIVE SUMMARY

The research objectives of this study were to:

(1) Estimate survival and travel time for run-of-river yearling Chinook salmon during transit through the lower Columbia River and estuary (LRE);
(2) Produce information on fish health/pathology to help understand (i) the timing and trends of mortality in groups of fish with different outmigration histories as they migrate through the Columbia River and estuary and (ii) potential net pen effects that may influence the comparison of transported and in-river fish; and
(3) Integrate survival, travel time, and physical and environmental factors to estimate the extent and potential causes of differential mortality of transported and in-river run-of-river yearling Chinook salmon in the lower Columbia River and estuary.

Methods. In this study, a total of 4310 run-of-river yearling Chinook salmon were surgically implanted with acoustic tags. At Lower Granite Dam, 2165 yearling Chinook salmon were surgically implanted with acoustic tags and transported by barge through the hydropower system. A total of 884 of these barged fish (herein referred to as the Barge treatment group) were offloaded at Bonneville Dam and deposited into two estuary net pen sites (river kilometer [RKM] 7, Sand Island; and RKM 29, Tongue Point) and held for 28 days; the remaining 1281 fish were released below Bonneville Dam at the barge-release site near Skamania Landing (RKM 227) to complete their outmigration through the LRE. An additional 1249 run-of-river outmigrant yearling Chinook salmon were surgically implanted with acoustic tags at Lower Granite Dam and released to migrate in the river system. A subgroup of these fish were re-collected at Bonneville Dam and John Day dams in the sort-by-code collection systems (herein referred to as the In-River treatment group) and deposited into net pens at the Sand Island site. An additional 896 run-of-river outmigrant yearling Chinook salmon were collected and surgically implanted with acoustic tags at Bonneville Dam (herein referred to as the Bonneville treatment group) and transported to the two net pen sites in the estuary for a 28-day holding period. Finally, 1080 yearling Chinook salmon raised at the Newport Research Station's (NRS) Fish Disease Laboratory (FDL) from Rapid River Hatchery stock were deposited in net pens at Tongue Point and Sand Island to serve as reference fish (herein referred to as the Reference treatment group); Reference fish were not surgically implanted with an acoustic tag.

Survival and Travel Time Analysis. Travel times and survival probabilities of transported and in-river yearling Chinook salmon in the lower Columbia River and estuary (LRE) were estimated using Juvenile Salmon Acoustic Telemetry System (JSATS) acoustic tags and concomitant detection arrays. The detection arrays were deployed as part of other on-going studies.

In-river fish took from 10 ($\widehat{SE}$ = 0.14) to 19 ($\widehat{SE}$ = 0.20) days to transit Reach 1, which consisted of travel from Lower Granite Dam to just below Bonneville Dam (RKM 202). Overall survival of in-river fish within this Reach was 53% ($\widehat{SE}$ = 0.01). Survival probabilities were not correlated with travel times. Barged fish transited the majority of Reach 1 in a barge hold over roughly a 36-hour period; the mean survival probability was 94.5% ($\widehat{SE}$ = 0.01).

In-river fish took slightly over 2 days to transit the subsequent two Reaches below Bonneville Dam to the mouth of the estuary at RKM 8.3, a distance of 197 RKM. Within the LRE, travel speeds were slowest for in-river fish in Reach 3, which encompasses the last 27 RKM prior to ocean entry. The mean probability of survival of in-river fish in the LRE was 86% ($\widehat{SE}$ = 0.02) for the entire outmigration season, with specific values during the early, middle, and late periods of the outmigration season of 83 ($\widehat{SE}$ = 0.03), 86 ($\widehat{SE}$ = 0.04), and 89% ($\widehat{SE}$ = 0.04), respectively. Travel time and survival of in-river fish through the LRE did not vary significantly over the outmigration season, with maximum travel time differences of 5 hours and survival differences of 6% between early, middle, and late periods.

For barged fish, travel times between RKM 202, 25 km downstream of the barge release site at RKM 227, to the mouth of the estuary (Reach 2 and 3) were longer than those of in-river fish, with mean values progressively decreasing from 8 ($\widehat{SE}$ = 0.19) to 3 ($\widehat{SE}$ = 0.03) days over the outmigration season. Within the LRE, travel speeds were slowest for barged fish in Reach 3. The mean probability of survival of barged fish in the LRE was 72% ($\widehat{SE}$ = 0.02) for the entire outmigration season, with specific values during the early, middle, and late periods of the outmigration season of 64 ($\widehat{SE}$ = 0.03), 70 ($\widehat{SE}$ = 0.03), and 83% ($\widehat{SE}$ = 0.02), respectively. Despite the lower survival probabilities of barged fish through Reaches 2 and 3, the overall survival from Lower Granite Dam to river kilometer 8.3 was higher (68%, $\widehat{SE}$ = 0.02) than for in-river fish (46%, $\widehat{SE}$ = 0.02).

The extent of differential mortality between barged and in-river fish was assessed in terms of the Barge to In-River Survival Ratio ($\widehat{BI}$ ratio), with values greater than 1 indicative of a higher survival of barged fish relative to in-river fish, and vice versa. Estimates of $\widehat{BI}$ for treatment groups pooled over the season were 1.78 ($\widehat{SE}$ = 0.05) for Reach 1 (spanning from Lower Granite Dam to just below Bonneville Dam in which barged fish spent the majority of transit distance in a barge hold) and 0.84 ($\widehat{SE}$ = 0.03) for Reaches 2 and 3 (spanning from RKM 202 to 8.3 in which both treatment groups actively migrated), with an estimate of 1.50 ($\widehat{SE}$ = 0.07) for the entire study area (Lower Granite Dam to RKM 8.3). The pooled $\widehat{BI}$ ratio between RKM 202 and 8.3 (0.84) was statistically different than a ratio of 1. The non-pooled $\widehat{BI}$ ratios were 0.77 ($\widehat{SE}$ = 0.05), 0.81 ($\widehat{SE}$ = 0.05), and 0.94 ($\widehat{SE}$ = 0.05) for fish transiting RKM 202 to 8.3 (collectively Reaches 2 and 3) during the early, middle, and late periods of the outmigration season, respectively. The non-pooled $\widehat{BI}$ ratio of 0.77 was statistically different than a ratio of 1. The values of the $\widehat{BI}$ ratios suggest differential mortality in the LRE, with a higher incidence of mortality in barged fish than in-river fish.

Net Pen Mortality. Morbid individuals were collected daily from each of the net pen holding sites. Daily counts of morbid fish collected at each site were used to estimate statistical differences in the cumulative incidence of mortality between the following treatment groups: (1) Barged, Bonneville, In-River and Reference at Sand Island; (2) Barged, Bonneville, and Reference at Tongue Point (no in-river fish were held at Tongue Point); (3) Early, Middle, and Late passage cohorts of Barged and Reference groups at both net pen locations; and (4) Bonneville at both net pen locations.

Location of the net pen site was the main factor influencing net pen mortality. For all treatment groups held at both net pen locations, mortality was significantly greater at Tongue Point (freshwater site) relative to Sand Island (saline-influenced site). Very low mortality in reference fish at both net pen sites suggested that the net pens themselves were not significantly contributing to the observed incidence of net pen mortality in acoustic-tagged fish from all treatment groups. Hence, the elevated incidence of mortality of both barged and in-river fish at Tongue Point, relative to Sand Island, would suggest that fish arrived at Bonneville Dam in a compromised condition that decreased their probability of survival during extended freshwater transit time.

The Barged treatment group experienced significantly greater mortality in the beginning of the net pen holding period relative to fish with an in-river outmigration history (Bonneville treatment group) at Tongue Point, while during the last days of holding this trend reversed. Furthermore, mortality of barged fish was higher in the Early passage cohort than the Late cohort. Trends in net pen mortality would suggest that barged fish, as a population, are not as healthy as in-river fish entering the LRE, and that fish barged late in the season are healthier upon entry into the LRE than fish barged early in the season.

The mean travel times of actively migrating JSATS-tagged in-river and barged fish in the LRE were 2 and 3-8 days, respectively. Mortality of barged fish actively migrating through the LRE was compared statistically to mortality of barged fish held in the net pens at Tongue Point during the respective days. This comparison was not made with in-river fish due to the fact that this treatment group was only held in net pens at Sand Island (saline-influenced site), and the majority of the LRE is freshwater. Survival of the Barged treatment group in the net pens at Tongue Point was approximately 10% higher than the survival of actively migrating barged fish between Skamania (RKM 227) and RKM 8.3. When adding estimated piscivore predation and the true extent of avian predation to the mortality observed in the net pens, the resulting value falls into the range of survival of actively migrating barged fish in the LRE. This analysis highlights the potential value of using estuary net pens to study the extent and possible causes of health-related mortality of actively migrating fish in the LRE.

Pathology. Histopathological analyses were performed on both fish destructively sampled throughout selected collection sites in the FCRPS, as well as all morbid fish in the net pens. For destructively sampled run-of-river yearling Chinook salmon collected at Lower Granite and John Day dams, the prevalence of clinical signs of disease was very low: at Lower Granite Dam, the prevalence of Bacterial Kidney Disease (BKD) was 5%, while at John Day Dam no clinical signs of disease were detected in any fish sampled. However, following 28 days of net pen holding, no treatment group was free of clinical signs of disease, and the most prevalent diseases were BKD, fungal (mycotic) infections, ceratomyxosis, and metabolic lesions. For those groups of fish with an outmigration history involving river migration (e.g. In-River and Bonneville treatment groups), the prevalence of ceratomyxosis after net pen holding was approximately 92-94%. In contrast, barged and reference fish had a prevalence rate of 34% and 17%, respectively. These results would suggest that fish migrating within the Columbia River are at an increased risk of contracting this parasite. Reference fish with initially no indication of ceratomyxa infection at the population level showed low population prevalence of the disease (17%) after 28 days of holding, indicating that disease transmission may have occurred between fish held within the net

pens, and/or the estuary itself was a contributing factor. The prevalence of mycotic infections in fish following 28 days of holding was very low, and was detected only in the Barged and Reference groups at the Tongue Point net pen site. The prevalence of metabolic lesions was low in groups sampled at Lower Granite and John Day dams (between 10-28%), but increased during 28 days of holding (34-45%). The prevalence of these lesions in reference fish was 2%.

The majority of mortalities during net pen holding were diagnosed with mycotic infections and ceratomyxosis. The Bonneville treatment group had a considerably higher prevalence of mycotic infections than in-river or barged fish. The prevalence of mycotic infections in morbid fish was higher at the freshwater net pen site (Tongue Point) than at the saline-influenced site (Sand Island). Absence of ceratomyxosis in barged fish may be explained by the temporally and spatially reduced exposure of these fish to the habitat which promotes the transmission of this disease as compared to the Bonneville fish. Bonneville fish may have arrived from different natal hatcheries and presumably spent more time outmigrating than did barged fish and hence had an elevated risk of contracting the disease. The analyses of mortalities among Bonneville fish over the whole course of holding indicates favorable conditions for contracting and spreading of ceratomyxosis.

Severe metabolic lesions associated with infectious disease and other stressors were highly prevalent in morbid fish in the Barged treatment group. Typically, metabolic lesions are found in stressed, diseased, and/or anorexic fish. The extent to which these lesions were caused by or connect to mycotic infections or stressors such as collection, transport, and release is currently unknown and beyond the scope of this study. Regardless of the cause of metabolic lesions, the prevalence of these lesions in morbid barged fish increased over the first 7 days of net pen holding; the prevalence rate then stabilized in morbid fish for the duration of net pen holding. The initial spike in prevalence of metabolic lesions in morbid fish may be due to the mortality of severely anorexic or otherwise stressed fish arriving at the net pens after barge transport.

Pathogen Prevalence. Fish tissues and water samples were surveyed for eight salmonid pathogens by the detection of their genetic material with polymerase chain reaction (PCR): *Renibacterium salmoninarum*, *Aeromonas hydrophila, Aeromonas salmonicida, Listonella anguillarum, Yersinia ruckeri*, Infectious Hematopoietic Necrosis Virus (IHNV), Viral Hemorrhagic Septicemia (VHSV), and the fungal family Saprolegniacea. In contrast to the histopathology, PCR analysis does not differentiate between clinical and sub-clinical signs of disease.

The most common pathogen detected among all experimental treatment groups was *R. salmoninarum* with a prevalence of up to 22%. The second most commonly detected pathogen was Saprolegniaceae (3-10%). Since PCR surveys were limited to kidney tissue, tests likely detected only those fish with systemic Saprolegniasis infections. Saprolegniaceae PCR detection was greater among fish held at Sand Island than Tongue Point, despite Saprolegniaceae detections in two water samples and an overall higher prevalence of mycotic infections detected by histopathology at Tongue Point. Fewer PCR detections at the conclusion of 28-day holding period at Tongue Point may have been due to more severely diseased fish dying in those net pens during holding, leaving fewer infected fish to sample.

Covariates Effects Analysis for Survival and Travel Time. A variety of measures of migration timing, size at tagging, handling at Lower Granite Dam, and environmental conditions were examined as covariates of survival and travel time of migrating fish. Covariates of migration timing included collection, tagging, and river release dates. Length and weight were used as covariates of size at the time of tagging. Collection source and holding duration at Lower Granite Dam were included as covariates representing handling. Collection source was tabulated for all fish, but varied only for barged fish. Covariates describing environmental conditions consisted of the average daily discharge at both Lower Granite and Bonneville dams.

For the in-river fish in Reach 1 (Lower Granite Dam to RKM 202), larger fish (fork length and weight) that were held for the shortest amount of time prior to release below Lower Granite Dam had the highest probability of survival. Travel times were shorter in Reach 1 for larger fish, as well as for fish collected later in the season. In Reaches 2 and 3 (RKM 202 to 8.3), the measured physical and environmental covariates did not explain variation in survival. For Reaches below Bonneville Dam (2 and 3), travel times decreased with increased discharge at Bonneville Dam. In Reach 2, larger fish size (fork length and weight) was associated with shorter travel times, but in Reach 3 weight and length were not significantly related to travel time.

For barged fish, in the Reach downstream from Bonneville Dam to RKM 35.6 (Reach 2), fish that were held longer at Lower Granite Dam or arrived at times of higher discharge at Bonneville Dam tended to travel faster. In the last Reach (Reach 3; RKM 35.6 to 8.3), barged fish that were released at times of higher discharge at Bonneville Dam tended to travel faster than fish that experienced lower discharge. Discharge at Bonneville Dam was the main factor for increased survival probabilities of barged fish in the LRE.

Conclusions

In this study, there was evidence of differential mortality between barged and in-river outmigrant yearling Chinook salmon in the LRE. Over the course of the entire outmigration season, there was a 19% elevated incidence of mortality between RKM 202 and 8.3 in fish barged to the estuary relative to fish that actively migrated to the estuary in-river. This finding should not necessarily be interpreted as providing evidence for leaving fish in-river during outmigration: the overall survival of barged fish from Lower Granite Dam to river kilometer 8.3 was higher (68%) than for in-river fish (46%).

Differential mortality was greatest in fish arriving at the estuary early in the outmigration season. This finding supports a barging strategy that leaves fish in the river early in the outmigration season, and increases the number of transported fish later in the outmigration season. Seasonal variation in differential mortality in the LRE could feasibly be used to further optimize transport schedules.

Predation was responsible, in part, for the observed differential mortality between barged and in-river fish in the LRE. Bird predation estimates below Bonneville Dam on East Sand Island were considerably higher in barged study fish (7%) than in-river study fish (4%).

The incidence of mortality in estuary net pens as well as the prevalence of specific diseases would suggest that both barged and in-river fish are present in the LRE in a compromised state that decreases their probability of survival during extended freshwater transit time. Additionally, based on data from the net pens, the extent of mortality of barged fish in freshwater is significantly greater than in a saline-influenced environment, and this trend is particularly pronounced early in the outmigration season. Results from the net pen study also suggest that mortality of Barged and In-River groups is not significantly different once fish enter water with salinity nearly equivalent to seawater. Given that the transit time in the LRE of barged fish is greater than in-river fish (data based on actively migrating JSATS-tagged barged and in-river fish in the LRE), one might hypothesize that fish health is contributing to the differences in differential mortality observed in actively migrating JSATS-tagged barged and in-river fish in the LRE. However, this study did not attempt to make specific statistically significant linkages between measures of fish health and survival of actively migrating barged and in-river fish because of logistical difficulties in study implementation associated with unusually high river flows in the 2008 outmigration season.

The Draft Final version of this report underwent a regional external peer-review directed by the USACE Walla Walla District. As part of this external peer-review, it became evident that some individuals within the Region believe no direct inference can be made between the extent of mortality in the net pens and that observed in fish actively migrating through the LRE. Others within the region believe the extent of mortality observed in the estuary net pens over a time period commiserate with LRE outmigration (2 and 3-8 days for in-river and barged fish, respectively) represents a component of the overall mortality observed in actively migrating JSATS-tagged fish in the LRE. For the latter individuals, the mortality of actively migrating barged fish in the LRE was 10% greater than the mortality observed in fish held in the net pens at Tongue Point. The 10% difference likely reflects, in part, piscivore and avian predation. Using reported values of piscivore and avian predation in the LRE with mortality observed in the estuary net pens, the overall mortality of barged fish actively migrating in the LRE can be subdivided as: 7-11.8% related to causes identified in morbid net pen fish which were largely associated with infectious diseases; 2.2-9.2% minimum related to avian predation; and 5% minimum related to piscivore predation.

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1.0 INTRODUCTION

The Columbia River Basin provides critical habitat for threatened and endangered salmon species in the Pacific Northwest. Thirteen stocks, or evolutionarily significant units (ESUs; Waples 1991) from this region are threatened or endangered; Chinook (*Oncorhynchus tshawytscha*) salmon ESUs include Snake River spring/summer and fall run, Lower Columbia River, Upper Willamette River, and Upper Columbia River spring-run (NRC 1996). Factors contributing to the decline of salmon populations in the Pacific Northwest include habitat degradation, over harvest, hydropower operation, and hatchery production (NRC 1996). The Federal Columbia River Power System (FCRPS) is providing beneficial hydroelectric power, irrigation water, flood protection, navigation, and recreation for the region, but the FCRPS has also critically affected salmon migration and populations, with some of the Columbia River Basin ESUs migrating past as many as eight dams. In addition to restricting access to adult reproductive habitat (Raymond 1988), the FCRPS also contributes to stock losses in juveniles during river outmigration.

Direct and Delayed Mortality

Losses in outmigrating salmonid populations would occur regardless of the presence of the hydroelectric dams due to other causes of mortality. For example, in the absence of the FCRPS, juvenile salmonid mortality in the river and estuary during outmigration may occur due to predation, disease, chemical toxicity, water quality, nutrition, physiological stresses associated with smoltification, and injury (Figure 1a). However in the presence of the FCRPS, juvenile salmonid mortality may increase for all of these other causes, as well as for additional sources of direct mortality (Figure 1b). Direct mortality is considered to occur when death takes place during the same life stage as the stressor, and delayed mortality is considered to occur at a life stage subsequent to the stressor.

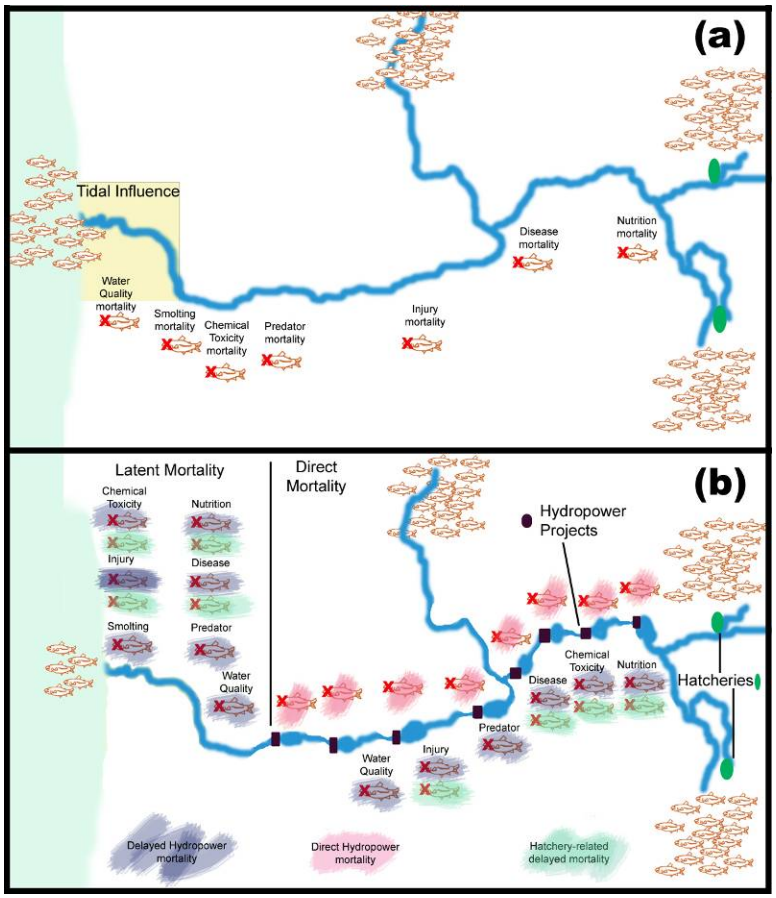

Figure 1. Non-hydrosystem (a) and hydrosystem (b) factors contributing to juvenile salmon mortality during outmigration.

Specific actions have arisen to mitigate the impacts of the FCRPS on salmon survival including: juvenile fish-passage and collection facilities; adult fish ladders; predator control; transportation (barging); flow augmentation; and reservoir drawdown (Muir et al. 2001; Ruckelshaus et al. 2002). However, the specific impacts (benefits and/or losses) of these remediation strategies on population numbers are currently not well quantified. Recent research has addressed this data gap with two types of studies, described here as 'juvenile life-stage' and 'adult return' studies. Juvenile life-stage studies, in part, focus on the short-term, direct effects of specific interactions between the FCRPS and juvenile fish during outmigration, e.g. the incidence of gas-bubble trauma. Adult return studies, in part, focus on the status of adult fish returns in reference to outmigration history differences, e.g. smolt-to-adult return (SAR) rates for fish that are barged versus fish that remain in the river during outmigration. Some of the former studies have shown that the remediation strategies themselves may induce levels of stress that exacerbate delayed health effects associated with predator response, disease susceptibility, and growth. For example, barging juvenile salmon was shown to induce stress associated with handling and crowding, and passage through bypass systems caused mechanical injuries such as bruising and descaling (Budy et al. 2002; NRC 1996). These results support some of the latter adult return studies that have shown decreasing SARs for increasing number of dams bypassed (Sandford and Smith 2002). In coupling results from both 'juvenile life-stage' and 'adult return' studies, one may conclude that the FCRPS mitigation strategies may not only induce direct mortality or harm to outmigrating smolts, but also contribute to the incidence of delayed mortality.

Quantifying the extent of direct mortality due to the presence of the FCRPS or a mitigative action is challenging, but tractable given that the mortality occurs at the specific location of interest that is being monitored (e.g., a turbine). In contrast, delayed mortality occurs in a subsequent life stage; and in the case of the FCRPS, this life stage can be in the estuary or ocean, where monitoring is limited. The D and T/C parameters are commonly used as gross measures of differential mortality associated with barging relative to an in-river outmigration strategy. The *T/C* ratio is a direct comparison of the SAR for smolts that are transported through the FCRPS to the SAR of In-River smolts experiencing 0–3 Columbia River bypasses during outmigration:

$$T/C = SAR_2(T_0)/SAR(C_0) \tag{1.1}$$

The differential mortality (*D*) is an alternative parameter for comparing transportation and In-River outmigration histories:

$$D = \frac{SAR_2(T_0)}{V_t} \Big/ \frac{SAR(C_0)}{V_c} \tag{1.2}$$

where V_c is the survival rate of In-River outmigrants from Lower Granite Dam to Bonneville Dam (ca. 51-54% for hatchery-reared Snake River spring/summer Chinook salmon), and V_t is the survival rate of transported juveniles from Lower Granite Dam to Bonneville Dam. The parameter V_t is defined as barge survival (assumed 98%) adjusted for in-river survival to barge collection sites downstream of Lower Granite Dam (Berggren et al. 2003). Therefore, D normalizes the SARs for direct mortalities of smolts that occur in the barge and during in-river outmigration to Bonneville Dam. Due to the large difference between values for V_t and V_c, the information provided from the T/C ratio and D parameter are not the same. The T/C ratio has been generally greater than 1.0 for hatchery-reared Snake River spring/summer Chinook salmon (Berggren et al. 2003), implying that transporting fish around the dams increases the return rate of adults relative to fish with an in-river outmigration history. In contrast, D values have been

generally less than 1.0 for hatchery-reared Snake River spring/summer Chinook salmon (Berggren et al. 2003), implying that transporting fish around the dams increases their post-FCRPS mortality relative to fish with an in-river outmigration history. The contrast in outcomes predicted by the T/C ratio and the D parameter has also been observed in three previous juvenile life stage studies that were completed in 2002, 2006, and 2007 for the United States Army Corps of Engineers (USACE) as part of the Anadromous Fish Evaluation Program (AFEP) in which barging was shown to mitigate some of the stressors associated with in-river outmigration through the FCRPS, but barged fish appeared to have greater mortality immediately after transport (Arkoosh et al. 2006; Dietrich et al. 2007). In the replicate studies, fish with in-river outmigration histories were more susceptible to disease during planned laboratory challenge with an infectious agent (e.g., *Listonella anguillarum*) than fish with barged outmigration histories (Arkoosh et al. 2006; Dietrich et al. 2007). However, in 2007 barged fish were observed to have a greater mortality rate prior to the laboratory pathogen challenge than in-river fish. The mortalities were associated with infectious diseases contracted prior to laboratory arrival, and the barged population that survived was shown to be more resistant to the laboratory-imposed disease challenge than fish with in-river outmigration histories (Dietrich et al. 2007).

Collectively, the results from these previous AFEP studies and the implications of the D parameter estimates are not mutually exclusive, but rather highlight the importance of understanding the relative significance of various components influencing the SAR rates, of which disease-induced mortality is only one. We found that in-river migration strategies are correlated with the incidence of post-FCRPS disease-induced mortality over barged fish; hence, for equal pathogen exposure in the estuary and ocean, greater disease-induced mortality would be expected in the in-river group. To offset the reduction in stock losses associated with in-river migration strategies, other factors influencing post-FCRPS mortality must preferentially impact fish with a barged outmigration history to satisfy estimates of D. Potential factors contributing to the difference between populations include: (a) increased injury or stress induced by barging operations; (b) barged smolts are not physiologically prepared for early saltwater entry due to the rapid migration downriver on the barge; (c) incomplete imprinting resulting in increased adult straying; (d) early estuary conditions to which transported fish are introduced, but the slower in-river migrants are not; (e) incomplete population selection within the FCRPS for the healthiest transported population that is completed in the estuary or early ocean transit; and (f) disease transmission in the barge hold and raceways resulting in increased delayed disease-induced mortality among transported smolts.

Acoustic Telemetry

The following study represents an incremental expansion of these three prior AFEP-funded delayed mortality studies that were conducted either in laboratory or estuary net pen settings. In this study we used acoustic tags to expand past lab and net pen research into the lower 235 km of the Columbia River and estuary (LRE) to further explore differential mortality. Prior telemetry studies have utilized acoustic tags and concomitant detection arrays to estimate survival, travel time, and ocean-entry timing of both run-of-river stream- and ocean-type juvenile salmonids through the lower Columbia River and estuary (McComas et al. 2007 and McComas et al. 2008). Run-of-river salmonids were tagged at Bonneville Dam and tracked for up to 30 days during transit to the mouth of the Columbia River. Based on data obtained in the 2006 outmigration season, 67% of run-of-river yearling juvenile salmon survived transit through the LRE

(McMichael et al. 2007). Stated alternatively, 33% of the run-of-river yearling juvenile salmon ceased migration or died in the LRE; the specific causes of mortality are unknown, with the exception of estimates of avian predation.

While the past three AFEP-funded delayed mortality studies provide a preliminary estimate of the incidence and cause of differential mortality in the LRE, how well this estimate reflects what is taking place in the open lower-river and estuary system was unknown for three main reasons. First, the actual LRE transit time of transported yearling Chinook was unknown. Although we do have estimates of transit time of in-river run-of-river yearling Chinook salmon over the past several years from Bonneville Dam to the mouth of the Columbia River (McComas et al. 2007 and McComas et al. 2008), we do not know how well this estimate reflects fish with a transportation outmigration history. An accurate estimate of travel time was needed to truncate the mortality in the estuary net pens to reflect the actual estuary transit time. Second, the mortality that occurred in the net pens represented causes associated with fish held quiescently in the estuary. There are a number of other possible causes of mortality that are not captured with survival estimates obtained from the net pens (e.g., avian and piscivorous predation). Hence, the significance of these other possible causes of mortality could only be assessed by comparing the incidence of mortality of fish transiting the LRE with the incidence of mortality of fish held in the net pens. Additionally, understanding the causes of mortality of fish held in the net pens may help further understand possible causes of mortality in fish transiting the LRE. Third, the mortality observed in the net pens may arise from the net pens themselves such that survival estimates obtained from the net pens may grossly underestimate survival of fish transiting the LRE. In such a situation, the cause of mortality in the estuary net pens may not accurately reflect mortality in fish transiting the LRE.

In response to this disconnect between fish movement studies and net pen mortality studies, we initiated an acoustic telemetry study in 2008 (Figure 2). In total, 4,310 run-of-river yearling Chinook salmon smolts were surgically implanted with acoustic tags. At Lower Granite Dam, 2,165 yearling Chinook salmon smolts were surgically implanted with acoustic tags and transported by barge through the hydropower system. A total of 884 of these barged acoustic-tagged fish were offloaded at Bonneville Dam and deposited into two estuary net pen sites (Sand Island and Tongue Point) and held for 28 days; the remaining 1,281 fish were released below Bonneville Dam at the barge release-site at Skamania Landing (RKM 227). Survival estimates of the barged fish released at Skamania were obtained with JSATS detection arrays deployed as part of another ongoing study entitled "A study to estimate juvenile salmonid survival from Bonneville Dam through Columbia River estuary using acoustic tags" (PIs: McComas and McMichael). An additional 1,249 run-of-river outmigrant yearling Chinook salmon were surgically implanted with acoustic tags at Lower Granite Dam and released back into the river system. A subgroup of these fish were re-collected at Bonneville and John Day dams and deposited into net pens at the Sand Island site. Finally, an additional 896 run-of-river outmigrant yearling Chinook salmon were surgically implanted with acoustic tags at Bonneville Dam and transported to the two net pen sites in the estuary for a 28-day holding period.

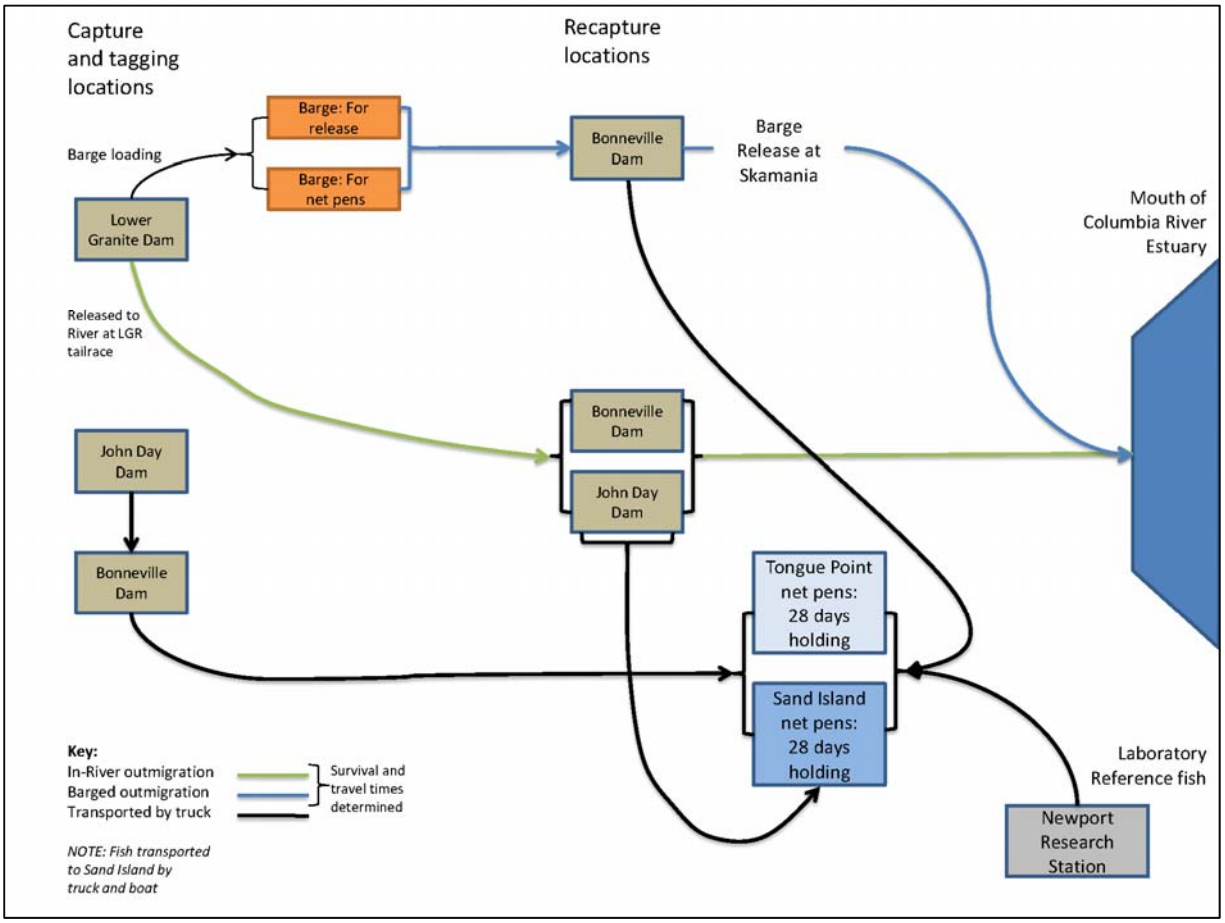

Figure 2. JSATS and estuary net pen study design.

Various health measures were obtained on both live and dead fish using a suite of techniques (histopathology, hematology, polymerase chain reaction) at different times and locations throughout the study area (Figure 3) to assess general condition and health, and to explore how measured covariates relate to estimated travel times and survival in the LRE. The subsequent integration of travel time, survival, and relevant environmental conditions and handling variables provided the basis for assessment of the extent and possible causes of differential mortality between barged and in-river fish in the LRE.

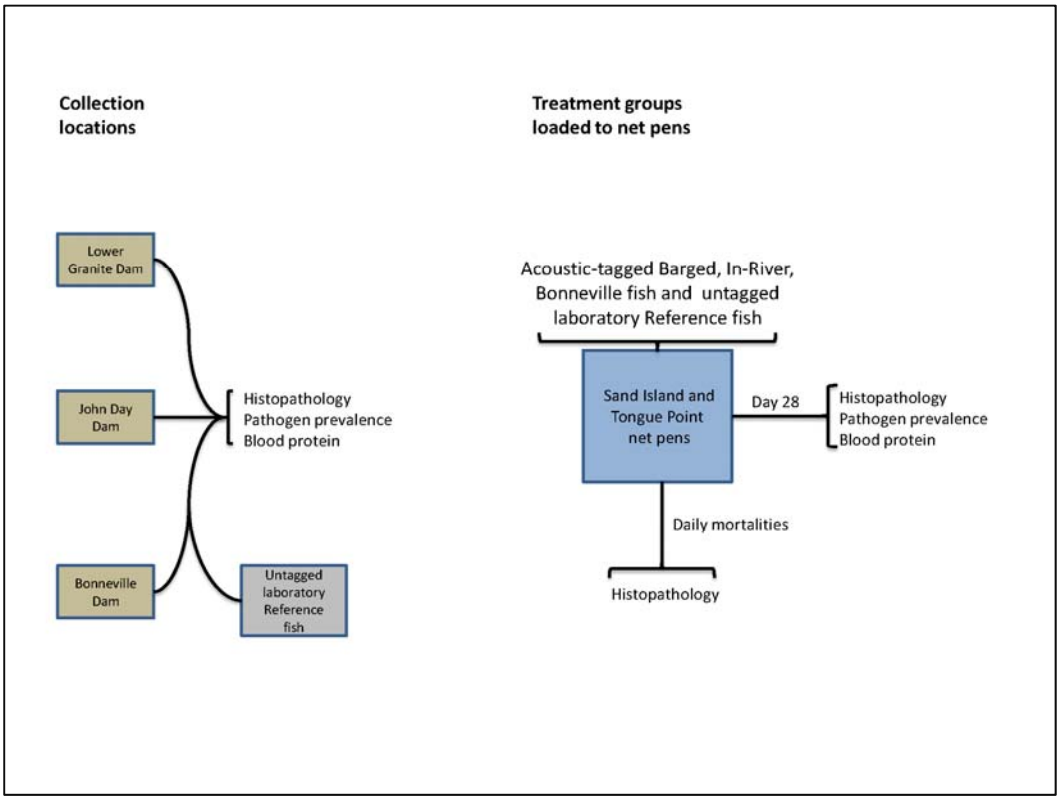

Figure 3. Sampling regimen at collection sites and estuary net pens.

Objectives

The objectives of the research reported herein were to: (1) estimate survival and travel time in JSATS-tagged run-of-river yearling Chinook salmon with different outmigration histories during transit through the LRE; (2) produce information on fish health/pathology to help understand (i) the timing and trends of mortality in groups of fish with different outmigration histories as they migrate through the Columbia River and estuary and (ii) potential net pen effects that may influence the comparison of transported and in-river fish; and (3) integrate survival, travel time, and physical and environmental factors to estimate the extent and potential causes of differential mortality of transported and in-river run-of-river yearling Chinook salmon in the LRE.

This report is subdivided into the following sections:

1. Methods (Chapter 2)
2. Objective 1 (Chapter 3)
3. Objective 2 (Chapters 4-6)
4. Objective 3 (Chapter 7)
5. Conclusions (Chapter 8)

The results and discussions are written in reference to each section with the conclusions section synthesizing material presented in all sections.

Project Area Description

The study area includes the spring/summer Chinook migration corridor from Lower Granite Dam (Snake River kilometer, SRKM, 173) to Sand Island (Columbia River kilometer, CRKM, 8.3) at the mouth of the Columbia River estuary. In-River outmigrants originating from the Snake River Basin encounter eight hydroelectric projects during their outmigration: four on the lower Snake River, including Lower Granite Dam (SRKM 173), Little Goose (SRKM 113), Lower Monumental (SRKM 67), and Ice Harbor (SRKM 15.6) dams; and four on the Columbia River, including McNary (CRKM 470), John Day (CRKM 347), The Dalles (CRKM 308), and Bonneville Dam (CRKM 235) dams.

Net Pen Site Descriptions

Net pens were located in the LRE at Sand Island and Tongue Point. The Sand Island net pen site was located just off the main channel of the Columbia River approximately 7 kilometers from the river mouth. The Tongue Point net pen site was located off the main channel of the Columbia River approximately 29 kilometers from the river mouth.

2.0 METHODOLOGY

Outmigration and Estuary Conditions

2008 Columbia and Snake River Conditions

According to data collected by the USACE Portland District Reservoir Regulation and Water Quality Section, flows for 2008 in the Snake and Columbia rivers experienced a marked increase from historical ten year averages in the beginning of May with high annual flow until early July (Figure 4). Compared to the outmigration conditions in 2007, the influent flows to Lower Granite Dam were similar in 2008 until this sustained peak flow occurred. In contrast, influent flows at Bonneville Dam in 2008 were, at times, 100 kcfs lower than 2007 until approximately May 5th. After May 5th, influent flow at Bonneville Dam increased and matched the 2007 flow, and then continued to increase, exceeding the 2007 flow by roughly 100 kcfs until early July. During the outmigration period and increased flows, the water temperatures in the Snake and Columbia Rivers steadily increased (Figure 5). Temperatures were roughly equal at all dams during the 2008 collection period (for the dams in which data was available). Measured temperatures during the 2008 collection period ranged from 9ºC in late-April to 13ºC in late-May at Bonneville Dam. Water temperatures between 10 and 15.6ºC are considered optimal for Chinook salmon, but the State of Oregon, Water Quality Standard for maximum temperature in salmonid migratory waters is 18ºC (ODEQ 1995). The water temperatures never exceed 18ºC during the collection period.

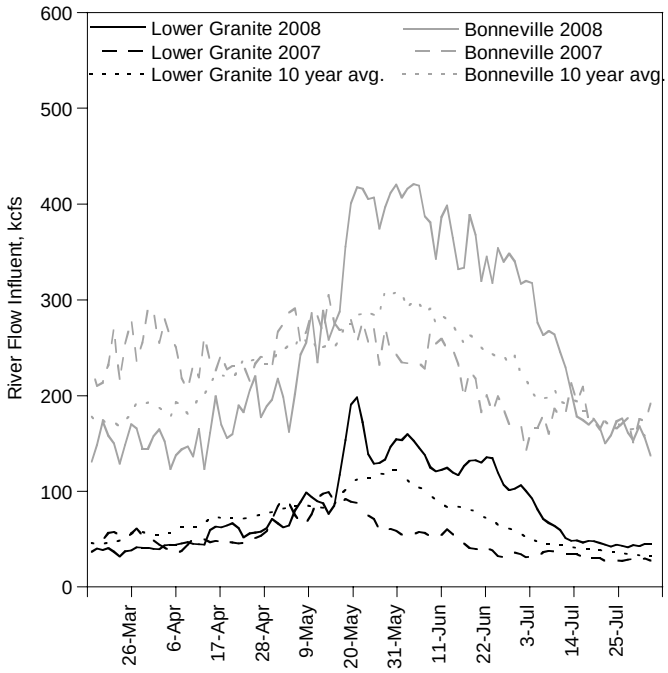

Figure 4. Influent flows of the Snake and Columbia rivers in 2007 and 2008, and ten year averages at Lower Granite and Bonneville dams, respectively.

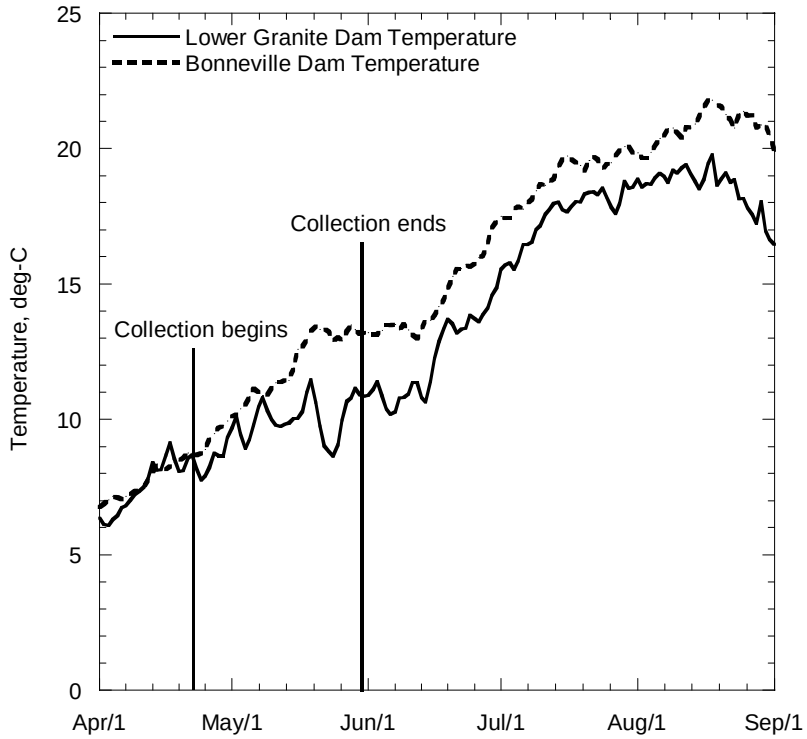

Figure 5. Water temperatures measured at Lower Granite and Bonneville dams during spring/summer Chinook outmigration in 2008.

Barging Conditions

USACE 8000 series barges were used during transport of barged fish. Barged fish assigned for the net pen study were separated from the general outmigrating run-of-river populations in the barge hold by suspended net pens. The experimental fish were exposed to the same water quality conditions, but physical contact with the other fish was prevented. During each transport trip water quality data were regularly collected in each barge hold along with counts of fish mortalities. Barge water quality parameters were recorded using a CTD multi-probe (YSI Inc.; Yellow Springs, OH) held within the suspended barge hold net pens. The CTD contained probes that recorded dissolved oxygen (DO), pH, temperature, and conductivity every 15 minutes. Barge hold water temperatures varied from 8 to 13ºC during transport of the Barged treatment group and gradually increased over the transport period. The dissolved oxygen concentrations were within acceptable ranges (8.5-14.0 mg/l) during all trips. On average, mortality of study fish during transport in net pens suspended in barge holds between Lower Granite and Bonneville dams was 0.79%.

Lower River and Estuary Conditions

Water quality parameters of temperature, dissolved oxygen, pH, and specific conductivity were collected at both net pen sites (Figures 6 and 7). The CTD recordings for Sand Island were not available after May 7 due to instrument failure. For the month of June, temperature and salinity values from the CORIE lower Sand Island node (http://www.ccalmr.ogi.edu/CORIE/) were used in place of CTD data for the Sand Island net pen location (Figures 6c and 6d); a high degree of correspondence was observed between CORIE and CTD data prior to May 7 (data not shown),

and hence, CORIE values after May 7 were assumed to reflect Sand Island net pen locations for the month of June. Data collection location for the CORIE node near Sand Island is located roughly seven meters below the surface, whereas the net pens were located approximately one meter below the surface. Given the differential depth and changes to the river hydrograph after May 7, a definitive assessment of the water quality conditions at the net pen site near Sand Island will require more detailed analyses of plume modeling data generated by Antonio Baptista, the PI of the CORIE system.

At Sand Island, maximum and minimum daily water temperatures and salinities ranged from 9 to 12°C and 2.1 to 28 ppt, respectively, early in the outmigration season, and 9 to 17°C and 1.1 to 33.2 ppt, respectively, late in the outmigration season. At Tongue Point, maximum and minimum daily water temperatures ranged from 11-12°C early in the outmigration season to 16-18°C late in the season. Salinity values at Tongue Point net pens were close to zero and exhibited little daily fluctuation. Mean daily water temperatures at Tongue Point increased from 8.7 to 19.2°C over the outmigration season, whereas the mean temperature at Sand Island increased from 9.9 to 12.3°C. At Sand Island, where fish were held from April 25 to June 30, 2008, the mean daily water temperature never exceeded 15.6°C, the upper limit of the Oregon Department of Fish and Wildlife (ODFW) optimal range for Chinook salmon. At Tongue Point, where fish were held from April 25 to June 28, 2008, the mean daily water temperature exceeded 15.6°C roughly 18% of the time. Values of dissolved oxygen and pH at both net pen locations (Figure 7) were well within acceptable ranges (13-10 mg/L DO, 8.5-7.5 pH) for Chinook salmon. The declining DO-values at Sand Island immediately prior to May 7 were attributed to CTD failure. Collectively, the two net pen sites span the range of water quality encountered by outmigrating spring/summer Chinook salmon in the LRE.

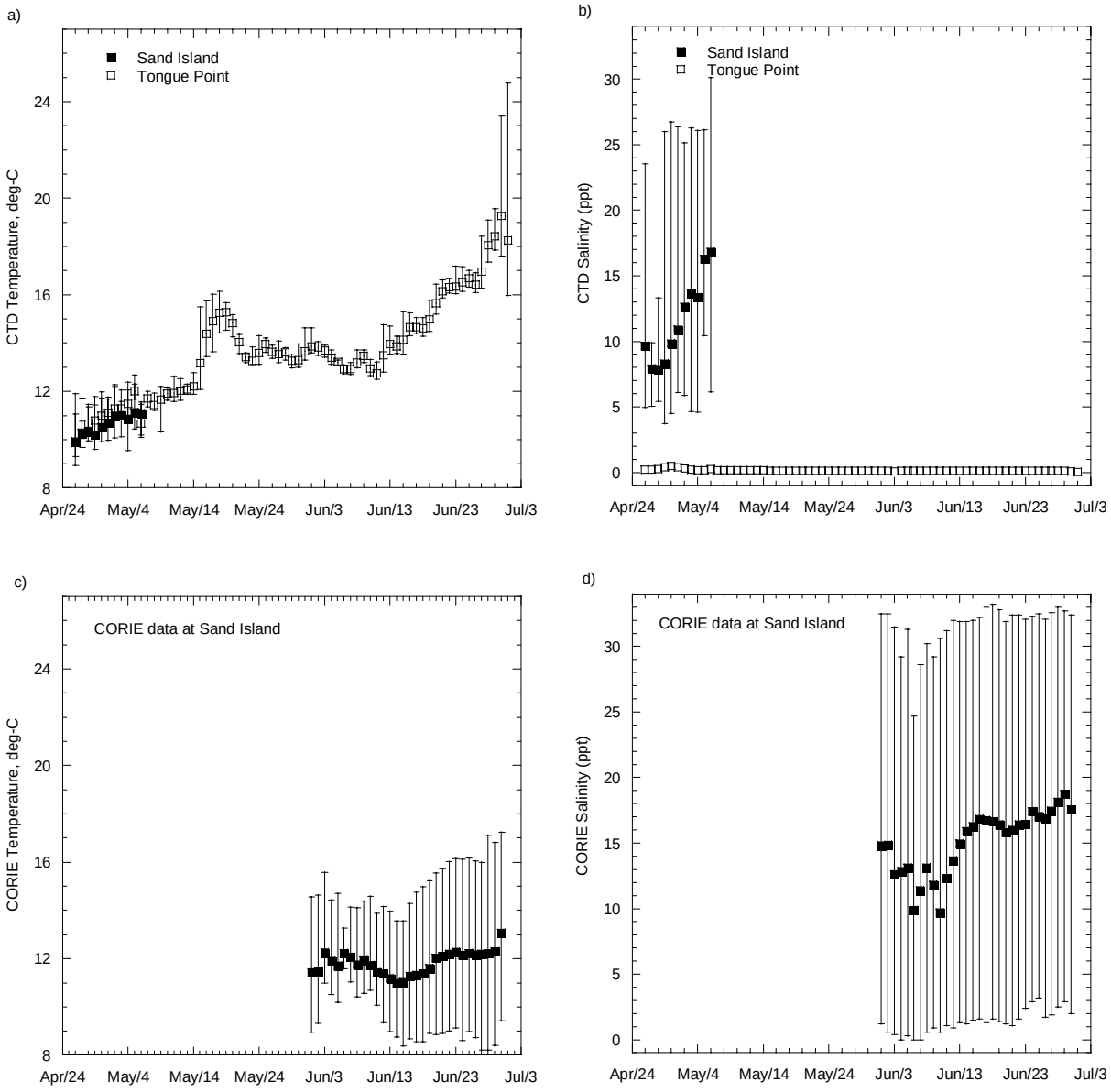

Figure 6. CTD measurements at Sand Island and Tongue Point net pens of a) water temperature and b) salinity. CORIE data for the month of June at the Lower Sand Island node of c) water temperature and d) salinity. The daily mean values and high/low ranges are shown for all figures.

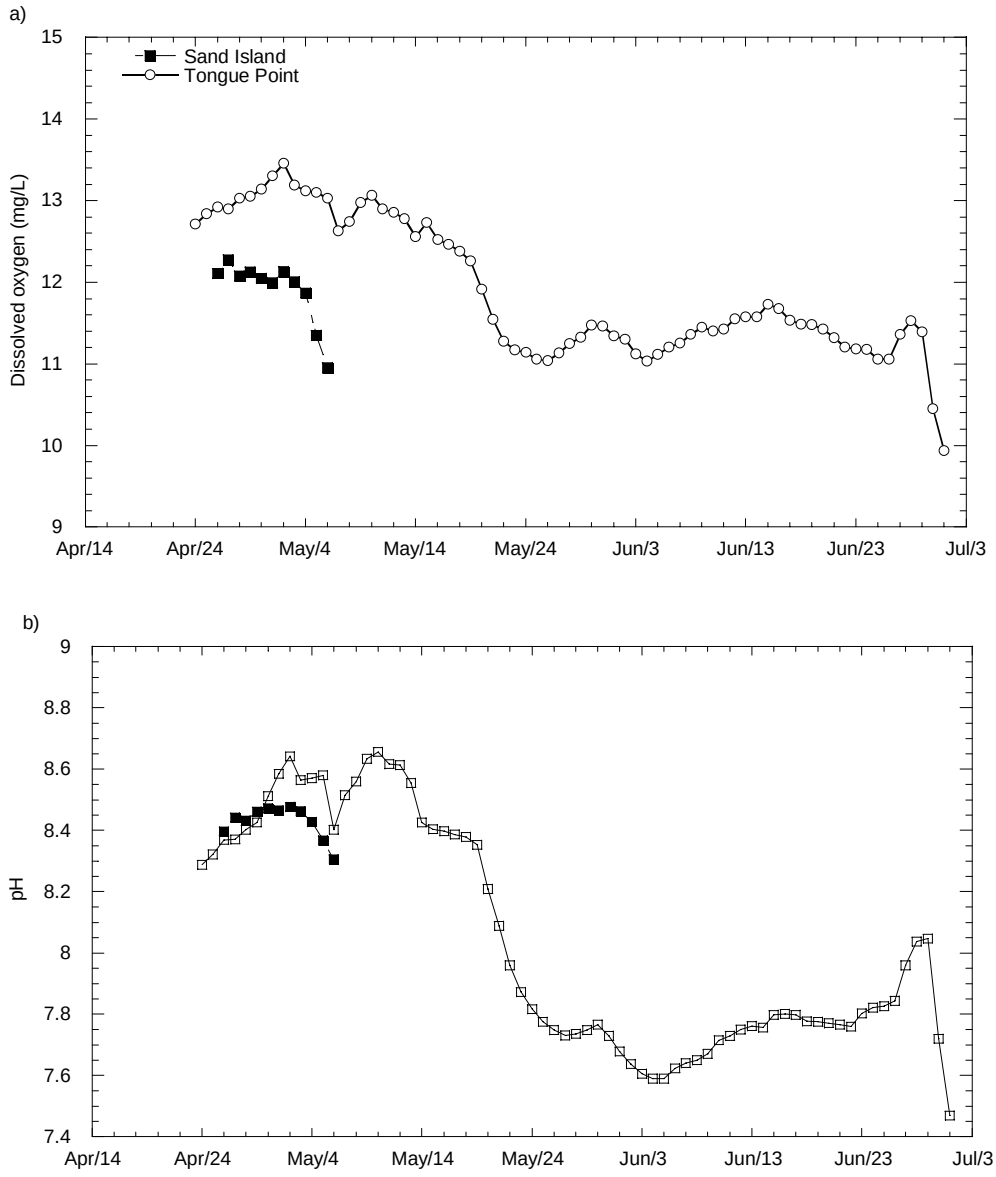

Figure 7. Mean CTD measurements of (a) dissolved oxygen and (b) pH at Sand Island and Tongue Point.

Fish Collection

Lower Granite Dam Collection

Run-of-river hatchery yearling Chinook salmon were collected from one of three locations in the Lower Granite Dam juvenile bypass system on the Snake River (RKM 695 from the mouth of the Columbia River, Snake River RKM 173) from April 21 to May 19, 2008. The majority of

study fish were collected from the juvenile fish facility sample room (N = 2999) with additional fish being obtained from the collection raceway (N = 440) and the separation by code (SbyC) tanks (N = 28; Table 1). Hatchery yearling Chinook salmon 95 mm or longer and not previously implanted with a Passive Integrated Transponder (PIT) tag were used for this study. Fish were transferred water-to-water from the collection site to holding tanks (width 0.91m, length 0.61m, depth 0.61m, volume 505L) supplied with flow-through river water and located inside an enclosed surgery trailer. Pre-surgery holding time ranged from 24 to 48 h. Temperature in the tanks throughout the tagging period ranged from 10.3-10.9°C.

Bonneville Dam and John Day Dam Collection

Run-of-river hatchery yearling Chinook salmon were collected from the juvenile fish bypass system at Bonneville Dam (RKM 235) and John Day Dam (RKM 348) for tag implantation at Bonneville Dam from May 22 to May 30, 2008. The majority of study fish were collected from the juvenile fish facility sample room at John Day Dam (N = 466) and Bonneville Dam (N = 180) with additional fish being obtained from the Bonneville Dam SbyC tanks (N = 172) and John Day Dam SbyC tanks (N = 78) (Table 3). Hatchery yearling Chinook salmon 95 mm or longer and not previously PIT-tagged were used for this study. Fish collected at the Bonneville Dam juvenile fish facility were transferred water-to-water from the collection site to holding tanks (width 0.91m, length 1.82m, depth 0.91m, volume 1,510 L) supplied with flow-through river water. Fish collected at the John Day Dam juvenile fish facility were transported by truck (transport tank dimensions: width 1.2m, length 1.3m, depth 1.2m, volume 1,893L) to the holding tanks at Bonneville Dam and supplied with flow-through river water. Truck transport time ranged from 1 to 2 hours from John Day Dam to Bonneville Dam. Pre-surgery holding time ranged from 24 to 48 h. Collection at John Day Dam, and the narrow window of time that fish were collected, was due to the high river flow conditions in 2008.

Tagging

Lower Granite Dam Tagging

Surgeries to implant 3,414 yearling Chinook salmon with tags (described in detail below) were performed on 12 dates from April 23 through May 20, 2008. Study fish were assigned to one of three treatment groups: Barged for net pens, Barged for release, or In-River. Not all treatment groups were represented on every tagging date. Sample sizes ranged from 12 to 380 per treatment per tagging date (Table 1). Daily tagging treatments were selected based on the juvenile fish transportation (barging) schedule. Surgeries were typically performed in blocks by treatment group. All treatments were handled the same during pre-surgery holding, tagging, and post-surgery holding.

Table 1. Numbers of yearling Chinook salmon tagged at Lower Granite Dam by collection location, treatment, and tagging date (2008).

	Sample Room			Collection Raceway			Separation by Code Tank		
Tagging Date	Barged for net pens	Barged for release	In-River	Barged for net pens	Barged for release	In-River	Barged for net pens	Barged for release	In-River
April 23	98	0	0	78	257	0	0	0	0
April 24	0	0	259	0	0	0	0	0	0
April 29	57	0	0	105	0	0	0	0	0
April 30	0	204	0	0	0	0	12	16	0
May 1	0	0	254	0	0	0	0	0	0
May 6	178	258	0	0	0	0	0	0	0
May 7	177	71	0	0	0	0	0	0	0
May 13	179	0	0	0	0	0	0	0	0
May 14	0	0	253	0	0	0	0	0	0
May 16	0	0	245	0	0	0	0	0	0
May 18	0	380	0	0	0	0	0	0	0
May 20	0	95	291	0	0	0	0	0	0

Pre-surgery

All fish were anesthetized with an 80 mg/L dose of Tricaine methanesulfonate (MS-222). As fish reached stage 4 anesthesia, they were transported in a pitcher of anesthetizing water (80 mg/L MS-222) to a technician who obtained pre-surgery fish measurements. Fish fork length (nearest mm) and weight (nearest 0.1 g) were measured and recorded (Table 2). Lipid content (FM 992, manufacturer: Distell) was also measured in pre-surgery fish (this data is not reported due to the poor performance of the equipment on the study fish). Each fish was implanted with a uniquely-coded PIT tag and a uniquely-coded acoustic transmitter which were disinfected with 70% ethyl alcohol and rinsed with distilled water prior to implantation. The PIT tags (Destron Fearing model TX1411SST; 12.5 mm X 2.1 mm, 0.1020 g) were read with a Destron Fearing FS2001F-ISO Reader. Advanced Telemetry Systems (ATS) Pinger Dish II was used to read the acoustic transmitter code and to verify that the transmitter was functioning prior to implantation. Juvenile Salmon Acoustic Telemetry System (JSATS) transmitters (ATS model SS160; 12.00 mm X 5.21 mm X 3.77 mm; 0.282 g weight in water) weighing 0.435 g in air (SE 0.001, n=30) were programmed with a ping rate of 5 sec. Tag life of these transmitters was 32.0 d (the point at which >10% of the tags failed).

Following collection of physical data, fish were delivered in the pitcher of anesthetizing water to one of four surgeons. The tags (PIT and JSATS) assigned to each fish were delivered in a separate cup at the same time. The surgeon placed the fish ventral side up into a v-shaped groove in a foam rubber pad and inserted a piece of latex tubing (outside diameter = 4.8 mm) into the fish's mouth. Two gravity-fed buckets connected to the tubing were situated above each surgeon. One bucket contained fresh water with a dose of 40 mg/L MS-222 and the second bucket contained fresh water. Surgeons were able to provide appropriate maintenance anesthesia dosing during the surgery by adjusting the valves on each bucket.

Table 2. Descriptive statistics by treatment and tagging date for yearling Chinook salmon tagged at Lower Granite Dam.

			Length (mm)			Weight (g)			Fulton Condition Factor		Tag Burden (%)	
Treatment	Tagging Date	*N*	Mean	Min	Max	Mean	Min	Max	Mean	SE	Mean	SE
Barged for release	April 23	257	125	101	161	19.4	8.5	40.6	1.0	0.00	3.0	0.06
	April 30	220	141	103	187	28.5	9.5	72.0	1.0	0.01	2.2	0.06
	May 6	258	142	104	170	29.6	11.7	50.5	1.0	0.00	1.9	0.04
	May 7	71	144	109	171	31.0	14.6	52.6	1.0	0.01	1.8	0.06
	May 18	380	143	109	174	28.1	11.3	55.3	0.9	0.00	2.0	0.02
	May 20	95	142	116	175	28.1	13.4	54.7	1.0	0.01	2.0	0.05
In-River	April 24	259	126	95	156	19.8	7.0	37.1	0.9	0.01	3.0	0.07
	May 1	254	138	100	197	26.7	9.2	75.6	1.0	0.01	2.3	0.06
	May 14	253	143	116	174	28.9	15.4	50.2	1.0	0.00	1.9	0.03
	May 16	245	142	106	167	28.2	12.1	47.5	1.0	0.00	2.0	0.03
	May 20	291	144	118	171	29.0	15.9	55.3	1.0	0.00	1.9	0.02
Barged for net pens	April 23	176	128	95	177	21.4	7.0	61.3	1.0	0.00	2.8	0.08
	April 29	162	132	98	207	23.6	7.4	95.8	1.0	0.01	2.6	0.08
	April 30	12	146	101	160	32.8	9.2	46.2	1.0	0.02	2.0	0.38
	May 6	179	141	105	177	29.5	12.6	54.0	1.0	0.01	2.0	0.05
	May 7	176	142	108	179	29.4	13.5	52.6	1.0	0.00	1.9	0.04
	May 13	179	141	113	178	27.5	13.0	61.6	1.0	0.00	2.0	0.04

Surgery

To implant the tag, an incision approximately 7 mm long was made 3 mm lateral and parallel to the linea alba (ventral midline) between the pectoral fin and the pelvic girdle using a BD Beaver Micro-Sharp 1.5 mm-long blade. The PIT tag was inserted into the peritoneal cavity followed by the acoustic transmitter. The incision was closed with two simple interrupted sutures (5-0 Monocryl suture; a monofilament manufactured by Ethicon) using reinforced surgeon's knots. Surgical instruments were autoclaved daily prior to use. Between surgeries, instruments and suture needles were disinfected with ethyl alcohol and rinsed with distilled water.

Lower Granite Dam Tagging Recovery

Tagged yearling Chinook salmon were placed by treatment group into one of three tanks (width 0.91m, length 0.61m, depth 0.61m, volume 505L) immediately after surgery where they were allowed to recover with flow-through river water for 24 to 48 h prior to placement in the release tank, barge net pens, or general barge fish transport holds. Throughout the season there were 38 post-surgery holding mortalities (1.1%). Mortality ranged from 0 to 4 fish per treatment group per day except on one day when there were 14. This increase in mortality was attributed to an elevated dosing of one surgeon's anesthetic maintenance bucket. Overnight fish mortalities were removed from the tanks prior to release.

Bonneville Dam Tagging

Surgeries to implant yearling Chinook salmon with tags (tagging methods were identical to those described in detail above) were performed on four dates from May 22 through May 30, 2008. These study fish tagged at Bonneville Dam were all assigned to the Bonneville net pen treatment group. Numbers of fish and the specific collection location are specified in Table 3. Sample sizes ranged from 78 to 274, and daily tagging sessions were selected based on juvenile fish availability. All fish tagged at Bonneville Dam were handled the same during pre-surgery holding and tagging as fish at Lower Granite Dam.

Bonneville Dam Tagging Recovery

Immediately after surgery, tagged yearling Chinook salmon were placed into perforated, covered 18.9 L (5 gallon) buckets housed within one of several tanks (width 2.44 m, length 1.52 m, depth 0.61m, volume 2260 L) which were supplied with flow-through river water. Experimental fish were allowed to recover for 24 to 48 h prior to placement in the transport truck. Over the tagging period, only two overnight mortalities were recorded (0.2%). Overnight fish mortalities were removed from the tanks prior to release.

Table 3. Numbers of yearling Chinook salmon tagged at Bonneville Dam by collection location (2008).

Tagging Date	Bonneville Dam Sample Room	Bonneville Dam SbyC Tanks	John Day Dam Sample Room	John Day Dam SbyC Tanks
May 22	180	0	0	0
May 23	0	172	0	0
May 29	0	0	192	78
May 30	0	0	274	0
Total	180	172	466	78

Fish Releases

Lower Granite Dam Fish Releases

Following post-surgery holding, Barged treatment groups were transferred to the barge general holding area approximately 1 h prior to the 9:00 am (Pacific Standard Time; [PST]) barge departure from Lower Granite Dam. Fish travelled within the barge hold for 32 to 44 h until their release to the Columbia River near Skamania Landing (RKM 227), which is downstream of Bonneville Dam (Table 4). Barged treatment groups designated for estuary net pens were transferred to the barge at Lower Granite Dam and placed into suspended net pens within the general barge holding area approximately 1 h prior to barge departure. Fish travelled within these segregated net pens (1 m by 1 m by 1.2 m) in the barge hold for 32 to 44 h at which time fish were removed from the barge at Bonneville Dam (RKM 235) and transported on a truck (transport tank dimensions: width 1.2 m, length 1.3 m, depth 1.2 m, volume 1,893L) for an additional 3 to 5 h to the estuary net pen site at Tongue Point (RKM 29). The water quality in the transport tank (i.e. temperature, dissolved oxygen, salinity, pH, carbon dioxide, ammonia, nitrite, and chloride) was periodically monitored during transit, and never exceeded recommended levels. Water temperature was controlled through the periodic addition of ice. Fish density in the transport tank never exceeded 1 fish/liter. Upon reaching Tongue Point, a portion of the fish were placed in net pen structures and held for approximately 28 days. The

remaining fish were placed on a boat (fish hold volume approximately 1,514L) and transported to Sand Island (RKM 7; 1-2 h additional transport time), where they were placed in estuary net pen structures and held for 28 days.

In-River treatment groups were transferred from the post-surgery holding tank to the 18,500 L release tank supplied with flow-through river water approximately 1 h after completion of surgery session. Following overnight recovery, In-River groups were released at 6:00 am (PST) into the Lower Granite Dam tailrace through a hose connecting the release tank to the juvenile bypass outflow pipe.

Table 4. Date, time, location, and number of fish released per Barged group (2008).

Lower Granite Dam Load Date	Fish Loaded	Barge Release Date	Barge Release Time (PST)	Release Location (RKM)
April 24	257	April 25	1605	227
May 1	220	May 2	1610	227
May 8	329	May 9	1840	227
May 19	380	May 20	1720	227
May 21	95	May 22	1705	227

Bonneville Dam Fish Releases

Following post-surgery holding, fish were transported 3 to 5 h by truck to the estuary net pen site at Tongue Point (RKM 29). Upon Reaching Tongue Point, a portion of the fish were placed in net pen structures and held for approximately 28 days. The remaining fish were placed on a boat and transported to Sand Island (RKM 7; 1-2 h additional transport time) where they were placed in net pen structures and held for approximately 28 days. The water quality in the transport tank (i.e. temperature, dissolved oxygen, salinity, pH, carbon dioxide, ammonia, nitrite, and chloride) was periodically monitored during transit, and never exceeded recommended levels. Water temperature was controlled through the periodic addition of ice. Fish density in the transport tank never exceeded 1 fish/liter.

In-River Recollection

A subgroup of fish (In-River for net pens) implanted with PIT and JSATS tags at Lower Granite Dam, and released back into the river at the Lower Granite Dam tailrace, were recollected at Bonneville and John Day dams using the separation by code (SbyC) system (Table 5). The SbyC systems were programmed to identify and collect the study fish based on data contained in the Columbia Basin PIT Tag Information System (PTAGIS). Fish diverted from the juvenile bypass system into temporary holding tanks were scanned for the presence of PIT-tags and verified as study fish tagged at Lower Granite Dam. Other fish inadvertently collected by the SbyC system were removed and released back into the river. Each tagged experimental fish was anesthetized with a non-lethal dose of tricaine methane sulphonate (80 mg/L MS222; Sigma-Aldrich) and the length and weight were recorded. The fish were allowed to recover in tanks supplied with flow-through river water at an approximate density of 0.5 to 1 fish/L for one to two days. After the recovery period, fish were transported by truck to Ilwaco Bay (3-5 h transport time) and then by boat (1-2 hour transport time) to the estuary net pens located near Sand Island. The water quality in the transport tank (i.e. temperature, dissolved oxygen, salinity, pH, carbon dioxide, ammonia, nitrite, and chloride) was periodically monitored during transit, and never

exceeded recommended levels. Water temperature was controlled through the periodic addition of ice. Fish density in the transport tank never exceeded 1 fish/liter.

Table 5. Numbers of yearling Chinook recollected by location (2008).

Recollection Date	Bonneville Dam SbyC Tanks	John Day Dam SbyC Tanks
May 12	1	
May 17	9	
May 19	2	
May 26		1
May 27		4
May 28		3
May 29		14
May 30		10
May 31		5
June 1		5
Total	*12*	*42*

Treatment groups for the net pen study

The number of live fish loaded into the net pens (day 0) by treatment group and net pen location is shown in Table 6. Numbers of fish per treatment group differed from numbers of fish collected/tagged due to mortalities during transport to the net pens. The In-River treatment group for the net pen study contained actively migrating fish recollected at Bonneville or John Day dams that were subsequently trucked to the Sand Island net pen site only. The Barged treatment group contained fish that were collected and tagged at Lower Granite Dam, transported in net pens suspended in barge holds to Bonneville Dam, and subsequently transported by truck and boat to the estuary net pen sites. The Bonneville treatment group was collected at John Day or Bonneville dams, tagged at Bonneville Dam, and subsequently transported by truck and boat to the estuary net pen sites. Barged, Bonneville, and In-River treatment groups experienced identical travel times from Bonneville Dam to the estuary net pen sites in the truck transportation tank (ca. 3-5 hours) and in the boat tank (ca. 1-2 hours) under approximately equal density conditions. Yearling juvenile salmon obtained as eggs from Rapid River Hatchery broodstock and raised at the NRS-FDL comprised the Reference group; these fish served as a reference to assess possible net pen effects on the incidence and cause of mortality observed in the Barged, In-River, and Bonneville study fish. Reference fish were transported under similar density conditions in the truck tank mentioned above, and travel time from the laboratory to estuary net pens was approximately 3-4 hours. Reference fish were transported and placed into the net pens at three times during the study period to match the timing of seasonal cohorts of Barged and In-River study fish. Reference fish were not tagged with either acoustic or PIT tags.

Table 6. Treatment group and net pen locations (Sand Island vs. Tongue Point) for the net pen study.

	Net Pen Location	
Treatment group	**Sand Island**	**Tongue Point**
In-River (n=51)	yes	no
Barged (n=868)	yes	yes
Bonneville (n=894)	yes	yes
Reference (n=1080)	yes	yes

Seasonal Cohorts

A total of 3467 run-of-river hatchery yearling Chinook salmon were collected and surgically implanted with acoustic tags at Lower Granite Dam. Efforts were made to collect run-of-river fish for this study during the early, middle, and late migration periods. Passage histories of PIT-tagged hatchery spring/summer Chinook salmon detected at the Lower Granite Dam Juvenile Bypass System were established as a reference population. The dates and times of PIT-tag detections were compiled by Pacific States Marine Fisheries Commission (PSMFC 2008). During the 2008 outmigration season, 79990 spring/summer Chinook salmon from 11 hatcheries were detected at the Lower Granite Dam Juvenile Bypass System between March 28 and December 13. The present study focuses on the outmigration history of yearling spring/summer Chinook salmon after release, therefore the selection of seasonal cohorts for collected fish was based on the release date of treatment groups. Collection, tagging, and release dates of all treatment groups and subsequent placement into seasonal cohorts are summarized in Table 7.

Table 7. Collection, tagging, and release dates of treatment groups, and placement into seasonal cohorts Early, Middle, and Late.

Treatment	Collection Date	Tagging Date	Release Date	Cumulative percent of PIT-tagged outmigrants during Release Date	Seasonal Cohort
Barged for release	April 22	April 23	April 25	4.0 – 4.4	Early
	April 29	April 30	May 2	9.8 – 12.5	Early
	May 5	May 6	May 9	43.6 – 50.9	Middle
	May 6	May 7	May 9	43.6 – 50.9	Middle
	May 16/17	May 18	May 20	94.9 – 96.1	Late
	May 18/19	May 20	May 22	96.6 – 96.9	Late
In-River	April 22	April 24	April 25	4.0 – 4.4	Early
	April 30	May 1	May 2	9.8 – 12.5	Early
	May 12/13	May 14	May 15	80.2 – 82.3	Middle
	May 14/15	May 16	May 17	84.1 – 87.3	Late
	May 18	May 20	May 21	96.1 – 96.5	Late
Barged for net pens	April 21/22	April 23	April 25	4.0 – 4.4	Early
	April 27/28	April 29	May 2	9.8 – 12.5	Early
	April 29	April 30	May 2	9.8 – 12.5	Early
	May 4/5	May 6	May 9	43.6 – 50.9	Middle
	May 6	May 7	May 9	43.6 – 50.9	Middle
	May 11	May 13	May 16	82.3 – 84.1	Late

Acoustic Receivers

Acoustic-tagged fish were detected using autonomous acoustic receivers (Sonic Concepts, Inc., model N201) deployed for other concurrent studies using the same tag technology. The receivers were arranged into transects across the river, or arrays, ranging from Bonneville Dam forebay to RKM 2.8 at the mouth of the Columbia River. Nine arrays were used to calculate

travel and survival statistics for this study. Descriptions of these arrays can be found in Table 8. Receivers were recovered and data were downloaded every two to four weeks, depending on the requirements of the primary study for which the receivers were deployed. A detailed description of the equipment and deployment strategy can be found in McMichael et al. (2008).

Table 8. Description of acoustic receiving arrays used to calculate travel time and survival.

Array Name	River Kilometer	Array Location Description	Receivers
CR237.0	237	Bonneville Dam forebay array	4
CR202.0	202	Bonneville Dam tailrace primary, Reed Island	9
CR193.0	193	Bonneville Dam tailrace secondary, Lady Island	6
CR113.0	113	Kalama Primary array	6
CR086.2	86.2	Oak Point Array	4
CR049.6	49.6	Three-Tree Point array	3
CR035.6	35.6	Rice Island array	4
CR008.3	8.3	Estuary primary array, East Sand Island to Clatsop Spit	22
CR002.8	2.8	Estuary secondary array, between North and South Jetties	31

Survival and Travel Time Analysis

Survival and travel time for actively migrating acoustic-tagged fish were measured for various release groups through three Reaches, demarcated by the location of key acoustic telemetry receivers (Table 9). The first Reach included the section of the river between release at Lower Granite Dam (either to the river or into a barge) and RKM 202 below Bonneville Dam. The second Reach spanned between RKMs 202 and 35.6, at Rice Island, a point near where previous research showed a decrease in survival estimates (McMichael 2007). Reach 3 covered the section between RKMs 35.6 and 8.3, at East Sand Island, and the last array to which survival could be calculated. Release groups were categorized by treatment group (Barged or In-River at Lower Granite Dam) and season (Early, Middle, Late, or Pooled; Table 7).

Table 9. Definition of Reaches used for survival and travel time analyses.

	Upstream Boundary		Downstream Boundary	
Reach	Name	RKM	Name	RKM
1	Lower Granite Dam	695	CR202.0	202
2	CR202.0	202	CR035.6	35.6
3	CR035.6	35.6	CR008.3	8.3

Survival was estimated for each Reach and release group using the Cormack-Jolly-Seber release-recapture model (Cormack 1964; Jolly 1965; Seber 1965). Estimated survival was corrected for the probability of tag failure, and standard errors were computed using the bootstrap with 10,000 bootstrap iterations, following the methodology of Townsend et al. (2006). The result was an estimated conditional probability of survival through each Reach for each release group. In addition, cumulative survival from Lower Granite Dam to RKM 8.3 was estimated for pooled releases of Barged or In-River groups (x) as follows:

$$\hat{S}_x = \prod_{i=1}^{3} \hat{S}_{xi} \; . \qquad (2.1)$$

Standard errors were estimated using the Delta Method (Seber 2002: pp. 7-9). For each Reach, the ratio ($\widehat{BI}$) of the survival of the Barged treatment group to the In-River treatment group was estimated as:

$$\widehat{BI} = \frac{\hat{S}_B}{\hat{S}_I}, \tag{2.2}$$

with variance estimator

$$\widehat{Var}\left(\widehat{BI}\right) = \widehat{BI}^2 \left[\frac{\widehat{Var}\left(\hat{S}_B\right)}{\hat{S}_B^2} + \frac{\widehat{Var}\left(\hat{S}_I\right)}{\hat{S}_I^2}\right]. \tag{2.3}$$

Travel time was measured for each Reach for all tagged fish that were detected at both the upstream and downstream boundary receiver arrays for the Reach in question. Travel time was calculated using the time of first detection on the boundary receiver arrays or from the time of release to the river at Lower Granite Dam for Reach 1. In addition, travel time from release at Skamania Landing (RKM 227; Table 4) to the first array (RKM 202) was determined for the acoustic-tagged Barged treatment group. Average travel times for each release group were computed using the harmonic mean.

Fish Care at Estuary Net Pens

The objective of fish care at the estuary net pens was to provide animal care that minimized stress and injury for the duration of holding. This involved daily fish feeding, removal of mortalities, and regular removal of algal growth from the nets. Fish were fed a diet of dry food pellets (Bio-Oregon; Warrenton, OR) once per day.

Net Pen Mortality Analysis

Mortalities were collected daily from each of the net pen holding sites. For each dead fish, PIT tag identifications were gathered using a Destron-Fearing portable transceiver (Model FS2001F-ISO) mounted with an antenna system coupled to the PC-based "P3" data entry and validation program published by the Pacific States Marine Fisheries Commission. Dead fish collected at each site were used to estimate statistical differences in the cumulative incidence of mortality between the following treatment groups: (1) Barged, In-River, Bonneville, and Reference at Sand Island; (2) Barged, Bonneville, and Reference at Tongue Point; (3) Barged Early, Middle, and Late passage cohorts and Reference cohorts at both net pen locations, and (4) Bonneville at both net pen locations. Statistically significant differences in the cumulative mortality between the groups of experimental fish were based on the time varying standard errors of the cumulative mortality estimated using Number Cruncher Statistical Systems (NCSS) based on a non-parametric approach outlined in Marubini and Valsecchi (1995). Statistical differences in the incidence of mortality between treatment groups were assessed for each day of holding using a two sided t-test.

Histopathology Examinations

Collection and description of treatment groups

Morbid fish were removed from the net pens on a daily basis for histological analyses. Additionally, untagged live fish were destructively sampled at selected locations and times coinciding with tagging for both Lower Granite and John Day dams. Live fish from all treatment groups were also destructively sampled at the end of net pen holding (day 28), as well as reference fish at the start and end of net pen holding. All mortalities collected for histological analyses were stored a maximum of one hour at 4°C before processing.

Pathology Processing

The gills and internal viscera (kidney, liver, spleen, heart, and gastrointestinal tract) were dissected from each fish and placed into a biopsy bag prior to immersion in 10% neutral-buffered formalin with a final minimum volume of 1 part tissue to 10 parts formalin (Bancroft J.D. and Stevens 1994; Hopwood 1990; Presnell J.K. and Schreibman 1997). Additional scrapings or sections of the integument including the fins were also collected as warranted. Tissues were fixed for a minimum of 72 hours prior to further dissection and submission for routine histological processing including paraffin embedding, sectioning at 5 microns, and staining with hematoxylin and eosin reagents (Bancroft J.D. and Stevens 1994; Presnell J.K. and Schreibman 1997).

Calculation of Disease Prevalence

Significant findings from histopathological examination were grouped according to specific diseases, metabolic lesions, and combination of diseases. A percent prevalence was calculated by dividing the number of positives for the given diagnosis by the total number of examined fish, multiplying the result by 100.

Pathogen Prevalence Survey

Pathogen Selection and PCR References

Nine salmonid pathogens, encompassing viral, fungal, and bacterial microorganisms, were surveyed in fish tissues and water samples, by the detection of their genetic material with polymerase chain reaction (PCR). The pathogens are listed in Table 10 along with the respective reference used for PCR primers and reaction conditions. Several dilutions of each sample nucleic acid were tested to reduce effects of PCR inhibitory compounds present in the water sample nucleic acid extracts (Rajal V.B. et al. 2007a). Each PCR assay was optimized for the study design and all samples subsequently screened using the optimized method.

Table 10. Methods used in pathogen PCR analyses.

Pathogen	PCR Reference
Aeromonas hydrophila	(Dorsh M. 1994)
Listonella anguillarum	(Hong G.E. et al. 2007)
Flavobacterium columnare	(Welker et al. 2005)
Yersinia ruckeri	(Del Cerro et al. 2002)
Renibacterium salmoninarum	(Modified from Chase and Pascho, 1998)
Aeromonas salmonicida	(Del Cerro et al. 2002)
Infectious hematopoietic necrosis virus (IHNV)	(Williams K et al. 1999)
Viral hemorrhagic septicemia virus (VHSV)	(Williams K et al. 1999)
Saprolegniaceae	(Dieguez et al. 2007)

Location of Sample Collection

A portion of untagged fish collected at Lower Granite and John Day dams were immediately sacrificed for pathogen prevalence analyses. Samples collected from run-of-river fish at Lower Granite Dam occurred on multiple days throughout the outmigration period, whereas samples collected at John Day Dam only occurred on one day at the conclusion of the field season (Table 11). Additionally, a portion of the live fish surviving the 28-day net pen holding period were destructively sampled on the last day of holding prior to release for pathogen prevalence analysis.

Table 11. Location and number of samples collected for pathogen prevalence analysis.

Location	Number of Fish Collected
Lower Granite Dam	179
John Day Dam	39
After Estuary Net Pen Holding	
Sand Island	
Barged	50
In-River from Lower Granite Dam	
Collected at John Day	34
Collected at Bonneville Dam	10
Bonneville from John Day	30
Bonneville from Bonneville Dam	20
Tongue Point	
Barged	50
Bonneville from John Day	30
Bonneville from Bonneville Dam	20

Sample Collection and Preparation

Both water and fish tissue samples were collected and analyzed during the survey of pathogens prevalent in the FCRPS during hatchery spring/summer Chinook outmigration.

Water samples

Water samples were collected along the Snake and Columbia River Chinook salmon migration corridor where fish were routinely held (barge loading raceways, barge holds, sort-by-code holding tanks at Lower Granite and Bonneville dams, and estuary net pens; Table 12). Twenty-liter grab samples of water were collected at the sites and stored at 4ºC before processing within 48 hours at NRS. As per Rajal et al. (2007a), the samples were filtered and concentrated to an

approximate volume of 70 mL using a hollow fiber ultrafiltration system and the resulting samples were stored at -20ºC until recovery and PCR analyses. Each water sample was spiked with a benign surrogate virus (bacteriophage PP7) and subsequently analyzed to calculate the microorganism recovery effectiveness for that particular sample as per Rajal et al. (2007a).

Table 12. Water sample locations and dates of collection.

Location and Description	2008 Collection Dates							
Barge Sampling								
Lower Granite Dam JFF raceway	NA[a]	5/8	5/17					
Barge hold at loading	4/24	5/8	5/17					
Barge hold at unloading	4/25	5/9	5/18					
Sort-By-Code Holding Tanks								
Lower Granite Dam	4/24							
John Day Dam	6/4							
Bonneville Dam	5/18							
Estuary Net Pens								
Tongue Point	4/25	5/9	5/18	5/26	6/5	6/13	6/19	6/27
Sand Island	4/25	5/10	5/19	5/26	6/5		6/19	6/27

[a]Not Applicable. There was no general transport of the bypassed fish at Lower Granite Dam prior to May 2.

Fish tissue samples

Fish were lethally harvested and a small (ca. 40 mg) piece of anterior head kidney was aseptically collected from each fish and placed into 1.5ml tubes containing the preservative RNAlater (Qiagen, Valencia, CA). Kidney samples were stored at -20°C prior to nucleic acid extraction. The prevalence of pathogens in fish tissues was investigated using semi-automated, high-throughput PCR described below.

Nucleic Acid Extraction

Water samples

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

Tissue Samples

DNA and RNA were extracted from a maximum of 25mg (DNA) or 30mg (RNA) kidney tissue in 96-well format following the manufacturers' directions for animal tissues (Qiagen: DNeasy 96 Blood and Tissue kit, RNeasy 96 kit). Appropriate controls were included in the extraction process. The purified RNA was immediately converted to cDNA using the High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). DNA and cDNA was diluted 1:10 using RNase-free water. Purified nucleic acids were stored at -20°C prior to PCR.

Polymerase Chain Reactions

Water samples

For each pathogen listed in Table 11, five PCR reactions were performed for each sample (undiluted DNA, 1:2, 1:10, negative control, positive control). In general, each 25 µL reaction contained the following mix (Applied Biosystems, Foster City, CA): 1.5 to 5 mM $MgCl_2$, 10X Buffer II, 800 nM each primer, 800 µM dNTP's, 1.25 U of AmpliTaq Gold DNA polymerase, and 5 µL of the nucleic acid. Amplification was performed using a GeneAmp 9700 thermalcycler (Applied Biosystems, Foster City, CA) according to the referenced literature. Amplified DNA was stored at 4°C prior to gel electrophoresis.

Fish tissues

For each pathogen listed in Table 11, a 10 µL reaction contained the following mix (Applied Biosystems, Foster City, CA): 1.5 to 5 mM $MgCl_2$, 10X Buffer II, 800 nM each primer, 800 µM dNTP's, 0.5 U of AmpliTaq Gold DNA polymerase, and 3 µL DNA or cDNA (non-dilute and 1:10). Primers were labeled with either FAM, VIC, or NED fluorescent dye (Applied Biosystems, Foster City, CA) for later detection by capillary electrophoresis. Reactions were run on a 384-well platform, and each plate contained the following controls: 2 positive controls (for amplification and fragment analysis), and 12 negative controls (no DNA and extraction controls). Amplification was performed using a GeneAmp 9700 thermalcycler (Applied Biosystems, Foster City, CA) according to the referenced literature and optimized protocol. Amplified DNA was stored at 4°C prior to fragment analysis.

Analysis of PCR Results

Water samples: gel electrophoresis

All reactions were screened for positive PCR amplification using a 1.5% agarose gel in 0.5X TAE buffer subsequently stained with ethidium bromide for visualization of PCR products.

Tissue samples: capillary electrophoresis

All fragment analyses were performed on an Applied Biosystems DNA analyzer 3730xl. Up to four PCR products were added to a sequencing cocktail consisting of GeneScan LIZ1200size standard (Applied Biosystems; Foster City, CA) and Hi-Di Formamide (Applied Biosystems; Foster City, CA), and denatured for 5minutes prior to fragment analysis using a custom run module (8kV and 6,200 second run time).

Raw data output from the DNA analyzer was imported into GeneMapper (v3.7, Applied Biosystems, Foster City, CA) software and analyzed for the presence of size-specific peaks, which represent positive PCR products of target pathogens. Peaks within 2 base pairs of the anticipated PCR product size and at least twice the intensity of the background noise were scored as positive.

Covariates Effects on Survival and Travel Time Analysis

The variation in survival rates and travel times of migrating fish were analyzed in relation to numerous measures of migration timing, fish condition at tagging, handling at Lower Granite Dam, and environmental conditions (Table 13). Measures of migration timing (collection,

tagging, and river release dates) and length/weight of fish were used in survival and travel time models for both the In-River and Barged treatment groups and for all three Reaches. Handling covariates were collection source and holding duration. Collection source was measured for all fish, but varied only for barged fish (Table 1). Thus, collection source was used in effects analyses only for the Barged treatment group. Because relatively few fish were collected from either the Raceway or Sort-by-Code tank compared with the Sample room at Lower Granite Dam, collection source was represented as a binary measure: 1, if the fish was collected from the Sample room and 0 otherwise. Holding duration was defined as the time difference between the collection date and the river release date. Covariates describing environmental conditions consisted of the average daily discharge at both Lower Granite and Bonneville dams. Discharge at Lower Granite Dam was measured at the time of in-river fish release at Lower Granite Dam, and was used in models of survival and travel time through Reach 1 for this group only. Discharge at Bonneville Dam was measured at the time of arrival at RKM 202.0 (the first acoustic array downstream of Bonneville Dam), and was used in models of survival and travel time through Reaches 2 and 3 for both In-River and barged fish.

Table 13. Covariates used in survival and travel time effects analysis for acoustic-tagged fish.

Category	Covariate	Definition	Reach	Treatment group
Migration Timing	Collection Date	Date of collection at Lower Granite Dam (day of year)	1,2,3	In-River, Barged
	Tagging Date	Date of tagging (day of year)	1,2,3	In-River, Barged
	River Release Date	Date of release to river at Lower Granite Dam (In-River fish) or Skamania (Barged fish) (day of year)	1,2,3	In-River, Barged
Fish Condition at Tagging	Weight	Fish weight at tagging	1,2,3	In-River, Barged
	Length	Fish fork length at tagging	1,2,3	In-River, Barged
Handling	Collection Source	0 or 1; 0 = Raceway or Sort-by-Code tank at Lower Granite Dam; 1 = Sample room at Lower Granite Dam	1,2,3	Barged
	Holding Duration	Difference between River Release Date and Collection Date	1,2,3	In-River, Barged
Environmental Condition	Discharge at Lower Granite Dam	Average daily KCFS on day of river release at Lower Granite Dam	1	In-River
	Discharge at Bonneville Dam	Average daily KCFS on day of arrival at CR202.0	2,3	In-River, Barged

Individual regressions were performed using each covariate separately, after first accounting for the effect of barging on either survival or travel time, as appropriate. Multivariate regressions were performed using the covariates seen to be significant at the 10% level (i.e., $P<0.100$) from the individual regressions. Because of high correlation among some individual covariates (e.g., the migration timing covariates), not all combinations of covariates were considered for the multivariate regressions. In particular, for the multivariate regressions, only a single date covariate was considered, and either length or weight, but not both.

Environmental Effects on Survival

The probability of survival (S) of acoustic-tagged fish through the three Reaches (i.e., from Lower Granite Dam to RKM 8.3) was analyzed using a proportional hazards model and individual-based covariates (Table 13) using the program SURPH (Smith et al. 1994). Reach 1 was analyzed separately, and Reaches 2 and 3 were analyzed concurrently. The In-River and Barged treatment groups were analyzed separately. A proportional hazards link was used, where for each Reach i and individual j:

$$E\left(S_{ij}\right) = S_{0i}^{\exp\left(\beta_i x_{ij}\right)} \tag{2.4}$$

where $E\left(S_{ij}\right)$ is the expected survival probability through Reach i for individual j; S_{0i} is the probability of survival through Reach i, averaged across all individuals; and β_i is the regression coefficient for covariate x for Reach i and individual j. All models estimated unique detection probabilities for each Reach. Model selection was performed using likelihood ratio tests and forward selection at a P-value=0.10.

Environmental Effects on Travel Time

Travel time of acoustic-tagged fish through the three Reaches was regressed against individual-based covariates (Table 13). Separate regressions were performed for the In-River and Barged treatment groups for travel time through each Reach. Travel times were log-transformed to generate normally distributed errors:

$$\ln\left(T_{ij}\right) = \beta_{0i} + \beta_{1i} x_j + \varepsilon_{ij}\,, \tag{2.5}$$

where T_{ij} is travel time through Reach i for individual j, β_{0i} is the average travel time (on the log scale) through Reach i, β_{1i} is the regression coefficient for covariate x through Reach i, x_j is the observed value of covariate x for individual j, and ε_{ij} is the error term for individual j in Reach i. Model selection was performed using ANOVA and forward selection with a P-value of 0.10.

3.0 SURVIVAL AND TRAVEL TIME ANALYSIS

Introduction

Mortality in the LRE and ocean comprises a significant portion of the overall mortality experienced by salmon throughout their lifecycle, and seasonal and annual fluctuations in salmonid mortality in these environments are a significant source of recruitment variability (Bradford 1995). The JSATS is providing researchers with acoustic transmitters and detection systems to estimate juvenile salmonid survival through the LRE. Results from a recent multi-year project established travel times and survival probabilities for subyearling and yearling run-of-river Chinook salmon with an in-river outmigration history, between the Bonneville Dam juvenile bypass facility outfall (RKM 231.3) and an array located at RKM 8.3. Based on data obtained in the 2005 and 2006 outmigration season, mean travel time for yearlings were 3.0 and 4.1 days, respectively, with 75% and 66% surviving transit through this river segment, respectively (McComas et al. 2008, McComas et al. 2007). To-date, little is known on the survival and travel time of barged yearling Chinook salmon in the LRE.

Results

Tagged Release Groups

A total of 1249 acoustic-tagged in-river fish designated for this survival study were released at Lower Granite Dam spanning the period of time from April 25, 2008 to May 21, 2008.That number of fish was comprised of the following passage cohorts (Figure 8): 500 Early (released to the Snake River April 25 and May 2), 245 Middle (released May 15), and 504 Late (released May 17 and 21). These fish arrived at the first acoustic array (RKM 202) located just downstream of Bonneville Dam from May 12 through June 17, 2008 as shown in Figure 8.

Additionally, a total of 1281 acoustic-tagged fish were loaded into barges at Lower Granite Dam and subsequently released to the river at Skamania Landing (RKM 227). Release of the Barged treatment group commenced on April 25, 2008 and finished with the barge arriving at Skamania Landing on May 22, 2008. The total number of fish was comprised of the following cohorts (Figure 8): 477 Early (released at Skamania April 25 to May 2), 329 Middle (released May 9), and 475 Late (released May 20 and 22). These fish arrived at the first acoustic array from April 25 through May 23, 2008 as shown in Figure 8.

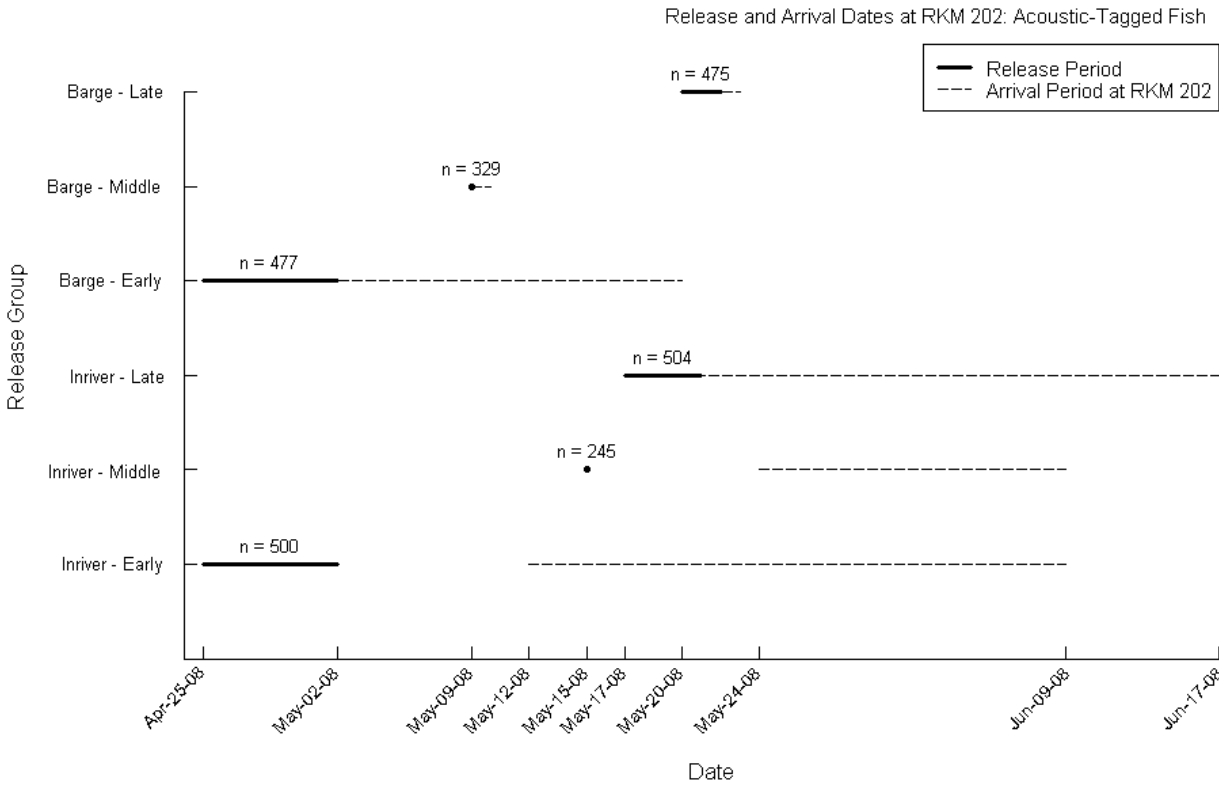

Figure 8. Release dates, arrival dates, and size of the release groups (n) at RKM 202 of acoustic-tagged fish used in survival and travel time analyses. For the In-River treatment group, release date = date of release to river at Lower Granite Dam. For the Barged treatment group, release date = date of release to river at Skamania Landing (RKM 227). The extended arrival date for the Early Barged cohort is due to a single fish, while the rest of this group arrived at RKM 202 no later than May-08-08.

Survival Estimates of Acoustic-Tagged Fish

Survival of In-River Fish

The overall estimated survival for In-River treatment groups of acoustic-tagged fish from Lower Granite Dam to the last array at RKM 8.3 was 0.4562 (Table 14). Early group of in-river fish had the lowest estimated survival (0.4078; $\widehat{SE}$ = 0.0249), while the Middle group had the highest estimated survival (0.5442; $\widehat{SE}$ = 0.0353). The same pattern among the seasonal groups was seen for the first two individual Reaches, with the Early group having the lowest survival and the Middle group having the highest. In the last Reach, the Early group had the highest estimated survival (0.9166; $\widehat{SE}$ = 0.0364), and the Middle group had the lowest estimated survival (0.8619; $\widehat{SE}$ = 0.0370).

Survival of Barged Fish

The overall estimated survival for Barged treatment groups from Lower Granite Dam to the array at RKM 8.3 was 0.6824 (Table 14). The Early group had the lowest estimated survival to RKM 8.3 (0.6188), and the Late group had the highest estimated survival (0.7406). The individual Reaches showed a different pattern of survival over the season. For Reach 1, which included 468 river kilometers of travel while in the barge (from Lower Granite Dam to Skamania

Landing), and 25 kilometers of active migration in the river from Skamania Landing to RKM 202, the Late group had the lowest estimated survival (0.8922), and the Middle group had the highest estimated survival (0.9879). For Reach 2, estimated survival ranged from 0.8598 for the Middle group, to 0.9421 for the Late group. For Reach 3, estimated survival for the Barged treatment group ranged from 0.7210 for the Early group to 0.8811 for the Late group.

Table 14. Estimated survival (standard error) of acoustic-tagged fish by treatment group, cohort, and Reach.

Treatment group	Cohort	Reach 1	Reach 1 + Reach 2	Reach 1 + Reach 2 + Reach 3	Reach 2	Reach 2 + Reach 3	Reach 3
In-River	Pooled	0.5313	0.5150	0.4562	0.9693	0.8586	0.8858
		(0.0142)	(0.0161)	(0.0163)	(0.0161)	(0.0207)	(0.0241)
	Early	0.4928	0.4449	0.4078	0.9027	0.8274	0.9166
		(0.0226)	(0.0240)	(0.0249)	(0.0270)	(0.0343)	(0.0364)
	Middle	0.6314	0.6314	0.5442	1.0000	0.8619	0.8619
		(0.0310)	(0.0310)	(0.0353)	(0.0000)	(0.0370)	(0.0370)
	Late	0.5228	0.5147	0.4632	0.9846	0.8860	0.8999
		(0.0224)	(0.0285)	(0.0302)	(0.0352)	(0.0440)	(0.0367)
Barged	Pooled	0.9448	0.8709	0.6824	0.9218	0.7223	0.7836
		(0.0066)	(0.0241)	(0.0170)	(0.0248)	(0.0174)	(0.0266)
	Early	0.9691	0.8583	0.6188	0.8856	0.6386	0.7210
		(0.0080)	(0.0557)	(0.0315)	(0.0570)	(0.0321)	(0.0560)
	Middle	0.9879	0.8494	0.6916	0.8598	0.7001	0.8142
		(0.0068)	(0.0404)	(0.0327)	(0.0407)	(0.0329)	(0.0476)
	Late	0.8922	0.8405	0.7406	0.9421	0.8301	0.8811
		(0.0148)	(0.0355)	(0.0245)	(0.0371)	(0.0244)	(0.0402)

Barged-In-River survival ratio, $\widehat{BI}$

Values of $\widehat{BI}$ greater than 1.0 indicate that the Barged treatment group had higher survival than the In-River treatment group, while the opposite is true for values of $\widehat{BI}$ less than 1.0. Estimates of $\widehat{BI}$ for treatment groups pooled over the season ranged from 1.78 ($\widehat{SE}$ = 0.05) for Reach 1(in which barged fish spent the majority of the RKM in barges) to 0.88 ($\widehat{SE}$ = 0.04) for Reach 3 (in which both treatment groups migrated actively), with an estimate of 1.50 ($\widehat{SE}$ = 0.07) for the entire study area (Lower Granite Dam to RKM 8.3) (Table 15).The $\widehat{BI}$ ratio was greater than 1 for fish through each Reach beginning at Lower Granite Dam, and the ratio decreased as the distance traveled increased. The $\widehat{BI}$ ratio was less than 1 for each Reach beginning at RKM 202, although the estimated ratio was significantly less than 1 only for the Middle cohort in Reach 2 (P=0.0007). The lowest pooled $\widehat{BI}$ ratio estimate was from RKM 202 to 8.3, and in this stretch of estuary the ratio increased from the Early to Late cohorts. The $\widehat{BI}$ ratio estimate for the Early and Pooled cohorts were significantly less than 1 for this Reach (P=0.0030 and P=0.0024, respectively), but not for the Middle and Late cohorts (P>0.2 in each case). The highest $\widehat{BI}$ ratio estimated for the pooled groups after RKM 202 was detected for Reach 2.

Table 15. Estimated Barged-In-River survival ratio, *BI*, (standard error) of acoustic-tagged fish by Reach and cohort.

Cohort	Reach 1	Reach 1 + Reach 2	Reach 1 + Reach 2 + Reach 3	Reach 2	Reach 2 + Reach 3	Reach 3
Pooled	1.7783	1.6911	1.4960	0.9510	0.8412	0.8846
	(0.0492)	(0.0705)	(0.0652)	(0.0301)	(0.0286)	(0.0385)
Early	1.9665	1.9293	1.5176	0.9811	0.7717	0.7866
	(0.0918)	(0.1626)	(0.1207)	(0.0696)	(0.0503)	(0.0687)
Middle	1.5646	1.3453	1.2709	0.8598	0.8123	0.9447
	(0.0775)	(0.0920)	(0.1020)	(0.0407)	(0.0517)	(0.0686)
Late	1.7066	1.6329	1.5988	0.9569	0.9369	0.9791
	(0.0784)	(0.1137)	(0.1169)	(0.0509)	(0.0540)	(0.0599)

Travel Time of Acoustic-Tagged Fish

In-River treatment group

Average travel time of acoustic-tagged fish in the In-River treatment group from Lower Granite Dam to RKM 8.3 ranged from 11.84 ($\widehat{SE}$ = 0.12) days for the Late release cohort to 21.74 ($\widehat{SE}$ = 0.21) days for the Early release cohort (Table 16). The Late cohort moved faster than the earlier cohorts in Reaches 1 and 2, while the Middle cohort of in-river fish moved the fastest through Reach 3. In every Reach, the Early cohort moved the slowest of all In-River cohorts. In the distance-scaled Figure 9, an increase in the travel rate for each In-River cohort was seen beginning at RKM 202, and continued at roughly the same rate to the mouth of the estuary. The only exception to this was the Late cohort, in which travel time increased slightly in Reach 3.

Barged treatment group

Average travel time of acoustic-tagged fish in the Barged treatment group from Lower Granite Dam to RKM 8.3 ranged from 4.80 ($\widehat{SE}$ = 0.03) days for the Late release groups to 10.52 ($\widehat{SE}$ = 0.22) days for the Early release groups (Table 16). In all three Reaches, travel times were lowest for the Late group, on average, while the Early groups had the longest travel times. The Barged treatment group moved faster than the In-River group through Reach 1, which consisted mostly of travel on the barge (Table 16, Figure 9). However, the Barged group traveled slower than the In-River group through all Reaches downstream of RKM 202. Travel times of barged fish through these Reaches decreased from Early to Late cohorts. Figure 9 shows the same phenomenon but scaled over distance travelled. All Barged passage cohorts slowed in Reach 3, resulting in a considerable increase in travel time starting at RKM 35.6.

Estuary transit rates

The speed at which barged and in-river fish travelled through the estuary decreased as they neared the last array (Table 17). From RKM 202 to 35.6, in-river fish travelled 98.4 Km/day, and barged fish 49.3 Km/day. In the last Reach (RKM 35.6 to 8.3), in-river and barged fish slowed to 53.5 Km/day and 27.8 Km/day, respectively. Both treatment groups travelled slowest in the final 27 Km of estuary, slowing to just over half their speed upstream of the array at RKM 35.6.

Table 16. Average travel time (standard error) in days of acoustic-tagged fish by treatment group, cohort, and Reach. The harmonic mean is reported.

Treatment group	Cohort	Reach 1	Reach 1 + Reach 2	Reach 1 + Reach 2 + Reach 3	Reach 2	Reach 2 + Reach 3	Reach 3
In-River	Pooled	12.39 (0.14)	14.01 (0.14)	14.74 (0.13)	1.69 (0.01)	2.29 (0.01)	0.51 (0.01)
	Early	19.14 (0.20)	21.30 (0.19)	21.74 (0.21)	1.78 (0.01)	2.41 (0.02)	0.55 (0.01)
	Middle	11.47 (0.09)	13.33 (0.11)	13.51 (0.09)	1.66 (0.01)	2.21 (0.02)	0.47 (0.01)
	Late	9.68 (0.14)	11.05 (0.12)	11.84 (0.12)	1.63 (0.01)	2.25 (0.01)	0.51 (0.01)
Barged	Pooled	1.81 (0.01)	5.35 (0.05)	6.25 (0.06)	3.37 (0.04)	4.27 (0.05)	0.98 (0.02)
	Early	1.95 (0.02)	7.68 (0.13)	10.52 (0.22)	5.33 (0.11)	7.91 (0.19)	1.50 (0.05)
	Middle	1.75 (0.004)	4.74 (0.04)	6.43 (0.08)	2.95 (0.04)	4.56 (0.07)	1.18 (0.03)
	Late	1.71 (0.004)	3.90 (0.02)	4.80 (0.03)	2.17 (0.01)	3.06 (0.03)	0.64 (0.01)

Table 17. Average speed (Km/day) travelled by treatment groups in the LRE.

	Reach 2	Reach 3
Treatment group	**CR202.0-CR035.6**	**CR035.6-CR008.3**
In-River (Pooled)	98.4	53.5
Barged (Pooled)	49.3	27.8

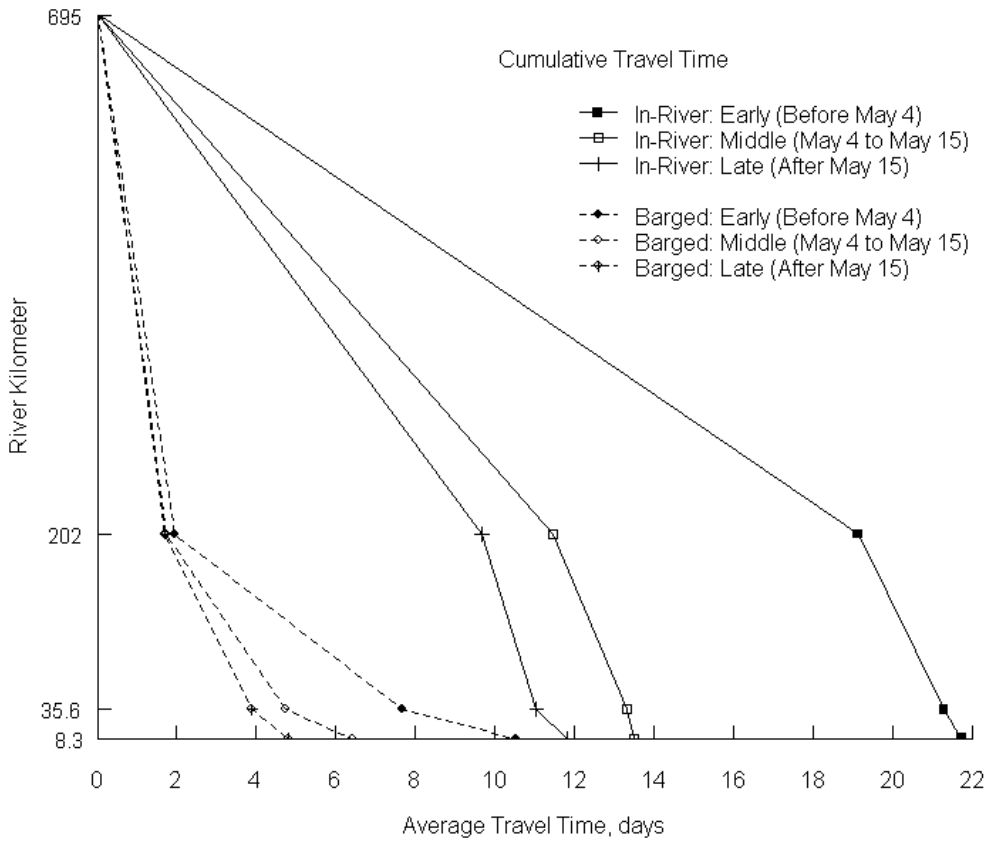

Figure 9. Average (harmonic mean) cumulative travel time of acoustic-tagged fish from Lower Granite Dam (RKM 695) to RKM 8.3 for In-River and Barged fish.

Avian Predation

Bird predation estimates from the East Sand Island (ESI) bird colony were determined from recovered PIT tags versus the total number of JSATS study fish detected below Bonneville Dam. Predation rates were adjusted for detection efficiencies for Caspian terns and double-crested cormorants, the two largest breeding colonies on East Sand Island with the greatest impact on the survival of juvenile salmonids from the Columbia and Snake rivers, with the average colony-wide estimate of 74.9% determined for 2008 (Collis et al. 2009). Bird predation was higher in the pooled Barged treatment group (7.06%) than the pooled In-River treatment group (3.99%) (Table 18). In-River cohorts experienced similar bird predation in all seasonal cohorts, while the Early and Middle Barged cohorts were considerably more preyed upon than the Late Barged cohort (Table 18).

Table 18. Summary of bird predation from East Sand Island (ESI) bird colony. Bird Predation Estimate is the percentage of total fish detected below Bonneville Dam that were recovered at the ESI Bird Colony divided by the average colony-wide estimate of detection efficiency (0.749).

	Early IR	Early Barge	Middle IR	Middle Barge	Late IR	Late Barge	Pooled IR	Pooled Barge	Pooled IR & Barge
Number of Recoveries (PIT) at ESI Bird Colony	8	32	3	22	7	8	18	62	80
Number of Fish Detected (JSATS) below Bonneville Dam	229	463	141	317	232	391	602	1171	1773
Bird Predation Estimate (%)	4.66	9.22	2.84	9.26	4.02	2.73	3.99	7.06	6.02

Discussion

The barge release site at Skamania Landing is located approximately 25 Km upstream of the array at RKM 202. Survival and travel time reported for barged fish within Reach 1 reflected both transportation within the barge hold and active outmigration in the LRE from Skamania Landing to RKM 202. The survival for Early and Middle cohorts over Reach 1 was 97-99% while only 89% for the Late cohort. The specific cause of the considerably higher mortality in the Late Barged cohort in Reach 1 is unknown. Tagging records indicate that the Late Barged cohort had considerably greater post-surgery mortality (14%) in comparison to the seasonal average of 1.1% during the 24-hour recovery phase. Statistical analyses were performed to evaluate if survival in Reaches 1 through 3 was significantly different for fish tagged by a specific surgeon, and it was concluded that none of the surgeons had a significant effect on survival of the Late Barged cohort in any of the Reaches.

Survival of barged fish was roughly 50% greater than that of fish with in-river outmigration history for the entire study area from Lower Granite Dam to the acoustic receiver array at RKM 8.3 in the estuary. As expected, greatest differential survival between barged and in-river fish occurred between Lower Granite and Bonneville dams (Reach 1, $\widehat{BI}$ = 1.78), indicating that transport initially increased the survival probability of yearling Chinook salmon. However, exclusively in the LRE (encompassing Reaches 2 and 3), where both groups of fish are actively migrating, this trend was reversed and survival of in-river fish was 19% greater than that of transported fish.

Mean travel time for in-river fish between release at Lower Granite Dam and detection at the RKM 202 (Reach 1) was 12.4 days, which is similar to estimates of 13.0 days for run-of-river yearling Chinook salmon determined by McComas et al. (2008). In Reach 1, travel times for the different in-river cohorts differed considerably. Early cohorts had the slowest travel time of approximately 19 days, while the Late group traveled twice as fast and covered the same distance in just over nine days. Estimated survival rates for Reach 1 were lowest for the Early cohort, followed by the Late cohort, while the Middle cohort had the highest probability of survival. Thus, cohorts actively migrating between Lower Granite and Bonneville dams had much shorter travel times as the season progressed, but this did not necessarily increase their probability of survival.

From RKM 202, in-river fish took slightly over 2 days to reach the array at RKM 8.3. Travel times and survival probabilities for Reach 2 and 3 combined were generally very similar for all in-river cohorts, and comparable to those determined in previous studies for run-of-river yearling Chinook salmon (McComas et al. 2008, McComas et al. 2007). Survival probabilities of in-river cohorts increased from Early to Late, but differences were not significant. Although the Early in-river cohort had significantly longer travel times than the Middle and Late cohorts, travel times between the fastest (Middle) and slowest (Early) in-river cohorts differed by less than 5 hours for Reach 2 and 3 combined.

Both treatment groups experienced the lowest survival rates in the last reach between RKM 35.6 and 8.3. At the same time, barged cohorts, especially Early and Middle, exhibited statistically significantly longer travel times and lower survival throughout the estuary compared to in-river cohorts. In the LRE (Reaches 2 and 3 combined), the longest mean travel time recorded (7.9 days) corresponded with the lowest estimated cohort survival of 64% (Early Barged cohort). In this region, the Early passage cohort of transported fish with the slowest travel times experienced the highest mortality, while the Late cohort migration was fastest and showed the highest survival probability, suggesting a correlation between LRE transit time and mortality for barged fish. Travel times and survival probabilities of Early and Late Barged cohorts were statistically significantly different.

It is important to note that the upper limit of salt water incursion in the estuary coincides with the beginning of Reach 2 where travel times decreased dramatically (~ km 36; Sherwood et al. 1990). Results of a study investigating the smoltification status for Rapid River and Clearwater Hatchery spring Chinook salmon yearlings with different outmigration strategies (Eder et al. 2009) indicate that fish barged early in the season had lower smoltification status than fish barged later in the season. Advanced smoltification, in turn, has been shown to increase directed downstream swimming speed in juvenile salmonids (Giorgi et al. 1991, Lundqvist et al. 1985).

As indicated above, barged fish spend statistically significantly longer time in the LRE when compared to the in-river fish. Barged fish spent between 19 hours (Late cohort) and 5.5 days (Early cohort) more to cover the distance between RKM 202 (25 km below release) and the array at RKM 8.3 than in-river fish. Net pen mortality results demonstrated that survival of barged fish held in the estuary were negatively influenced by transit time in freshwater, which is consistent with the decreased survival probabilities for the slower travelling barged fish.

The longer transit times of the barged fish may have increased their risk of predation. Although no estimate is available for piscivorous predation, avian predation estimates below Bonneville Dam associated with East Sand Island bird colonies were considerably higher in the pooled Barged treatment group (7.06%) than the pooled In-River treatment group (3.99%) (Table 18). Avian predation for the in-river fish was similar to findings in recent reports (McComas et al. 2007, 2008), but was more than doubled that for Early and Middle Barged cohorts, thus suggesting a correlation between estuary transit time and avian predation. Seasonal trends of increased travel times correspond with increased bird predation and overall decreased survival probabilities in barged fish. The shortest travel times (3.06 days), highest survival estimates (0.830), and lowest bird predation estimates (2.73%) all occur in the Barge Late passage group between release and RKM 8.3 (Tables 14, 16, and 18). However, the late Barged Cohort was

less preyed upon than the respective In-River cohort, despite travelling slower through Reach 3. While bird predation estimates for Early and Middle Barged cohorts seem to correlate with increased travel time and lower survival probabilities, these findings are based on various assumptions and it is possible that other, unknown factors may be responsible for these results. First, detection efficiencies are based on PIT-tagged fish, and egestion, regurgitation, and subsequent detection of double-tagged (PIT and JSATS) fish may differ from single-tagged fish. Second, it is difficult to determine exactly which reach is most impacted by avian predation since tag recoveries are related to all fish detected below Bonneville Dam. Third, bird predation estimates are based on the assumption of common tag deposition and recovery rates throughout the season. Fourth, travel time data was only received from fish that were not preyed upon. Thus, this data is based on the assumption that their travel speeds are representative of all fish migrating through the river at the same time. Finally, it is important to recognize that avian predation estimates based on on-colony detection efficiency are minimum estimates that do not account for off-colony tag deposition.

4.0 NET PEN MORTALITY

Introduction

The extent and putative cause of mortality were evaluated in estuary net pens for (a) fish collected and tagged with acoustic transmitters at Lower Granite Dam, barged to Bonneville Dam, and transported to the net pen sites (Barged treatment group); (b) fish collected and tagged at Bonneville and John Day dams and transported to net pen sites (Bonneville treatment group); (c) fish collected and tagged at Lower Granite Dam, released to travel in-river, and recollected at Bonneville and John Day dams for transport to net pen sites (In-River treatment group); and (d) a group of reference fish comprised of laboratory-reared juvenile spring Chinook salmon from Rapid River Hatchery broodstock transported to the estuary net pen sites and placed into the net pens at three times during the study period (Reference treatment group).

All treatment groups were comprised of run-of-river hatchery-reared (adipose fin-clipped) yearling Chinook salmon. Treatment groups were held at two estuary net pen locations, Tongue Point and Sand Island, representing different temperature and salinity conditions. The treatment groups Barged (n = 868), Bonneville (n = 894), and Reference (n = 1080) had similar numbers of fish held in net pens, while the In-River treatment group was significantly smaller (n = 51) due to re-collecting issues at Bonneville and John Day dams associated with high river flow in 2008. In addition, because of the small sample size, the In-River treatment group was held in estuary net pens only at Sand Island and thus only compared to other groups held at that location.

Results

Differences in Net Pen Mortality among Treatment Groups

Cumulative net pen mortality of fish from all treatment groups (Barged, Bonneville, and In-River) was significantly greater than that of reference fish at the Sand Island site (P-values $\leq$ 0.048 to 0.001; Figure 10a). While the cumulative mortality in the Reference group amounted to only 1.7% after 28 days of holding, Barged (9.7%), Bonneville (11.5%), and In-River treatments (15.7%) experienced significantly greater mortality in Sand Island net pens. A significant difference in cumulative mortality between Reference and treatment groups was generally seen after the first week of holding and lasted until the end of the 28-day holding period. When comparing survival at Sand Island between treatment groups, in-river fish mortality did not differ significantly from Barged or Bonneville groups at any given day of the holding period. Although in-river fish experienced higher mortality after day 5 than any other group held at Sand Island, the small number of individuals in this group did not allow the identification of a clear statistical difference with other treatment groups. Trends in mortality for Barged and Bonneville treatment groups at the Sand Island net pen site were similar overall, but significantly greater cumulative mortality was detected in the Barged group during days 13 to 17 (P-values $\leq$ 0.050 to 0.023), with a peak difference of 3.5% on day 17 (SE = 0.0155) (Figure 10a).

Cumulative estuary net pen mortality at Tongue Point pooled across cohorts was significantly greater than at Sand Island for the Barged (days 5-28; P-values $\leq$ 0.033-0.001) and Bonneville (days 7-28; P-values $\leq$ 0.041-0.001) treatment groups, but not for the Reference group (Figure 10a, b). The Tongue Point net pen site had a significant effect on Bonneville fish, with greater

mortality relative to fish at Sand Island for the majority of the holding time (days 7 to 28), and a peak difference of 26.6% on day 24. Similarly, a significantly higher percentage of barged fish died at Tongue Point in comparison to Sand Island for the majority of the holding time (days 5 to 28), and a peak difference of 26.2% on day 28. Survival of reference fish over 28 days of holding was comparable at Tongue Point and Sand Island (2.2% and 1.7%, respectively).

Differential mortality between treatments and the Reference groups occurred earlier at Tongue Point than at Sand Island (day 4 for both treatments), and with greater peak differences (>30% for both treatments) (Figure 10b). Cumulative mortality of Barged and Bonneville groups was very similar at Tongue Point, and a significant difference was only seen on Day 10 (5.3%, P-value ≤ 0.021) (Figure 10b).

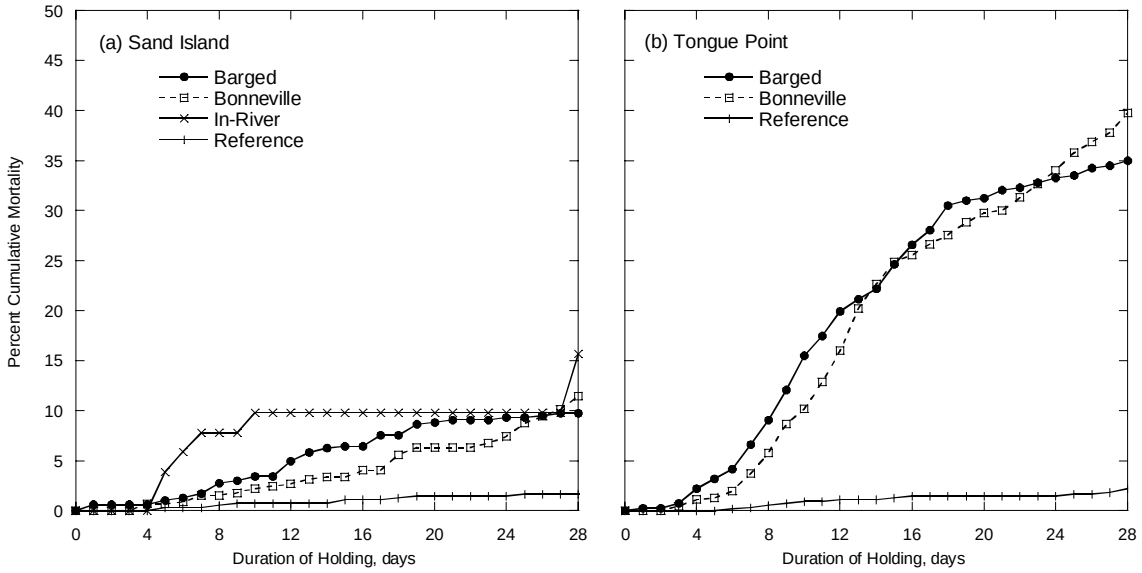

Figure 10. Cumulative mortality observed in Barged, Bonneville, In-River, and Reference treatment groups at different estuary net pen locations. Cumulative mortality at (a) Sand Island and (b) Tongue Point.

Differences in Net Pen Mortality for Barged and Reference Early, Middle, and Late Cohorts

Due to the significant differences in cumulative mortality between the two net pen locations (Tongue Point and Sand Island), differences in mortality between early, middle, and late cohorts of barged and reference fish were evaluated separately for each net pen location. A comparison of early, middle, and late passage cohorts between In-River and either Barged or Reference groups was not possible because too few in-river fish were re-collected to form seasonal cohorts. In addition, it was not possible to compare early, middle, and late passage cohorts of Bonneville fish with either barged or reference fish because Bonneville fish were collected at two different locations (Bonneville and John Day dams), and the cumulative incidence of mortality varied more between the location of collection (Bonneville Dam versus John Day Dam; Figure 12) than seasonally.

Cumulative mortality of Barged and Reference treatment groups was generally low at Sand Island. Mortality of Reference cohorts loaded into Sand Island net pens at an early, middle, and late time point differed only slightly. However, the Middle cohort experienced the greatest mortality at Sand Island, which differed significantly from mortality in the Early cohort during days 9-24 (P-values ≤ 0.044-0.013), and from mortality in the Late cohort during days 9-17 (P-values ≤ 0.044-0.013; Figure 11a). Barged passage cohorts had significantly greater mortality than the respective Reference cohorts throughout most of the holding period except for the Late cohort (only days 17, 27 and 28 were significantly different), and peak differences ranged from 12.5% (Early; *SE* = 0.0234) to 6.5% (Middle; *SE* = 0.0218) to 5% (Late; *SE* = 0.0244). Within the Barged group, the Early cohort experienced the greatest cumulative mortality at the Sand Island net pen site, whereas the Late cohort had the highest survival (Figure 11a). Mortality differed significantly between the Early and the Late cohort, but not the Middle cohort (P-values ≤ 0.024-0.008), and the greatest difference was 8.6% at the end of holding (*SE* = 0.0322). No significant difference in cumulative mortality was found between the Middle and the Late cohort in the Barged group at Sand Island.

At Tongue Point, the Early Barged cohort experienced the greatest mortality of all barged cohorts (Figure 11b). Differences between the Early and Late Barged cohorts were significant during the second half of holding (P-values ≤ 0.032-0.001), and greatest on the last day (20.4%; *SE* = 0.0621). Mortality of Early and Middle cohorts differed significantly only on two days (days 20 and 28; peak difference 12.1%; *SE* = 0.0551), and the Middle cohort had significantly greater mortality than the Late cohort early in the holding period (days 4 to 10, P-values ≤ 0.026-0.004).

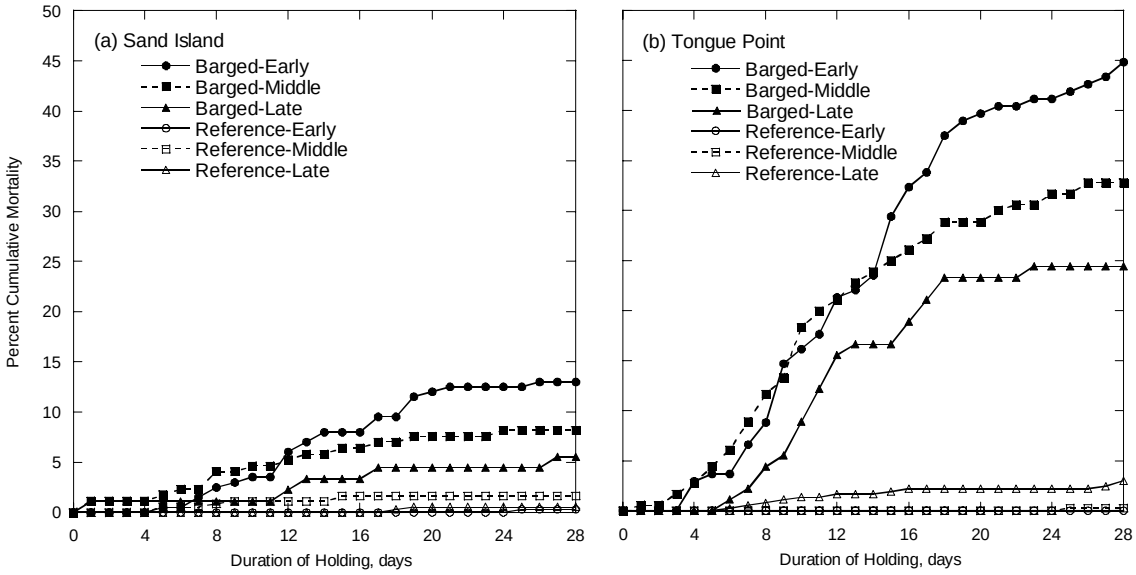

Figure 11. Cumulative mortality observed in Barged cohorts Early, Middle, and Late, and respective Reference cohorts at different estuary net pen locations. Cumulative mortality at (a) Sand Island and (b) Tongue Point.

Unlike any other treatment group in this study, fish comprising the Bonneville treatment group were collected at two different sites, Bonneville Dam and John Day Dam, which proved to affect

the cumulative mortality significantly. At the Sand Island net pen location, significantly more Bonneville Dam collected fish died than fish collected at John Day Dam during days 6 to 28 (P-values ≤ 0.043-0.001), and the greatest difference in cumulative mortality occurred on day 18 (9.8%; *SE* = 0.0257; Figure 12a). At the Tongue Point net pen location, cumulative mortality of Bonneville Dam collected fish was significantly greater than that of fish collected at John Day Dam during most of the holding period (days 7 to 28, P-values ≤ 0.005-0.001), with a peak difference of 27.8% on day 19 (*SE* = 0.0438) (Figure 12b).

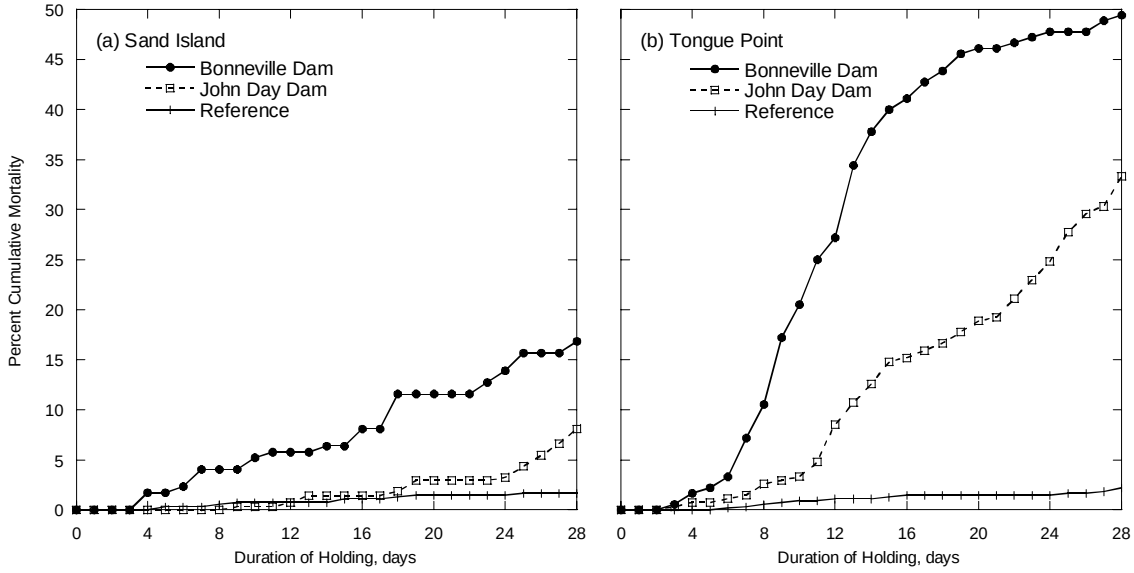

Figure 12. Cumulative mortality observed in fish collected at Bonneville Dam and John Day Dam and Reference groups at different estuary net pen locations. Cumulative mortality at (a) Sand Island and (b) Tongue Point.

Comparison of survival of net pen fish with fish actively migrating in LRE

Mortality of barged fish held in the net pens at Tongue Point was compared statistically to mortality of barged fish actively migrating through the LRE (Table 19). A similar comparison with in-river fish was not made: fish that actively migrated in-river through the FCRPS that were subsequently collected at Bonneville Dam were only held in net pens at Sand Island (saline influenced site), and the majority of the LRE is freshwater (~86%). Average avian predation for each Barged cohort (Table 18) was added to the mean survival of acoustic-tagged Barged cohorts from Skamania (RKM 227) to RKM 8.3 (Reach 2 and 3; Table 14) to account for bird predation estimates. Survival of respective Barged cohorts in Tongue Point net pens was determined for the average amount of days each cohort travelled through the LRE (Reach 2 and 3). Net pen survival of all barged cohorts was significantly higher than survival estimated for actively migrating cohorts after avian predation was accounted for (Table 19). The average difference between the upper and lower limit of the 95% confidence interval of survival estimates for actively migrating Barged cohorts and Barged cohorts held in net pens, respectively, was 9.7%, and increased from Early (7.0), to Middle (10.0), to Late cohorts (11.8; Table 19).

Table 19. Statistical comparison of the survival of barged fish held in the net pens with barged fish that actively migrated through the LRE.

Treatment group	Cohort	Estimated survival (SE) in the LRE	Bird predation (SE) [a]	95% Confidence interval for the mean	Net pen survival (SE)[b]	95% Confidence Interval for the mean	Minimum Difference[c]
Barged	Early	0.6386 (0.0321)	0.0922 (0.0028)	0.6677 – 0.7939	0.9118 (0.0243)	0.8642 - 0.9594	0.0703
	Middle	0.7001 (0.0329)	0.0926 (0.0028)	0.7597 – 0.8257	0.9556 (0.0154)	0.9254 - 0.9859	0.0997
	Late	0.8301 (0.0244)	0.0273 (0.0028)	0.8328 – 0.8820	1.0000 (n.a.)	1.0000	0.1180

[a]Estimates of bird predation reflect the number of tags recovered on East Sand Island specifically from our study fish adjusted to reflect the detection efficiency (Table 18). The values of avian predation reported here are minimum estimates of the true extent of avian predation in the LRE.
[b]Net pen survival for each cohort was determined for the average amount of days actively migrating cohorts travelled from Skamania (RKM 227) to RKM 8.3.
[c]Difference was calculated using the upper limit of the 95% confidence interval from the combined survival estimate of actively migrating fish and bird predation, and the lower limit of the 95% confidence interval from net pen survival.

Discussion

Net pen mortality of acoustic-tagged fish with different outmigration histories was assessed to produce covariate information on fish health that may be related to survival of acoustic-tagged yearling Chinook salmon in the LRE and to evaluate potential net pen effects. This is the second year of comparing the survival of barged and in-river yearling Chinook salmon using in-situ net pens, but the first time that net pen groups were employed with acoustic-tagged fish. Furthermore, including a new net pen site near Sand Island enabled fish to be held in water with salinity close to seawater and thus provided a comparison with barged fish held in freshwater at Tongue Point.

The location of the net pen site was the main factor influencing net pen mortality in non-reference fish. Significantly greater mortality at Tongue Point relative to Sand Island was detected for all pooled treatment groups, cohorts, or collection sites, held at both net pen locations. Significantly lower survival at Tongue Point in comparison to Sand Island was also observed in a parallel net pen study with PIT-tagged barged and in-river hatchery fish (Eder et al. 2009). Site differences were less pronounced between two net pen sites situated in different LRE freshwater locations (Dietrich et al. 2008). Interestingly, this net-pen-location-effect evident for treatment groups was not seen for reference fish. The low mortality in Reference groups at both net pen sites suggests that (a) there is only a minimal or no net pen effect, and (b) fish with a barged or in-river outmigration history arrive at Bonneville Dam in a condition that is not compatible with extended freshwater or saltwater residence time. Subsequent chapters present results from investigations of possible causes for net pen mortality.

Another trend observed in both the net pen and JSATS study was the greater cumulative mortality of the Early passage cohort of barged fish, followed by the Middle and Late cohort. The Early passage cohort of the Barged group experienced significantly greater mortality than the Late cohort at both net pen locations, with more pronounced differences at the Tongue Point site. Survival estimates of Barged cohorts actively migrating in the LRE were ranked the same, with greatest mortality in the Early cohort and highest probability of survival in the Late cohort. Although these differences manifested themselves much later in the net pens, it is worth noting

that mortality trends for Barged passage cohorts during net pen holding were similar to survival estimates of cohorts with a barged outmigration history actively migrating in the LRE.

Unfortunately, a direct estimate of differential mortality in the net pens between Barged and In-River treatment groups was not possible. Because of the small sample size of in-river fish recollected at the completion of outmigration (4.1%), these fish were only held at Sand Island. The small sample size did not permit splitting the fish into two groups, with one held at Tongue Point and the other Sand Island. Additionally, because of the strong net-pen location-effect apparent in the barged fish, we assumed such an effect would also occur with in-river fish, hence mortality of the in-river fish at Sand Island could not be used as a surrogate for the mortality of in-river fish at Tongue Point. An estimate of the mortality of in-river fish at Tongue Point would be necessary to quantify differential mortality in the LRE using data from the net pens.

There were two groups of fish with in-river outmigration histories within this study: (a) fish surgically implanted with acoustic tags at Lower Granite Dam (In-River treatment group) and (b) fish surgically implanted with acoustic tags at Bonneville Dam (Bonneville treatment group). The group tagged at Bonneville Dam was collected at two sites: the juvenile collection facility at Bonneville Dam and John Day Dam. The need to collect fish at John Day Dam arose during the study due to removal of all Submerged Traveling Screens (STS fish guidance screens) at Bonneville Dam Powerhouse 2 because of unmanageable debris accumulation on the vertical barrier screens, an action which effectively ended fish recollection. This change resulted in the collection of fish with shorter outmigration histories and different handling protocols, which were confounded with changes in the river conditions that may have impacted outmigration stressors such as water quality and pathogen exposure. Fish collected at Bonneville and John Day dams experienced significantly different cumulative net pen mortalities, indicating that collection site was a strong factor affecting survival in this study, and confounded the use of this group of fish for comparison to the Barged treatment group. A final confounding factor for any comparison of Barged and Bonneville treatment groups is stock origin, given the increased diversity of hatchery fish that may have been tagged as part of the run-of-river population in the Bonneville treatment group.

In addition to investigating causes of death of fish in net pens to better understand potential causes of death of fish actively migrating through the LRE, this study was intended to evaluate timing and trends of mortality in net pens as a surrogate for mortality of migrating smolts. Net pens were placed in the LRE in an environment that both barged and in-river yearling Chinook salmon traverse during outmigration. The net pens lacked important factors potentially affecting mortality rates of actively migrating fish such as, but not limited to, piscivore and avian predation, disorientation, and migratory stress. In addition, holding outmigrant fish in net pens may contribute to deteriorated water quality, disease transmission, or increased stress. To determine how survival estimates of actively migrating fish in the LRE were reflected in the net pen study, mortality of barged fish actively migrating through the LRE was compared statistically to mortality of barged fish held in the net pens at Tongue Point (Table 19). This comparison was not made with in-river fish because this treatment group was only held in net pens at Sand Island (saline influenced site), and the majority of the LRE is freshwater.

Comparison of survival observed in net pens of the Barged cohorts with survival of actively migrating fish revealed that estimates differed between 7.0% (Early cohort, day 8), 10.0% (Middle cohort, day 5) and 11.8% (Late cohort, day 3). In addition, a pattern of increased survival from Early to Middle to Late cohorts in actively migrating barged fish was also observed in the barged fish held in the net pens. Over the complete outmigration season, survival of the Barged treatment group in the net pens at Tongue Point was approximately 10% higher than the survival of actively migrating barged fish between Skamania (RKM 227) and RKM 8.3. Both piscivore and avian predation may be likely factors contributing to the discrepancy in mortality between actively migrating fish in the LRE and fish held in the net pens. Abundance and consumption of the northern squawfish, the predator with the greatest rate of juvenile salmonid consumption (Vigg et al. 1991), was reported to be highest downstream from Bonneville Dam in comparison to other parts of the Columbia River and lower Snake River (Ward et al. 1995). This predator, one of several piscivore predators in the Columbia River basin, was estimated to consume 9.7 million juvenile salmonids annually, or close to 5% of the annual juvenile salmonid outmigration in the LRE (Beamesderfer et al.1996). Additionally, the reported extent of avian predation in this study based on on-colony detection efficiency is a minimum estimate and values are likely much higher. Overall, if piscivore and the true extent of avian predation are added to the mortality observed in the net pens, the resulting value falls into the range of survival of actively migrating barged fish in the LRE. This analysis provides a basis for assessing the relative extent of possible causes of mortality of actively migrating barged fish in the LRE: 7-11.8% related to causes identified in morbid net pen fish which were largely associated with infectious diseases; 2.2-9.2% minimum related to avian predation; and 5% minimum related to piscivore predation. Additionally, this analysis highlights the potential value of using estuary net pens to study the extent and possible causes of health-related mortality of actively migrating fish in the LRE.

In summary, mortality trends observed in this net pen study were, in part, consistent with results from previous studies and provided evidence that net pen effects were minimal or non-existent. A quantitative linkage between the causes of mortality observed in the net pens with fish actively migrating through the LRE was only attempted for Barged cohorts due to inadequate re-collection numbers of in-river fish. This comparison demonstrated that net pens were not only a valuable tool to identify possible causes of mortality for in-river and barged fish in the LRE, but also to estimate the extent of mortality of actively migrating fish in the LRE. The following chapters will show that data obtained from net pen mortalities improved the understanding of potential causes of mortality related to the outmigration history of fish. Furthermore, the observed net-pen-location-effect provided important information for the discussion of survival and travel time differences estimated in actively migrating fish with a barged and in-river outmigration history (see *Discussion* under 3.0).

5.0 PATHOLOGY

Introduction

The manifestation of disease in study fish was assessed by histopathological examination of tissues of select specimen to determine possible causes of net pen mortality and the overall health condition of experimental fish. Three groups of fish were examined with histopathology: 1) destructively sampled untagged fish at tagging locations during the outmigration season; 2) all fish that died during the 28-day net pen holding period; and 3) a portion of the tagged fish that survived net pen holding. For the net pen mortalities, histopathological examinations were completed to determine the probable causes of death. In this context, histopathology examinations provided a direct association between a diagnosis and mortality. Significant causes of mortality were grouped according to infectious disease and metabolic (i.e. starvation) processes. For the destructively sampled fish the examinations yielded similar diagnoses, but findings provide only a baseline of health, and assisted in interpreting infectious or metabolic stressors occurring in the various analyses groups. The histopathological diagnoses presented in this chapter represent a population-level qualitative summary of observations. A detailed description of all histopathological diagnoses, diseases, and other health-related concerns are presented in Appendix B.

Results

Overview

The diseases listed below represent the most important findings from all pathology examinations. While fish from both groups (destructively sampled and net pen mortalities) were diagnosed with findings not listed below, these occurrences were deemed not important from a health standpoint nor did they seem to contribute to mortality. Some examples of these findings are the presence of various parasites (gill amoebae and skin, intestinal, kidney, heart, and gill trematodes) which are commonly found in migrating juvenile Chinook salmon.

Mycotic Infections

Mycotic infections (i.e. fungal or water mold infections) were defined as cutaneous or systemic depending upon the location of the infection. Mycotic infection of the integument and gill were considered as cutaneous mycosis for the purpose of these analyses and the similarity of the etiopathogenesis **(**development of a disease condition due to a specific causative agent). Systemic mycotic infections involved various organs with localization of mycotic elements within the vasculature, as well as colonization of the heart, liver, kidney, spleen, swim bladder, intestine and coelomic membranes.

Bacterial Kidney Disease (BKD)

Lesions consistent with a *Renibacterium salmoninarum* infection, the causative agent of BKD, were intermittently found in various treatment groups. The exams identified a multifocal, chronic, granulomatous inflammation of variable severity without the formation of discrete granulomas that primarily involved the kidney (or more specifically the renal interstitium), but also the heart, spleen, and liver, and the coelomic membranes including the pancreas.

Ceratomyxosis.

A common finding in all fish was severe enteric infections due to myxosporeans. Lesions were characterized by a prominent cellularity of the mucosa and submucosa of the anterior intestine and pyloric caeca, whereas the enlarged and multinucleated cells were consistent with the pre-spore developmental stages of the myxosporean *Ceratomyxa shasta*.

Metabolic Lesions

Two related but nonspecific severe lesions among experimental fish included atrophic steatopathy and lipid hepatopathy. Atrophic steatopathy is characterized by the condensation and loss (or atrophy) of the coelomic adipose tissue, whereas lipid hepatopathy refers to the intracytoplasmic localization of lipid vacuoles within the hepatocytes. The latter lesion is generally a consequence of the catabolism of the extrahepatic adipose tissue that is subsequently transported to the liver for utilization as an energy source. The lesions are nonspecific lesions that can occur with stress or any moribund condition including an infectious disease condition(s) that results in an increased energy demand that is exceeded by the normal intake of feed. The lesions may also be a manifestation of a terminal event in extremely moribund or anorexic fish that are unable to feed. Essentially, the adipose tissue is catabolized and transported to the liver in an attempt to meet this increased energy requirement. It should be noted that most teleost fishes, including salmonids, do not store lipid in the liver under ordinary conditions.

Table 20. Number of destructively sampled fish examined by pathology

Treatment Group and Location	Un-tagged[a]	Sand Island[b]	Tongue Point[b]
Lower Granite Dam	44		
Barged		20	21
In-River		16	
Bonneville			
John Day Dam	15	12	12
Bonneville Dam		6	6
Reference[c]		20	21

[a] These fish were from the run-of-river population of the identified treatment group, but were collected and examined without tagging.
[b] These groups were collected and examined after outmigration and 28 days of holding in the net pens.
[c] Reference fish were not tagged and never experienced outmigration.

Prevalence of Diseases in Destructively Sampled Fish Over Time

The total numbers of fish examined by histopathology are listed in Table 20. The prevalence of infectious diseases and metabolic lesions is presented below (Figure 13). The prevalence of various infections in sampled fish was low to non-existent in fish collected at Lower Granite and John Day dams. Due to problems associated with the low number of run-at-large fish available for acoustic tagging at John Day Dam, the number of fish available at this site for destructive sampling was reduced (n=15). With regard to specific infectious diseases (Figure 13a), the prevalence of ceratomyxosis after 28 days holding in the net pens was highest for the In-River and Bonneville groups (94% and 92%, respectively), lower for the Barged and Reference treatments (34% and 17%, respectively), and was not found in fish sampled at Lower Granite or John Day dams. After 28 days of holding, mycotic infections were detected only in the Barged and Reference groups at a prevalence of 1%. The incidence of BKD infections was low at Lower Granite Dam (4%) and higher at the end of net pen holding for Bonneville, Barged, and In-River groups (22%, 22%, and 12%, respectively). Sampled fish showed an increase in

metabolic lesions (Figure 13b) from Lower Granite Dam to John Day Dam (11% to 27%), while prevalence in treatment groups Bonneville, Barged, and In-River after 28 days of holding was even higher (33%, 44%, and 44%, respectively). In comparison, the prevalence of metabolic lesions in the Reference group was only 2%.

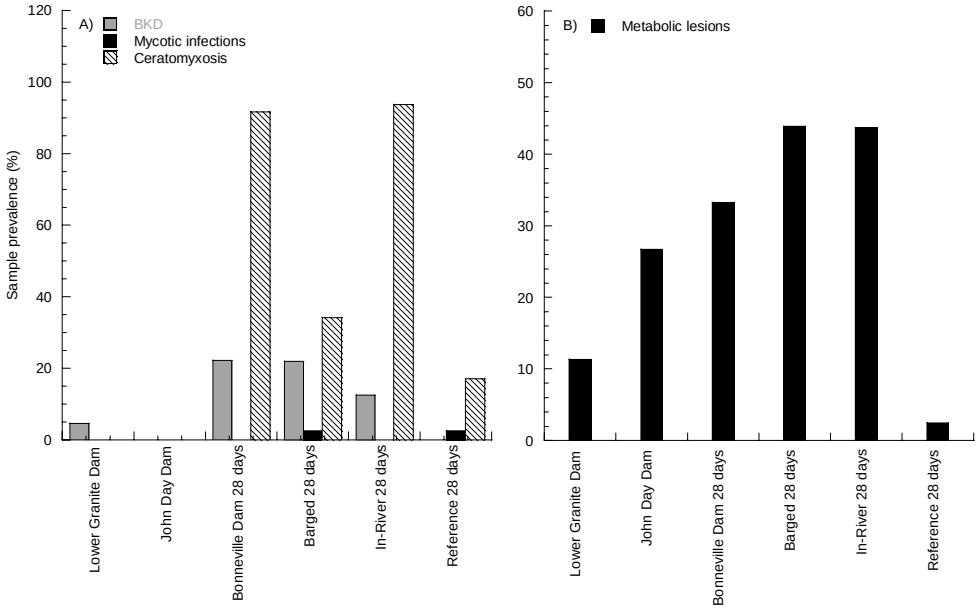

Figure 13. Prevalence of (A) infectious diseases and (B) metabolic lesions in cohorts destructively sampled before and after net pen holding.

When the prevalence of infectious disease at each net pen location after 28 days of holding in surviving fish was considered, all treatment groups showed higher disease prevalence at Tongue Point relative to Sand Island (Figure 14a). This comparison was not possible for the In-River treatment group since these fish were held at the Sand Island net pen location only. Prevalence rates for ceratomyxosis at Tongue Point were 94% for the Bonneville treatment group, 50% for the Barged treatment group, and 24% for the Reference treatment group. In comparison, the prevalence of ceratomyxosis was 89% for the Bonneville, 19% for the Barged, and 10% for Reference treatment groups at Sand Island, with the highest occurrence in in-river fish (94%). Mycotic infections were only seen at Tongue Point, and only in the treatment groups Barged and Reference (5% each). None of the fish sampled at Sand Island showed signs of this fungal infection. BKD was not seen in any of the reference fish, while the In-River group had a prevalence of 12% at Sand Island; the Bonneville group had a BKD prevalence of 5% at Sand Island in comparison to 39% at Tongue Point; and the Barged treatment group had a BKD prevalence rate of 19% at Sand Island and 25% for the Tongue Point. The prevalence of metabolic lesions (Figure 14b) was higher at Tongue Point than at Sand Island only for the Bonneville treatment group (39% and 28%, respectively) and the Reference group (5% and 0%, respectively), but lower at Tongue Point for barged fish (35% in comparison to 52% at Sand Island). The metabolic lesion prevalence was 44% among in-river fish at Sand Island.

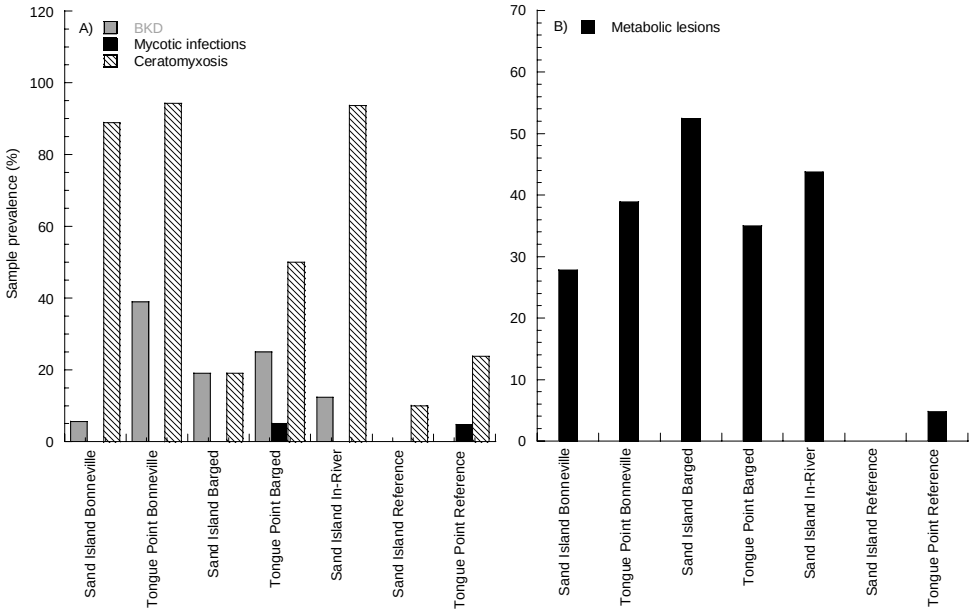

Figure 14. Prevalence of (A) specific infectious diseases and (B) metabolic lesions in destructively sampled cohorts at the end of net pen holding (28 days).

Prevalence of Diseases in Morbid Net Pen Fish Over Time

The cumulative daily prevalence of significant diseases causing death over the course of the 28-day net pen holding period for the Barged and Bonneville treatment groups are presented in Figure 15. For the Barged group (Figure 15a), the main diseases associated with mortality were metabolic lesions and mycotic infections. The diagnosis prevalence of ceratomyxosis in the Barged treatment group was virtually nonexistent (one fish at day 15, data not shown). For the Bonneville group, mycotic infections were the most frequently diagnosed cause of mortality, followed by ceratomyxosis and metabolic lesions (Figure 15b).

Mycotic infections reached a maximum prevalence of 42% at day 7 in morbid fish from the Barged group, followed by a steady decline to 24% at day 19, and a fairly constant prevalence level until the end of holding (Figure 15a). In comparison, a maximum of 67% of Bonneville morbid fish were diagnosed with mycotic infections at day 5, followed by a decline to 44% at day 11 (Figure 15b). The prevalence of this infection remained between 43-50% in Bonneville morbid fish for the rest of the holding period. The prevalence of metabolic lesions in morbid fish from the Barged group was considerably higher relative to Bonneville mortalities throughout the entire holding period. In Barged mortalities, the maximum prevalence rate of metabolic lesions reached 45% on day 4 and then declined steadily until day 12 to remain between 14-15% until the end of holding. In the Bonneville group, these lesions were first observed in 8% of mortalities collected on day 6. Prevalence of these lesions in Bonneville mortalities decreased until day 12 (2%), and remained low until the end of holding (1-2%). Notably, infections caused by ceratomyxa were much more prevalent in mortalities from the Bonneville treatment group, increasing steadily after approximately 18 days of holding until the end of net pen holding, with a maximum prevalence rate of 30% on day 28 (Figure 15b).

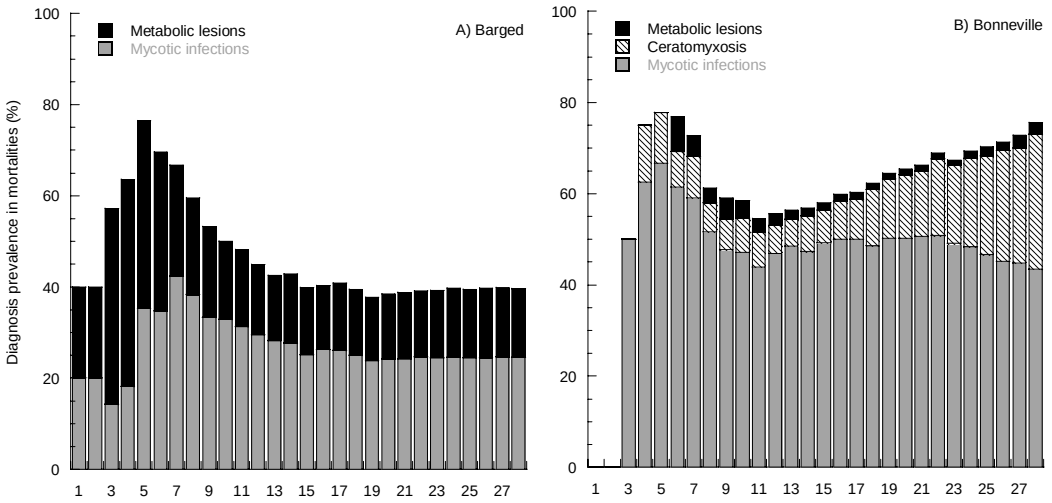

Figure 15. Cumulative diagnosis prevalence of major diseases in net pen mortalities over 28 days of holding for Barged (A) and Bonneville (B) treatment groups. Both net pen sites represented.

Progression of Disease During LRE Net Pen Holding

Disease progression in morbid Barged and Bonneville fish over the 28-day net pen holding period at both sites combined is graphically depicted in Figure 16. Disease categories associated with morbid barged fish included 'unknown causes', mycotic infections, and metabolic lesions. Mycotic infections and metabolic lesions were found to be associated with most of the mortality in the Barged treatment group until day 9, and all of the mortality from day 10 until the end of holding (Figure 16a). The prevalence of ceratomyxosis in this treatment group was virtually nonexistent (one fish, data not shown).

Disease categories associated with morbid Bonneville fish included 'unknown causes', mycotic infections, metabolic lesions, and ceratomyxosis. Mycotic infection prevalence in mortalities of the Bonneville treatment group was similar to the prevalence observed in fish that died in the Barged group both in magnitude and timing. However, infections caused by ceratomyxa were much more prevalent in mortalities of the Bonneville treatment group, increasing steadily after approximately 14 days of holding until the end of net pen holding, with a maximum prevalence rate of 7% on day 28 (Figure 16b). Metabolic lesions were associated less with morbid Bonneville fish than morbid barged fish, especially during the first three weeks of holding. Prevalence of these lesions increased in the last week of holding but remained comparatively low relative to the prevalence in morbid barged fish.

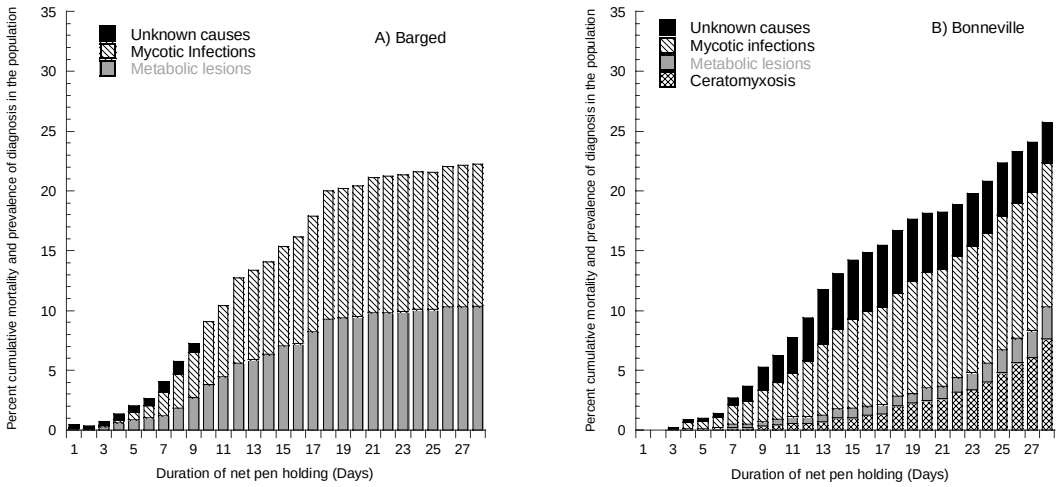

Figure 16. Population prevalence of diseases associated with morbid fish collected during net pen holding for (A) Barged, and (B) Bonneville treatment groups. Both net pen sites represented.

Prevalence of Disease in Mortalities by Site

The prevalence of infectious diseases and metabolic lesions was determined for daily mortalities collected for each net pen location (Figure 17). The prevalence of mycotic infections was higher in mortalities from Tongue Point relative to Sand Island for all treatment groups (except for In-River, which was only held at Sand Island). This was most evident for Bonneville, Barged, and Reference treatment groups, where mycotic infections were found in 53%, 65%, and 86% of mortalities at Tongue Point, respectively, but only in 18%, 22%, and 14% at Sand Island, respectively.

The ceratomyxosis diagnosis prevalence in mortalities from the Bonneville treatment group was 29% at Tongue Point in comparison to 47% at Sand Island. Ceratomyxosis was also seen in 25% of the mortalities that occurred in the In-River treatment group at Sand Island, but was only detected in one out of 138 mortalities from the Barged treatment group. The prevalence of metabolic lesions in mortalities did not vary significantly by net pen site; however, the Barged group had higher diagnosis prevalence (approximately 47% for both sites) than the Bonneville group (approximately 11%, both sites). Furthermore, these lesions were not found in mortalities of the Reference group at Tongue Point, but in 14% of the dead reference fish collected at Sand Island.

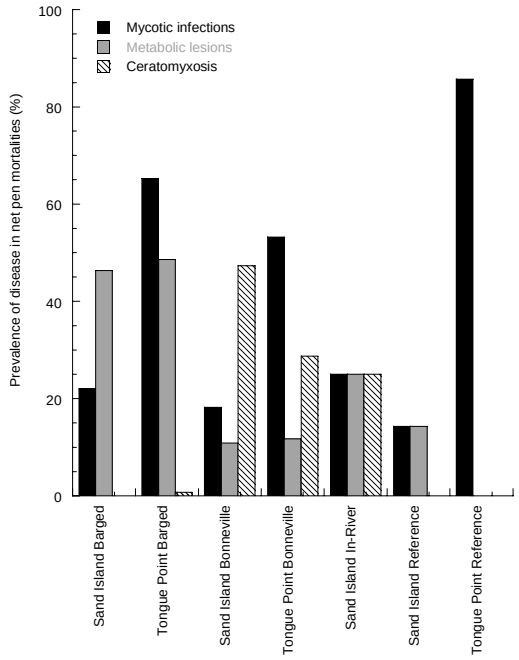

Figure 17. Prevalence of significant pathological findings in mortalities by treatment group and net pen location.

Discussion

Histopathological examinations of representative sub-samples of run-at-large Chinook and all net pen mortalities were completed, and the findings were reported by the likelihood of diagnosis-associated disease or cause of mortality in the respective population. The findings discussed below should not be confused with pathogen prevalence assessed with polymerase chain reaction (PCR) as described in Chapter 6.

Destructively Sampled Fish

With the exception of Bonneville and In-River treatment groups, relatively equal numbers of fish were sampled and subjected to histopathology exams. All surviving fish of the In-River treatment group were examined at the end of net pen holding. The low numbers of in-river fish recovered and sampled are in part due to the high cumulative incidence of mortality (70.5%) of fish held at Sand Island, as well as the removal of all Submerged Traveling Screens (STS fish guidance screens) at Bonneville Dam Powerhouse 2 because of unmanageable debris accumulation on the vertical barrier screens, an action which ultimately reduced fish recollections altogether. Fish recovery operations were subsequently moved to the John Day facility during peak migration, which prevented the collection of fish for more than a week.

In fish examined after 28 days of net pen holding, the prevalence of disease had increased relative to untagged fish collected prior to holding (Figure 13). Interestingly, the prevalence of ceratomyxosis was equally high in the Bonneville and In-River treatment groups. As mentioned above, the Bonneville group consisted of actively migrating run-of-river fish collected at

Bonneville or John Day dams, which were subsequently tagged and transported to the net pens. Thus, their outmigration history was similar to that of the In-River treatment group, except for the location of tagging and thus time the tag was in the fish. High prevalence of ceratomyxosis in these two groups may be explained by the ecology surrounding this pathogen and its dependence on host (salmon) and habitat. The myxosporean becomes infective for fish only after it has been ingested and subsequently expelled by a freshwater worm, *Manayunkia speciosa*. Results presented here indicate that passage of run-at-large smolts (Bonneville and In-River) through the Columbia River system between Lower Granite and Bonneville dams exposed fish to habitat which may be favorable for the intermediate freshwater host (Bartholomew et al. 1989; Stocking and Bartholomew 2007). Accordingly, a significantly lower incidence of ceratomyxosis was found in the Barged treatment group which was transported through this river segment much quicker, thus reducing the chances of contact and exposure time considerably. Infected fish are known to transmit the spore, provided the host worm is available (Bartholomew et al. 2004). Introduction of infected fish to the net pens may have promoted the spread of infections to the Reference group, and possibly the Barged group. This may explain how reference fish, with initially no indication of ceratomyxosis infection, show low prevalence of the disease after 28 days of holding.

The incidence of BKD, caused by the bacterium *Renibacterium salmoninarum*, also increased between Lower Granite and Bonneville dams, and with transit time in the estuary. Infections after 28 days of estuary net pen holding were similar in Bonneville, Barged, and In-River treatment groups. Handling stress associated with collection, tagging, transportation, and holding in the net pens may contribute to the probability that latent low-level infections become more severe (Mesa et al. 2000). The absence of infections in the reference fish after 28 days of net pen holding may be due to the lack of stressors associated with tagging that all the other treatment groups were exposed to, and suggests that this group did not have low level (subclinical) infection upon arrival at the net pens. The incidence of mycotic infections was low in all groups of sampled fish, which may be due to mortalities among fish with these infections and sampling of survivors (see below).

The incidence of metabolic lesions increased from early samples taken at Lower Granite Dam to samples taken downstream at John Day Dam, and the highest prevalence in fish collected at the end of holding in the net pens. Increased energy demand that is exceeded by the normal intake of feed is a likely explanation for fish actively migrating in the river (Bonneville and In-River treatment groups). In comparison, reference fish transported to the net pens had a very low prevalence of metabolic disease. The relatively high prevalence of metabolic lesions observed in the Barged treatment group is not expected to be caused by an increased energy demand associated with migration, but indicates that other stressors are preventing fish from this group to meet their energy demands, resulting in the abnormal storage and utilization of fat within the body (see *Appendix B*).

Estuary net pen location is the main factor affecting the survival of all treatment groups with the exception of the Reference group, and the analyses of pathogen prevalence of sampled fish support this finding. In general, the prevalence of pathogens was higher at Tongue Point for all treatment groups, and mycotic infections were detected only at Tongue Point. The fact that no mycotic infections were seen in fish sampled at Sand Island may be explained by the inability of

fungus to grow in saline conditions (Testrake 1959; Willoughby 1994). BKD and ceratomyxosis infections were more prevalent at the Tongue Point site relative to Sand Island, regardless of treatment group, suggesting that extended freshwater exposure increases the risk of contracting this infectious disease. The presence of ceratomyxosis infections at both net pen locations, Tongue Point and Sand Island, indicates that the intermediate host necessary for the transmission of the disease must be present in these habitats, and that conditions at Sand Island do not prevent the transmission of ceratomyxosis. Differences of environmental conditions at the two net pen sites also did not seem to exert an impact on fish metabolism, since the prevalence of metabolic lesions did not show a clear trend for fish held at either location.

Net Pen Mortalities

In order to increase the understanding of causes for differential mortality in the estuary for fish with different outmigration histories, all mortalities that occurred during 28 days of holding were evaluated by histopathology. The main infectious diseases found in mortalities were Saprolegnia species (mycotic infections) and ceratomyxosis. Mycotic infections and metabolic lesions were responsible for most of the mortalities in the Barged and Bonneville treatment groups, while a portion of the mortalities of the latter treatment was caused by ceratomyxosis. As mentioned above, absence of ceratomyxosis in barged fish may be explained by the temporally and spatially reduced exposure of these fish to the habitat which promotes the transmission of this disease as compared to the Bonneville fish. Although the hatchery of origin in the Bonneville fish is unknown, these fish presumably spent more time outmigrating than did barged fish and hence had an elevated risk of contracting the disease. The analyses of mortalities among Bonneville fish over the whole course of holding showed an increase in ceratomyxosis prevalence around day 18, which is fairly consistent with the maturation of this disease. Thereafter, prevalence of ceratomyxosis increased until the end of the net pen holding period, indicating favorable conditions for contracting and spreading of the disease.

The incidence of mortality due to mycotic infections was higher in the Bonneville compared to the Barged treatment group. The initial profile of diagnosis prevalence was similar in both treatment groups, with prevalence increasing during the first week (7 days Barged, 5 days Bonneville), followed by a decrease to a steady rate of infections in mortalities until the end of holding. The trend of increasing prevalence of mycotic infections in the first week followed by a decrease is consistent with the hypothesis that initial diagnoses are among ill fish arriving in the net pens after either barge transport or collection at Bonneville Dam. As mentioned previously, additional physical factors (debris on screens) associated with collection of the Bonneville fish resulted in descaling, thus compromising skin integrity and leading to increased risk of contracting mycotic infections. Thus, stressors associated with tagging, transport and collection likely resulted in the propensity for contracting mycotic infections in both Barged and Bonneville groups; however, the Bonneville group may have been in poorer overall condition due to concurrent infections with ceratomyxosis, which subsequently may have resulted in higher prevalence of mycotic infections following day 11.

The prevalence of metabolic lesions in Barged mortalities was higher throughout the course of net pen holding than for the Bonneville group, and was higher in fish held at Tongue Point relative to those at Sand Island. The increased prevalence of metabolic lesions in barged fish is consistent with results from sampled surviving fish from this group, and may be the result of

stressors associated with barging preventing fish to meet their energy demands. To which extent these lesions are caused by or are associated with mycotic infections detected in this group of fish, or are caused by stressors such as transport and handling is currently unknown and beyond the scope of this study.

The influence of net pen location on prevalence rates of the most prominent disease was much higher and more pronounced for mortalities than for the sampled fish. However, the trend of higher disease prevalence at Tongue Point in comparison to Sand Island for all treatment groups, with the exception of ceratomyxosis in Bonneville fish, was seen for sacrificed fish and for mortalities. It is likely that the significantly greater mortality at Tongue Point relative to Sand Island determined in the net pen mortality study is a consequence of the disease prevalence differences between the two net pen sites. An explanation as to why the prevalence of diseases in mortalities was enhanced relative to that in sampled surviving fish may be that mortalities were from the population over the course of holding, resulting in a surviving population at day 28 from which the most severely diseased fish had been removed. This would also explain the discrepancy between the high prevalence of mycotic infections in mortalities of some treatment groups (e.g. Barged), and the very low prevalence of these infections in the destructively sampled fish within the same treatment groups.

The prevalence of Saprolegnia in the Barged, Bonneville, and Reference treatment groups was considerably higher at Tongue Point than at Sand Island. Collectively, this may indicate that the differential site mortality observed in these treatment groups was driven by mycotic infections present in fish before net pen holding that resulted in mortality in only the severest cases at Sand Island, but may have thrived, as well as have been transmitted to other fish, in the freshwater environment of Tongue Point. Furthermore, the prevalence of ceratomyxosis was responsible for a substantial number of mortalities in those fish actively migrating in the river, but not in barged fish, suggesting that ceratomyxosis was one of the main causes of death in net pen fish with an In-River outmigration history. Higher prevalence of ceratomyxosis in Bonneville mortalities at Sand Island relative to Tongue Point may be due to the holding of infected in-river fish at Sand Island but not at Tongue Point. Mortalities diagnosed with metabolic lesions were similar across sites for Bonneville and Barged treatment groups, indicating that the physical environment did not affect the nutritional status of fish.

6.0 PATHOGEN PREVALENCE

Introduction

Knowledge of the spatial and temporal variation in pathogen prevalence is critical to understanding the ecology of infectious disease and ultimately the population dynamics of anadromous fishes. Disease associated with pathogens can lower the fitness of a population by increasing susceptibility to predation, reducing reproductive potential, and cause direct or delayed mortality (Schreck et al. 2006; Sindermann 1990). Pathogens can be transmitted by direct contact with infected fish, or by exposure to waterborne pathogens shed by infected fish. Our study fish are subjected to both modes of transmission during tagging and recovery, as well as during their outmigration. Finally, the fish exposed to stressors from the handling and crowding associated with barging or prolonged In-River outmigration, as well as during tagging surgery, may have decreased resistance to pathogens, hence increased mortality from disease.

A survey of the presence of multiple pathogen species in outmigrating run-of-river hatchery-reared spring/summer Chinook in the FCRPS has not been undertaken before, and the ecological distribution and impact of most of our target pathogens on fish survival is unknown. However, the prevalence of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), in wild and hatchery Chinook salmon collected during outmigration at dams in the Columbia River was examined between 1990 and 1998 and found to be high (86-100%) (Elliott et al. 1997). In addition, studies have found that 19-25% of hatchery and wild Chinook salmon collected 75 miles upstream from the Columbia River mouth were moderately to severely infected with *R. salmoninarum* as shown by ELISA (Meyers et al. 1993; Sanders et al. 1992). The most recent survey of pathogens in the FCRPS was completed with Dworshak and Rapid River hatchery fish during the 2007 outmigration, in which *R. salmoninarum* was detected in 44.4-60.6% of the fish with In-River and Barged outmigration histories, respectively (Dietrich et al. 2008).

This study examines the spatial and temporal prevalence of a suite of viral, fungal, and bacterial pathogens known to infect Chinook salmon in the Pacific Northwest in water and fish tissue samples collected from run-of-river hatchery-reared spring/summer Chinook with differing outmigration histories in the Snake and Columbia rivers. The pathogens targeted in this study include the following bacteria: *Renibacterium salmoninarum*, *Aeromonas hydrophila, Aeromonas salmonicida, Flavobacterium columnare, Listonella anguillarum, Yersinia ruckeri*; the following viruses: Infectious Hematopoietic Necrosis Virus (IHNV) and Viral Hemorrhagic Septicemia (VHSV); and the algal family Saprolegniaceae.

Results

Pathogen Detections in Water

Only the family Saprolegniaceae, the presumed causative agent of the mycotic infections, was found in the water samples. Two of the 8 samples (25%) collected from the Tongue Point net pen site were positive for Saprolegniaceae (dates 4/25 and 5/26). All water samples were tested for the presence of the pathogens listed in Table 10 using established PCR methods. The recovery of the surrogate virus (PP7) was within acceptable limits for all filtered samples and

these results coupled with the appropriate PCR controls ensured confidence in the low frequency of pathogens detected by PCR.

Pathogen Detections in Fish Tissues

Five pathogen species were detected in the run-of-river fish collected for the pathogen prevalence survey representing bacterial, virus, and algal family. *R. salmoninarum* and the water fungal family Saprolegniaceae were the most commonly found. In addition, IHNV, *Listonella anguillarum*, and *Yersinia ruckeri* were also found, but in isolated incidences. Their prevalence will be discussed in detail below in reference to the location at which the sample was collected and the outmigration history of the fish.

Bypassed run-of-river fish

R. salmoninarum (3.9%), Saprolegniaceae (1.7%), and IHNV (0.6%) were detected in the 179 samples collected from the Lower Granite Dam juvenile bypass (Table 21). In contrast, no pathogens were detected in any of the 39 fish collected at John Day Dam and sacrificed prior to tagging.

Barged fish

Saprolegniaceae was the only pathogen detected among fish that were barged from Lower Granite Dam and held in the estuary net pens. The prevalence of Saprolegniaceae after the 28-day holding period at Sand Island (8%) was two times greater than the prevalence at Tongue Point (4%), for an equal number of samples analyzed (Table 21).

Fish Tagged at Lower Granite Dam and Released to Travel In-River

Although the number of in-river fish originally tagged at Lower Granite Dam and collected downriver was small and they were held only at the Sand Island net pen site, a variety of pathogens were detected. In addition to Saprolegniaceae (6.8%), the bacterial pathogens *R. salmoninarum* (11.4%), *Listonella anguillarum* (2.3%), and *Yersinia ruckeri* (2.3%) were detected (Table 21). The detection data from this experimental treatment group was further parsed by the location at which the fish were collected downriver after tagging (Table 22). The samples collected from John Day Dam were found to have a higher prevalence of most of the detected pathogens than the fish collected at Bonneville Dam, including all incidences of *R. salmoninarum*, and *Y. ruckeri*, and the majority of Saprolegniaceae.

Table 21. Prevalence of detected pathogens.

	Number of Positive Fish per Number of Fish Collected (% detected)				
Location	***Renibacterium salmoninarum***	**Saprolegniaceae**	**IHNV**	***Listonella anguillarum***	***Yersinia ruckeri***
Lower Granite Dam	7/179 (3.9%)	3/179 (1.7%)	1/179 (0.6%)	0/179	0/179
John Day Dam	0/39	0/39	0/39	0/39	0/39
Estuary Net Pens[a]					
Sand Island					
Barged	0/50	4/50 (8.0%)	0/50	0/50	0/50
In-River from Lower Granite Dam[b]	5/44 (11.4%)	3/44 (6.8%)	0/44	1/44 (2.3%)	1/44 (2.3%)
Bonneville Dam[b]	11/50 (22.0%)	3/50 (6.0%)	1/50 (2%)	1/50 (2.0%)	0/50
Tongue Point					
Barged	0/50	2/50 (4.0%)	0/50	0/50	0/50
Bonneville[b]	11/50 (22.0%)	2/50 (4.0%)	0/50	0/50	0/50

[a] After 28 days of holding.
[b] Mixed samples collected at John Day and Bonneville dams.

Fish Tagged at Bonneville Dam

Fish collected from the John Day or Bonneville dam bypasses and tagged at Bonneville Dam were held at both Sand Island and Tongue Point with similar distributions of pathogen prevalence. The *R. salmoninarum* prevalence (22%) was identical at both locations, and only one more detection of Saprolegniaceae at Sand Island (6%) than Tongue Point (4%; Table 21). In addition, single incidences of IHNV and *L. anguillarum* were found at Sand Island, but not Tongue Point (Table 21). As was the case with in-river fish tagged at Lower Granite Dam, the samples collected from John Day Dam later in the outmigration were found to have a higher prevalence of most of the detected pathogens than the fish collected earlier at Bonneville Dam. The prevalence of *R. salmoninarum* was responsible for the greatest differences in collection location, regardless of the net pen site (Table 22). Only the prevalence of Saprolegniaceae was consistently greater in the fish originating from the Bonneville Dam bypass after holding at both net pen sites (Table 22).

Table 22. Prevalence of pathogens detected among In-River fish with different tagging and collection locations after net pen holding.

			Number of Positive Fish per Number of Fish Collected (% detected)				
Net Pen Site	**Tagging Site**	**Collection Site**	***Renibacterium salmoninarum***	**Sapro-legniaceae**	**IHNV**	***Listonella anguillarum***	***Yersinia ruckeri***
Sand Island							
	Lower Granite Dam						
		John Day Dam	5/34 (14.7%)	2/34 (5.9%)	0/34	0/34	1/34 (2.9%)
		Bonneville Dam	0/10	1/10 (10%)	0/10	1/10 (10.0%)	0/10
	Bonneville Dam						
		John Day Dam	10/30 (33.3%)	1/30 (3.3%)	0/30	1/30 (3.3%)	0/30
		Bonneville Dam	1/20 (5.0%)	2/20 (10.0%)	1/20 (5%)	0/20	0/20
Tongue Point							
	Bonneville Dam						
		John Day Dam	8/30 (26.7%)	1/30 (3.3%)	0/30	0/30	0/30
		Bonneville Dam	3/20 (15.0%)	1/20 (5.0%)	0/20	0/20	0/20

Discussion

At least one species from all pathogen classes (bacteria, virus, and algal) that were screened in this study were found in one or more fish kidneys, and the family Saprolegniaceae was found in two water samples. The most commonly detected pathogens were *Renibacterium salmoninarum* and the family Saprolegniaceae, both of which are considered endemic in the Columbia River basin (Pascho et al. 1988; Pascho and Elliott 1989; Elliott and Pascho 1991; Mueller 1994). All of the pathogen PCR tests are presence or absence assays and are not indicative of the severity of the pathogen infection, i.e. initial infection, asymptomatic carrier, or moribund.

Bacterial Kidney Disease (BKD) is a chronic, systemic infection of the kidney caused by *R. salmoninarum*, and has been repeatedly shown to be a significant disease affecting salmonids in the Pacific Northwest. In this survey, *R. salmoninarum* was the most commonly detected pathogen among all experimental treatment groups and locations (up to 22% prevalence) except for samples collected from the John Day Dam bypass and fish with a Barged outmigration history held at both net pens sites for 28 days. These detection rates are far less than those found in previous studies. Previous studies in the Columbia River and estuary that have found *R.*

salmoninarum in 86-100% (Elliott et al. 1997), 0-93% (Arkoosh et al. 2004), and 44-60% (Dietrich et al. 2008) of samples.

Unlike the other assays that target individual species, the assay for Saprolegniaceae targets the rDNA of the Family Saprolegniaceae. The decision to implement an assay for this large and diverse group of algal species was based on the high prevalence of infections observed using polymerase chain reaction (PCR) and histopathogical examination during a previous study (Dietrich et al. 2008). The most common isolates from fish are *Saprolegnia parasitica* and *S. diclina* (family Saprolegniaceae; Noga 1993), although the family Saprolegniaceae also includes other pathogenic oomycetes of fish such as *Achyla* sp. and *Aphanomyces* sp. (Roberts 2001). One study found that *Saprolegnia parasitica*, the primary agent found in lesions in Chinook and other salmon species collected in the Columbia River, was responsible for 91.0% of the Saprolegniasis cases in adult and juvenile salmon (Mueller 1994). Rare isolates of *Saprolegnia diclina* (1.0%) and *Saprolegnia ferax* (0.3%) were also found (Mueller 1994). In sum, the PCR assay detects all members of the family, but a definitive determination as to the species and strain resulting in mycotic infections cannot be determined with the present assay.

Members of the family Saprolegniaceae were the second most frequently detected pathogen by PCR and can represent a wide variety of pathogenic and non-pathogenic algal species that are ubiquitous in the environment (Mueller 1994). The PCR survey for Saprolegniaceae, however, was limited to kidney tissue, and thus likely detected only those fish with systemic Saprolegniasis infections in the kidney. Saprolegniasis is a chronic infection of freshwater fishes with a world-wide distribution, which generally leads to low but steady population mortality (Mueller 1994; Roberts 2001). The Saprolegniaceae prevalence by PCR in fish collected at both estuary net pen sites (3.3-10%) suggests a continuous infection rate among all experimental treatment groups throughout the 28-day holding period. Saprolegniaceae detection was greater among fish held at the Sand Island net pen site than Tongue Point, despite Saprolegniaceae detections in two water samples and an overall greater mortality at Tongue Point. In addition, the Sand Island site had higher salinity than Tongue Point (Figure 6), and salinity greater than 2.8% generally modulates Saprolegniasis infections (Testrake 1959; Willoughby 1994). This could suggest that fewer detections at the conclusion of holding fish at the Tongue Point site may be due to the more infected fish dying in those net pens during holding, leaving fewer infected fish to be sampled.

The presence of detected pathogens in the fish from different experimental treatment groups can be influenced by their outmigration history, handling, holding period, and location of collection and holding. In the current study, the fish collected had a high diversity of handling and outmigration experiences that was not matched with a high number of experimental and reference samples collected. The primary reasons for this discrepancy was the number of run-of-river fish available for tagging at Bonneville and John Day dams, and that collection operations had to be moved to John Day Dam after the start of the downriver collection due to debris-clogged bypass screens at Bonneville Dam. Regardless of the reasons, the discrepancy between high diversity in experimental treatment groups and low sample numbers complicates and restricts comparisons across experimental treatment groups. For example, run-of-river fish at Lower Granite Dam are hatchery-reared Chinook originating from the Snake River basin, while run-of-river fish collected at Bonneville and John Day dams are hatchery-reared Chinook

originating from the Columbia River basin, which includes the main-stem Columbia River stocks as well as the Snake River stocks. In addition, the shut-down of the juvenile bypass at Bonneville Dam at the start of high river flows prevented any sampling of Bonneville Dam bypass fish and forced collection to occur at John Day Dam. The latter effect had the consequence of collecting fish with shorter outmigration histories and different handling protocols, which were coincident with changes in the river conditions that could impact outmigration stressors, e.g. water quality and pathogen exposure. Finally, the pathogen prevalence data cannot be used to infer any pathogen transmission in the FCRPS because the 28 days of estuary holding and mortalities that occurred during that time period confounds any interpretation of the results. A more rigorous sampling effort will be required in the future in order to ascertain the distribution and transmission of pathogens among hatchery-reared run-of-river Chinook salmon with different outmigration histories as well as the impact of AT and PIT tagging has on the pathogen loading of the handled fish.

7.0 COVARIATES EFFECTS ANALYSIS FOR SURVIVAL AND TRAVEL TIME

Introduction

In order to increase the understanding of various physical and environmental factors influencing differences in the rates of mortality between groups of fish with barged and in-river outmigration strategies, the statistical significance of these metrics on river and estuary survival and travel time was explored. Travel time and survival through specific Reaches of the river and LRE was regressed on individual-based covariates representing migration timing (fish collection date, tagging date, and release date), size at tagging (fork length and weight), handling (holding duration, collection source at Lower Granite Dam), and environmental conditions (discharge at either Lower Granite Dam or Bonneville Dam). Identical comparisons and statistical analyses were performed for Barged and In-River treatment groups.

Results

Description of covariates

Migration timing covariates were highly correlated (Figure 18). Fork length and weight at tagging were also highly correlated (Figure 19), although both varied with migration timing (e.g., collection date). Collection source varied only for barged fish (Table 1), and holding duration varied considerably with collection date (Figure 20). Discharge at Lower Granite Dam increased throughout the season (Figure 21). Discharge at Bonneville Dam varied considerably early in the season, but was consistently elevated later in the season.

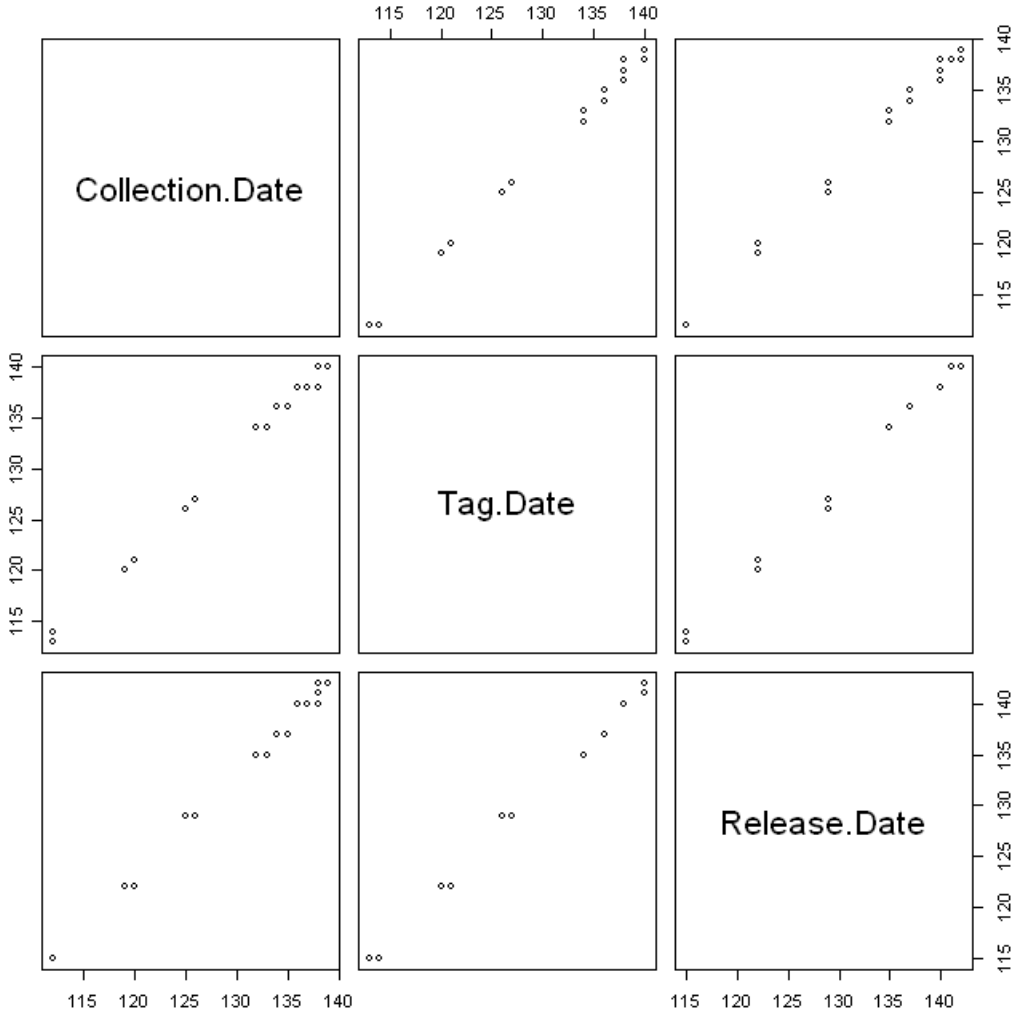

Figure 18. Observed collection date, tagging date, and river release date for acoustic-tagged In-River and Barged treatment groups, expressed as day of year (Day 115 = May 25, 2008).

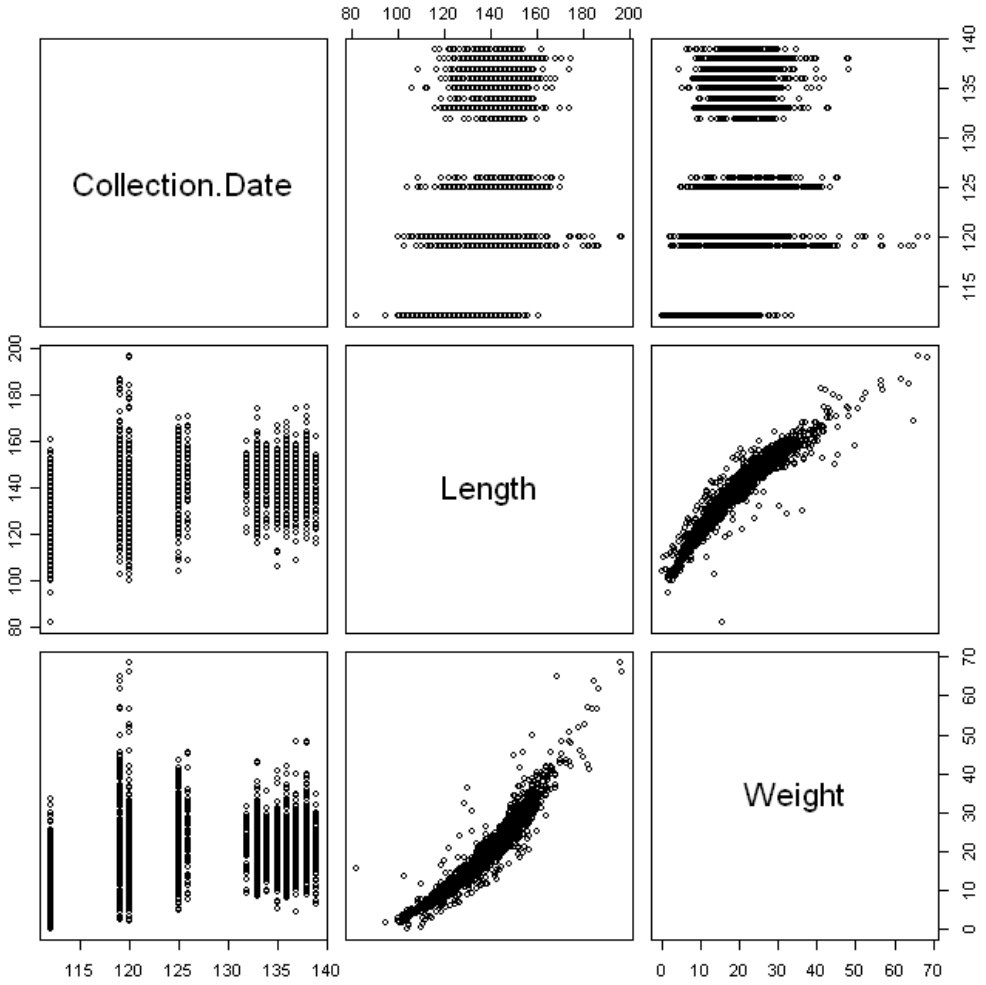

Figure 19. Observed fish fork length and weight at tagging, by collection date at Lower Granite Dam, for acoustic-tagged fish in the In-River and Barged treatment groups.

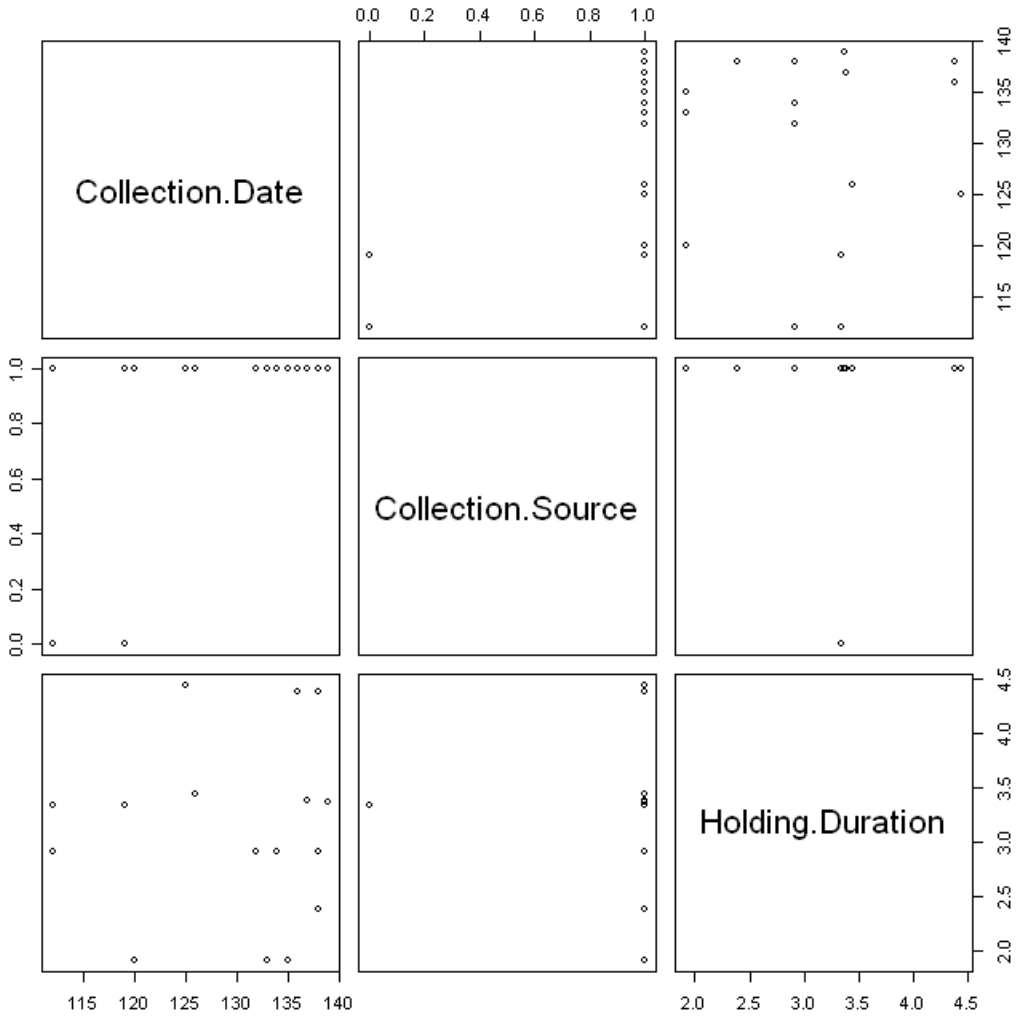

Figure 20. Collection date and collection source versus holding duration (time between collection date and release to river) for acoustic-tagged fish in the In-River and Barged treatment groups. Collection source was either 1 (Sample room at Lower Granite Dam) or 0 (Raceway or Sort-by-Code tank at Lower Granite Dam). All In-River fish were collected from the Sample room. Holding duration is expressed in days.

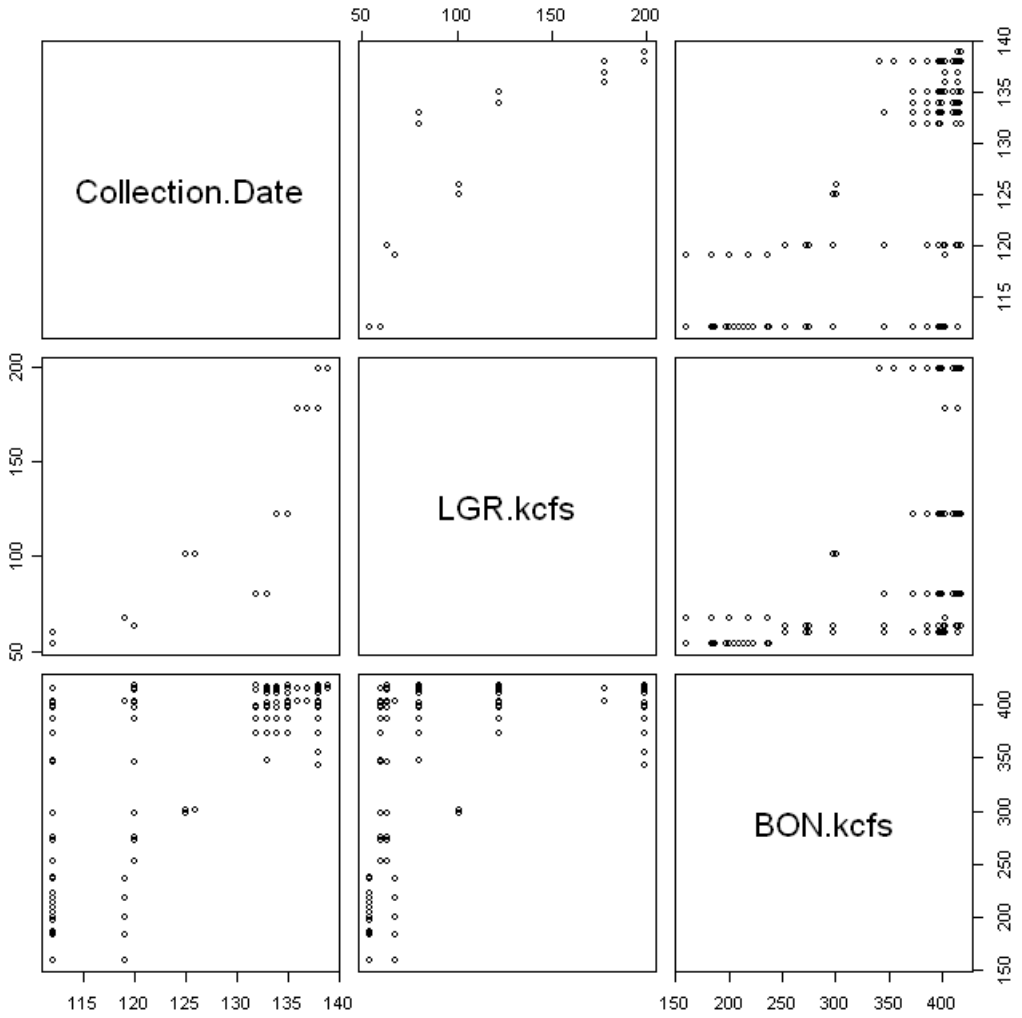

Figure 21. Discharge at Lower Granite Dam (LGR.kcfs) and Bonneville Dam (BON.kcfs) versus collection date at Lower Granite Dam for acoustic-tagged fish in the In-River and Barged treatment groups. Discharge at Lower Granite Dam was measured at the time of release to the river for the In-River treatment group, and at the time of barge loading for the Barged treatment group. Discharge at Bonneville Dam was measured at the time of first detection at RKM 202.

Survival Effects Analysis for Acoustic-Tagged Fish

In-River Treatment group

Proportional hazards modeling of survival from Lower Granite Dam to the acoustic array at RKM 202 (Reach 1) for the acoustic-tagged In-River treatment group found significant effects of collection date (P=0.0797), length (P=0.0007) and weight (P=0.0066) at tagging, and holding duration (i.e., the time difference between collection and release to the river, P=0.0189) (Table 23). Both tagging date and release date had marginally significant survival effects (P=0.1144 for each), while discharge at Lower Granite Dam had no discernable effect on survival (P=1.0). Because length and weight at tagging were highly correlated (Figure 19), only length was considered in multivariate models of in-river survival through Reach 1, along with collection date and holding duration. When both length at tagging and holding duration were accounted

for, the effect of collection date on survival was no longer significant (P=0.6088). Longer fish tended to have higher survival from Lower Granite Dam to RKM 202 when holding duration was also accounted for (P=0.0009), with the longest fish (FL=197.0 mm) having approximately twice the survival probability of the shortest fish (FL=95.0 mm; Figure 22). In-River fish that were held longer at Lower Granite Dam between collection and release to the river tended to have lower survival from Lower Granite Dam to RKM 202, when length at tagging was also accounted for (P=0.0235; Figure 23). Fish held for the maximum observed holding duration among in-river fish (2.92 days) had a survival probability that was 80% of the survival of fish held the minimum time (1.92 days). No covariate was detected to have a significant effect on survival (at the 10% level) through Reaches 2 and 3 (RKM 202 – 8.3) for the In-River treatment group (Table 24). The sample size for this analysis (n=530) was much smaller than for the analysis for Reach 1 (n=1244), due to upstream mortalities and detection efficiencies, resulting in lower power to detect effects.

Table 23. Results from single-variate analyses for the acoustic-tagged In-River treatment group for survival from Lower Granite Dam to RKM 202 (Reach 1). The G-statistic is the Likelihood Ratio Test statistic, with degrees of freedom (DF). Sample size = 1244. A proportional hazards link was used. The null model had AIC = 4664.77.

Category	Covariate	G-statistic	DF	P-value	AIC
Migration Timing	Collection Date	3.0711	1	0.0797	4663.70
	Tagging Date	2.4921	1	0.1144	4664.28
	Release Date	2.4921	1	0.1144	4664.28
Size	Length at Tagging	11.5151	1	0.0007	4655.26
	Weight at Tagging	7.3938	1	0.0066	4659.39
Handling	Holding Duration	5.5105	1	0.0189	4661.26
Environmental Condition	Discharge at Lower Granite Dam	0.0000	1	1.0000	4666.78

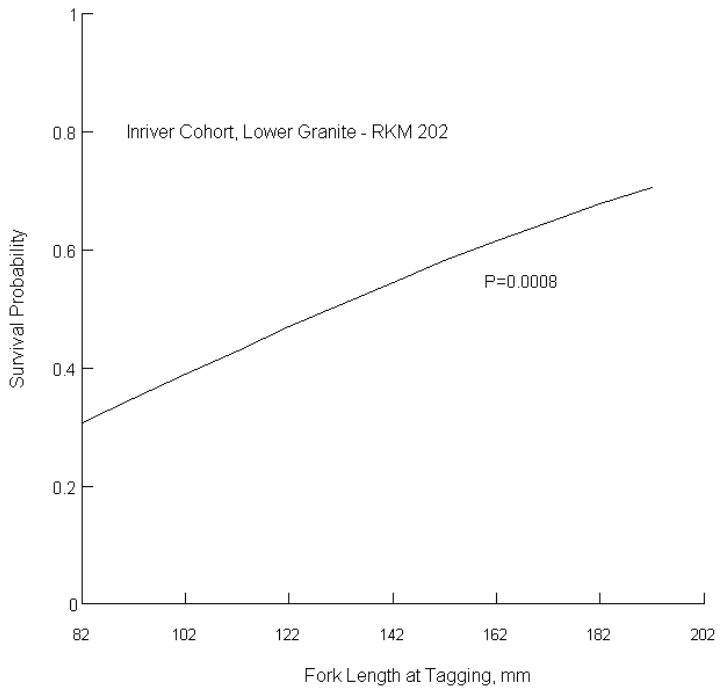

Figure 22. Fitted probability of surviving from Lower Granite Dam (RKM 695) to RKM 202 versus fork length at tagging for the acoustic-tagged In-River treatment group, with P-value of regression coefficient. Probability was evaluated at the average holding duration at Lower Granite Dam for In-River fish (2.45 days).

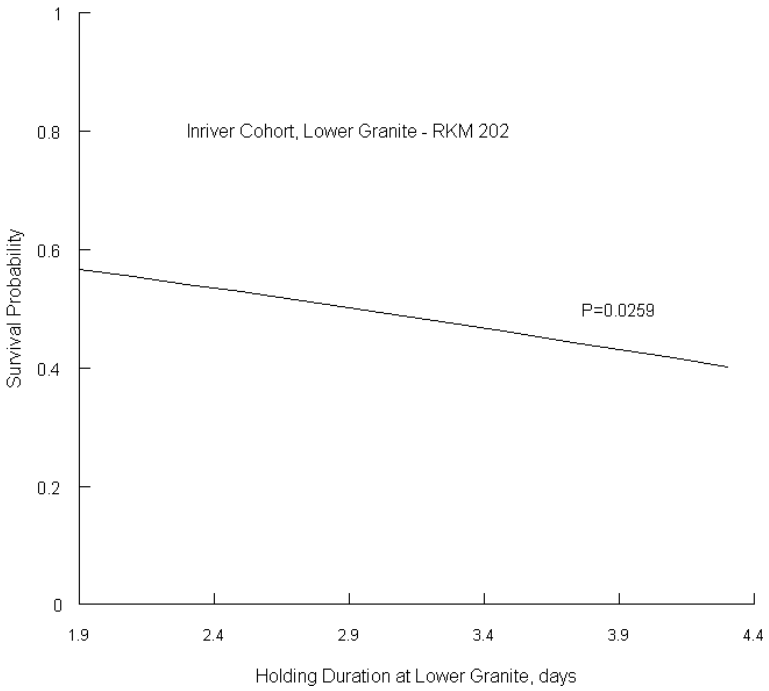

Figure 23. Fitted probability of surviving from Lower Granite Dam (RKM 695) to RKM 202 versus holding duration at Lower Granite Dam for the acoustic-tagged In-River treatment group, with P-value of regression coefficient. Probability was evaluated at the average fork length at tagging for In-River fish (138.5 mm).

Table 24. Results from single-variate analyses for the acoustic-tagged In-River treatment group for survival from RKM 202 to RKM 8.3 (Reaches 2 and 3). Models used a unique Reach effect for each covariate. The G-statistic is the Likelihood Ratio Test statistic, with degrees of freedom (DF). Sample size = 530. The null model had AIC = 1988.24.

Category	Covariate	G-statistic	DF	P-value	AIC
Migration Timing	Collection Date	0.2467	2	0.8840	1992.00
	Tagging Date	0.3928	2	0.8217	1991.85
	Release Date	0.3928	2	0.8217	1991.85
Size	Length at Tagging	0.0000	2	1.0000	1992.24
	Weight at Tagging	0.0000	2	1.0000	1992.24
Handling	Holding Duration	0.0000	2	1.0000	1992.24
Environmental Condition	Discharge at BON	2.4326	2	0.2963	1989.81

Barged Treatment group

Unlike the In-River treatment group, survival for the Barged treatment group through Reach 1 (Lower Granite Dam to RKM 202) was correlated with all of the covariates describing migration timing (collection date, tagging date, and release date, $P<0.0001$ for each), and not correlated to size (length and weight; Table 25). Similar to the In-River treatment group, survival for the Barged treatment group was correlated with the collection source (i.e., collected from Sample room vs. elsewhere; $P=0.0078$; Table 25). Because of the high degree of correlation among the

migration date covariates, only one migration date measure was used in multivariate modeling, along with collection source. Although tagging date had the smallest AIC, collection date was used because it is a more natural measure of migration timing, and the difference in AIC was small. With collection date accounted for, the effect of collection source was no longer significant (P=0.1416). This is not surprising, since all fish collected from the Raceway or Sort-by-Code tank were collected on the first two days of collection for the Barged treatment group, while fish were collected from the Sample room throughout the collection period (Table 1). There was a slight decrease in survival from Lower Granite Dam to RKM 202 for fish collected later in the collection period (P<0.0001). Barged acoustic-tagged fish collected at the end of the collection period (May 19, 2008) exhibited survival probabilities equal to approximately 90% of fish collected at the beginning of the collection period (April 22, 2008) (Figure 24).

Table 25. Results from single-variate analyses for the acoustic-tagged Barged treatment group for survival from Lower Granite Dam to RKM 202 (Reach 1). Collection source compared fish collected from the Sample room to fish from either the Raceway or the Sort-by-Code tank at Lower Granite Dam. The G-statistic is the Likelihood Ratio Test statistic, with degrees of freedom (DF). Sample size = 1278. A proportional hazards link was used. The null model had AIC = 1608.13.

Category	Covariate	G-statistic	DF	P-value	AIC
Migration Timing	Collection Date	28.6221	1	<0.0001	1581.51
	Tagging Date	28.9823	1	<0.0001	1581.15
	Release Date	27.8897	1	<0.0001	1582.24
Size	Length at Tagging	0.0000	1	1.0000	1610.13
	Weight at Tagging	0.0000	1	1.0000	1610.13
Handling	Holding Duration	0.0000	1	1.0000	1610.13
	Collection Source	7.0781	1	0.0078	1603.05

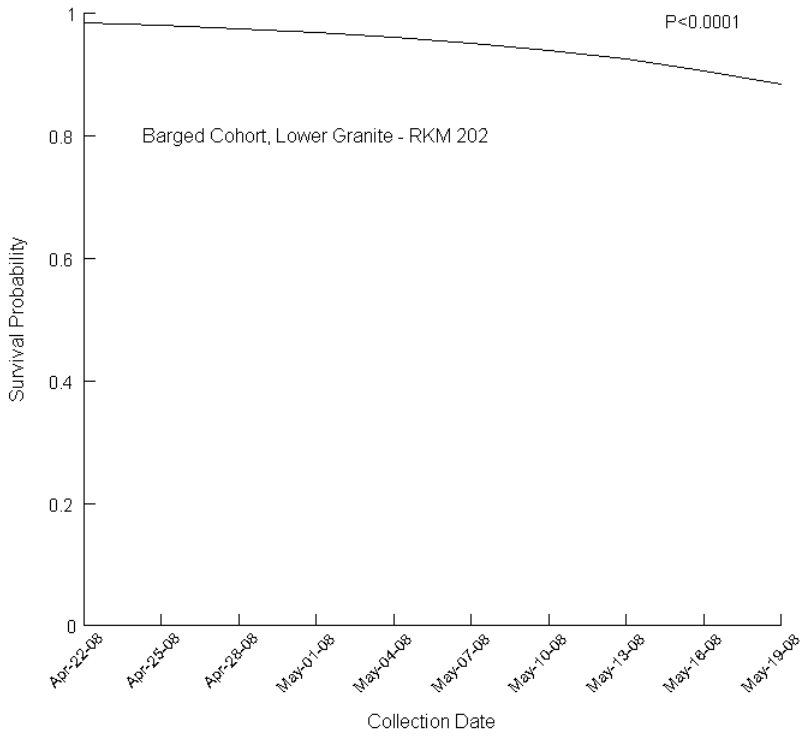

Figure 24. Fitted probability of surviving from Lower Granite Dam (RKM 695) to RKM 202 versus collection date at Lower Granite Dam for the acoustic-tagged Barged treatment group, with P-value of regression coefficient.

Survival of fish in the acoustic-tagged Barged treatment group through Reaches 2 and 3 (RKM 202 – RKM 8.3) was correlated with: all three measures of migration timing (collection date, tagging date, and release date, P<0.0001 for each); length (P=0.0220) and weight (P=0.0.0379) at tagging; holding duration at Lower Granite Dam (P=0.0103); and discharge at Bonneville Dam at the time of arrival at RKM 202 (P<0.0001; Table 26). Collection source was marginally correlated with survival from RKM 202 to RKM 8.3 (P=0.1510). Release date, length at tagging, holding duration, and discharge at Bonneville Dam were included in multivariate analyses. With discharge at Bonneville Dam accounted for, none of the other covariates had a significant effect on survival through Reaches 2 and 3 (P>0.25 for each). In general, fish that reached RKM 202 at times of higher discharge at Bonneville Dam had a higher probability of surviving from RKM 202 to RKM 8.3 (P<0.0001). The increase in survival through Reach 2 (RKM 202 – RKM 35.6) associated with discharge at Bonneville Dam was smaller than the analogous increase in survival through Reach 3 (RKM 35.6 – RKM 8.3), although survival was higher overall in Reach 2 (Figure 25).

Table 26. Results from single variate analyses for the acoustic-tagged Barged treatment group for survival from RKM 202 to RKM 8.3 (Reaches 2 and 3). Models used a unique Reach effect for each covariate. Collection source compared fish collected from the Sample room to fish from either the Raceway or the Sort-by-Code tank at Lower Granite Dam. The G-statistic is the Likelihood Ratio Test statistic, with degrees of freedom (DF). Sample size = 1084. The null model had AIC = 4041.30.

Category	Covariate	G-statistic	DF	P-value	AIC
Migration Timing	Collection Date	33.0204	2	<0.0001	4012.28
	Tagging Date	33.7093	2	<0.0001	4011.59
	Release Date	33.8339	2	<0.0001	4011.47
Size	Length at Tagging	7.6321	2	0.0220	4037.67
	Weight at Tagging	6.5466	2	0.0379	4038.76
Handling	Holding Duration	9.1447	2	0.0103	4036.16
	Collection Source	3.7811	2	0.1510	4041.52
Environmental Condition	Discharge at BON	46.7211	2	<0.0001	3998.58

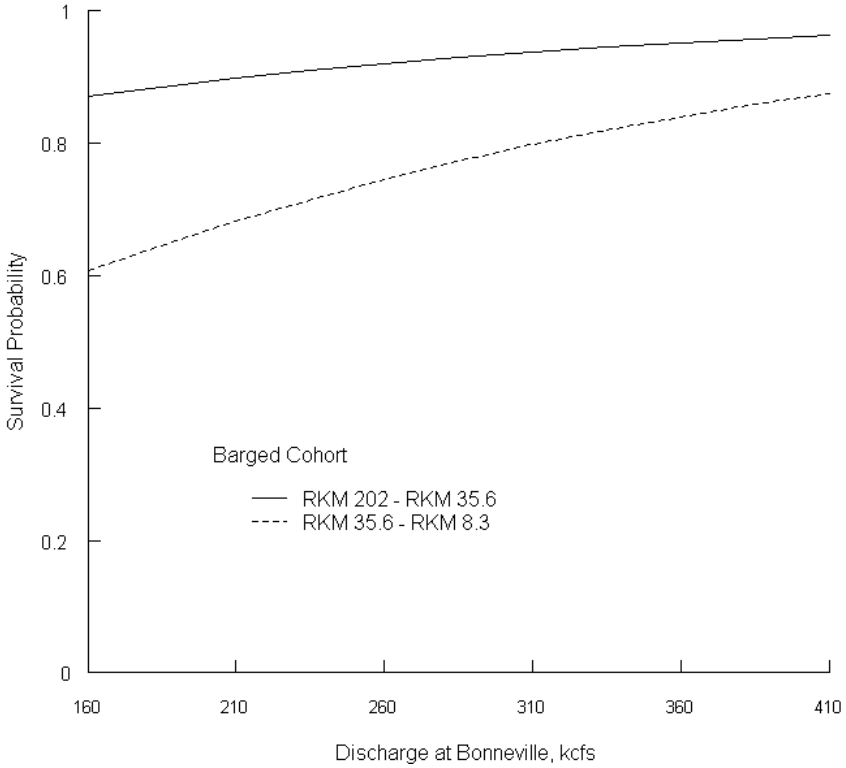

Figure 25. Fitted probability of surviving from RKM 202 to RKM 35.6 (Reach 2) and from RKM 35.6 to RKM 8.3 (Reach 3) versus discharged at Bonneville Dam measured at the time of arrival at RKM 202 for the acoustic-tagged Barged treatment group.

Travel Time Effects Analysis for Acoustic-Tagged Fish

In-River Treatment groups

Travel time of the acoustic-tagged In-River treatment group through Reach 1 (Lower Granite Dam to RKM 202) was correlated with all covariates considered (P<0.10 for each, Table 27), with collection date explaining the most variation in observed travel time (adjusted R_A^2=0.6830, Table 27). As expected from the high correlation among the migration timing covariates, both

tagging date and release explained nearly as much variation as collection date (adjusted R_A^2 =0.6779, Table 27). Discharge at Lower Granite Dam and length and weight at tagging each independently explained less than 40% of the variation in travel time from Lower Granite Dam to RKM 202. The effect of holding duration at Lower Granite Dam on travel time of the In-River treatment group was seen to be significant (P=0.0289), but holding duration nevertheless explained very little of the observed variation in travel time for in-river fish (adjusted R_A^2 =0.0071, Table 27).

The combined effects of the covariates were further analyzed using multiple regression. When collection date and length were accounted for, no other covariates had significant effects (at the 10% level) on travel time of in-river fish through Reach 1 (Table 28). Fish collected later tended to travel faster, as did fish that were longer at the time of tagging.

Table 27. Results of single-variate analyses for travel time from release at Lower Granite Dam to RKM 202 (Reach 1) for the acoustic-tagged In-River treatment group. The response variable was $y = \ln(\text{travel time})$. Covariates are ordered by P-value and adjusted R_A^2.

Covariate	Sample Size	P-value	R_A^2
Collection Date	531	<0.0001	0.6830
Tagging Date	531	<0.0001	0.6779
Release Date	531	<0.0001	0.6779
Discharge at Lower Granite Dam	531	<0.0001	0.3609
Length at Tagging	531	<0.0001	0.2345
Weight at Tagging	530	<0.0001	0.1851
Holding Duration	531	0.0289	0.0071

Table 28. Estimates of regression coefficients for travel time of the acoustic-tagged In-River treatment group from release at Lower Granite Dam to RKM 202 (Reach 1) against collection date and fork length at tagging. The response variable $y = \ln(\text{travel time})$. Multiple R^2=0.6991.

Coefficient	Estimate	S.E.	t	P-value
Intercept	3.3288	0.0457		
Collection Date	-0.0300	0.0011	-28.510	<0.0001
Length at Tagging	-0.0045	0.0009	-5.227	<0.0001

Travel times of the acoustic-tagged In-River treatment group through Reach 2 (RKM 202 – RKM 35.6) were related to all covariates except holding duration (P<0.10 for all covariates except holding duration; Table 29). While discharge at Bonneville Dam at the time of arrival at RKM 202 explained the most variation in travel time (adjusted R_A^2 =0.1573), relatively little of the variation in travel time through Reach 2 was explained by any of the covariates when

compared to travel time through Reach 1 (e.g., compare adjusted R_A^2 values from Table 27 and Table 29). Further analysis of the joint effects of these covariates found that discharge at Bonneville Dam and weight at tagging together explained the most variation in travel time (multiple R^2=0.1964), and that with these two covariates accounted for, no other covariates had significant effects at the 10% level. Fish that reached RKM 202 at times of higher discharge at Bonneville Dam traveled faster through Reach 2, as did fish with greater weight at tagging (Table 29).

Table 29. Results of single-variate analyses for travel time from RKM 202 to RKM 35.6 (Reach 2) for the acoustic-tagged In-River treatment group. The response variable was $y = \ln(\text{travel time})$. Covariates are ordered by P-value and adjusted R_A^2.

Covariate	Sample Size	P-value	R_A^2
Discharge at BON	184	<0.0001	0.1573
Release Date	184	<0.0001	0.1245
Tagging Date	184	<0.0001	0.1245
Collection Date	184	<0.0001	0.1235
Weight at Tagging	184	0.0008	0.0547
Length at Tagging	184	0.0053	0.0367
Holding Duration	184	0.7799	-0.0051

Table 30. Estimates of regression coefficients for travel time of the acoustic-tagged In-River treatment group from RKM 202 to RKM 35.6 (Reach 2) against discharge at Bonneville Dam and weight at tagging. The response variable $y = \ln(\text{travel time})$. Multiple R^2=0.1964.

Coefficient	Estimate	S.E.	t	P-value
Intercept	0.8162	0.0439		
Discharge at BON	-0.0010	0.0002	-5.545	<0.0001
Weight at Tagging	-0.0033	0.0012	-2.784	0.0059

Travel times of the acoustic-tagged In-River treatment group through Reach 3 (RKM 35.6 – RKM 8.3) were related to all covariates (P<0.10 for each; Table 31). As with Reach 2, no covariate explained much of the variation in observed travel time, with discharge at Bonneville Dam at the time of arrival at RKM 202 explaining the most (adjusted R_A^2=0.0841). Further analysis of the joint effects of these covariates found that discharge at Bonneville Dam and holding duration at Lower Granite Dam together accounted for the most variation in travel time (multiple R^2=0.1393). With these two covariates accounted for, no other covariates had significant effects at the 10% level. As with Reach 2, fish that reached RKM 202 at times of higher discharge at Bonneville Dam traveled faster through Reach 3 (Table 32). Fish that were

held longer at Lower Granite Dam before being released had longer travel times through Reach 3 (Table 32).

Table 31. Results of single-variate analyses for travel time from RKM 35.6 to RKM 8.3 (Reach 3) for the acoustic-tagged In-River treatment group. The response variable was $y = \ln(\text{travel time})$. Covariates are ordered by P-value and adjusted R_A^2.

Covariate	Sample Size	P-value	R_A^2
Discharge at BON	142	0.0003	0.0841
Collection Date	176	0.0031	0.0439
Release Date	176	0.0046	0.0398
Tagging Date	176	0.0046	0.0398
Holding Duration	176	0.0062	0.0367
Length at Tagging	176	0.0314	0.0208
Weight at Tagging	176	0.0445	0.0174

Table 32. Estimates of regression coefficients for travel time of the acoustic-tagged In-River treatment group from RKM 35.6 to RKM 8.3 (Reach 3) against discharge at Bonneville Dam and holding duration at Lower Granite Dam. The response variable $y = \ln(\text{travel time})$. Multiple R^2=0.1393.

Coefficient	Estimate	S.E.	t	P-value
Intercept	-0.2480	0.1244		
Discharge at BON	-0.0020	0.0005	-3.605	0.0004
Holding Duration	0.1428	0.0509	2.806	0.0057

Barged Treatment groups

Travel time of the acoustic-tagged Barged treatment group from release at Skamania Landing (RKM 227) to RKM 202 was regressed against the migration timing and size covariates used for the In-River treatment group, as well as collection source (Sample room or not), holding duration, and discharge at Bonneville Dam at the time of release from the barge at Skamania. Travel time through these 25 river kilometers was related to all covariates considered ($P<0.10$ for each; Table 33). Collection source explained the most variation in travel time (23%). The combined effects of these covariates were further analyzed using multiple regression. Together, collection source, discharge at Bonneville Dam, length at tagging, and holding duration at Lower Granite Dam explained the most variation in travel time (multiple R^2=0.2779), and no other covariates were significant at the 10% level. Barged fish that were collected from the Sample room at Lower Granite Dam traveled faster from Skamania to RKM 202 than fish that were collected from either the Raceway or the Sort-by-Code tank ($P<0.0001$; Table 34). Likewise, fish that were released from the barge at times of higher discharge at Bonneville Dam traveled faster upon release from the barge, while fish that were longer at tagging tended to travel more slowly. Unlike the In-River treatment group, barged fish held longer at Lower Granite Dam had

faster travel times upon release from the barge (P=0.0001; Table 34). However, the correlations among the covariates make direct interpretation of the regression coefficients risky.

Table 33. Results of single-variate analyses for travel time from release from the barge at Skamania Landing (RKM 227) to RKM 202 for the acoustic-tagged Barged treatment group. The response variable was $y = \ln(\text{travel time})$. Covariates are ordered by P-value and adjusted R_A^2.

Covariate	Sample Size	P-value	R_A^2
Collection Source	1087	<0.0001	0.2337
Release Date	1087	<0.0001	0.2059
Collection Date	1087	<0.0001	0.2059
Tagging Date	1087	<0.0001	0.1993
Discharge at BON	1087	<0.0001	0.1694
Holding Duration	1087	<0.0001	0.0876
Length at Tagging	1085	<0.0001	0.0176
Weight at Tagging	1085	0.0002	0.0119

Table 34. Estimates of regression coefficients for travel time of the acoustic-tagged Barged treatment group from RKM 227 to RKM 202 against collection source, discharge at Bonneville Dam, length at tagging, and holding duration at Lower Granite Dam. The response variable $y = \ln(\text{travel time})$. Multiple R^2=0.2779.

Coefficient	Estimate	S.E.	t	P-value
Intercept	-0.2228	0.0991		
Collection Source	-0.6282	0.0579	-10.847	<0.0001
Discharge at BON	-0.0015	0.0003	-5.610	<0.0001
Length at Tagging	0.0065	0.0014	4.647	<0.0001
Holding Duration	-0.1440	0.0371	-3.878	0.0001

For Reach 2 (RKM 202 – RKM 35.6), travel time of the acoustic-tagged Barged treatment group was significantly related to all covariates at the 10% level (Table 35). For this analysis, discharge at Bonneville Dam was measured at the time of arrival at RKM 202. Release date, collection date, and tagging date each explained nearly 60% of the variation in travel time, with release date explaining the most (adjusted R_A^2=0.5937; Table 35). Discharge at Bonneville Dam also explained over 50% of the variation in travel time, with collection source, holding duration, and length and weight at tagging accounting for less variation (Table 35). Further analysis of the joint effects of these covariates found that holding duration and collection source at Lower Granite Dam and discharge at Bonneville Dam together accounted for over 60% of the variation in travel time of barged fish through Reach 2 (multiple R^2=0.6190). With these covariates accounted for, no other covariate had a significant effect on travel time at the 10% level. Barged fish that were held longer or were collected from the Sample room at Lower Granite Dam, or

arrived at RKM 202 at times of higher discharge at Bonneville Dam, tended to travel faster through Reach 2 (Table 36).

Table 35. Results of single-variate analyses for travel time from RKM 202 to RKM 35.6 (Reach 2) for the acoustic-tagged Barged treatment group. The response variable was $y = \ln(\text{travel time})$. Covariates are ordered by P-value and adjusted R_A^2.

Covariate	Sample Size	P-value	R_A^2
Release Date	357	<0.0001	0.5937
Collection Date	357	<0.0001	0.5832
Tagging Date	357	<0.0001	0.5815
Discharge at BON	357	<0.0001	0.5030
Collection Source	357	<0.0001	0.4176
Holding Duration	357	<0.0001	0.1818
Length at Tagging	357	<0.0001	0.1301
Weight at Tagging	356	<0.0001	0.0790

Table 36. Estimates of regression coefficients for travel time of the acoustic-tagged Barged treatment group from RKM 202 to RKM 35.6 (Reach 2) against release date from the barge, holding duration, collection source, and discharge at Bonneville Dam. The response variable $y = \ln(\text{travel time})$. Multiple R^2=0.6190.

Coefficient	Estimate	S.E.	t	P-value
Intercept	2.2209	0.0681		
Holding Duration	-0.0716	0.0419	-1.709	0.0882
Collection Source	-0.4706	0.0513	-9.173	<0.0001
Discharge at BON	-0.0033	0.0003	-12.722	<0.0001

Travel time of the acoustic-tagged Barged treatment group through Reach 3 (RKM 35.6 – RKM 8.3) was significantly related to all covariates at the 10% level (Table 37), with discharge at Bonneville Dam (at the time of arrival at RKM 202) accounting for approximately 25% of the variation in travel time. Tagging date, release date, and collection date each accounted for approximately 24% of the variation in travel time, while collection source, length and weight at tagging, and holding duration at Lower Granite Dam each accounted for less than 10% of the variation (Table 37). With discharge at Bonneville Dam accounted for, no other covariate had a significant effect at the 10% level. Barged fish that reached RKM 202 at times of higher discharge tended to travel through Reach 3 faster than fish that experienced lower discharge (Table 38).

Table 37. Results of single-variate analyses for travel time from RKM 35.6 to RKM 8.3 (Reach 3) for the acoustic-tagged Barged treatment group. The response variable was $y = \ln(\text{travel time})$. Covariates are ordered by P-value and adjusted R_A^2.

Covariate	Sample Size	P-value	R_A^2
Discharge at BON	259	<0.0001	0.2524
Tagging Date	259	<0.0001	0.2388
Release Date	259	<0.0001	0.2383
Collection Date	259	<0.0001	0.2365
Collection Source	259	<0.0001	0.0836
Length at Tagging	259	0.0001	0.0579
Weight at Tagging	259	0.0016	0.0347
Holding Duration	259	0.0031	0.0298

Table 38. Estimates of regression coefficients for travel time of the acoustic-tagged Barged treatment group from RKM 35.6 to RKM 8.3 (Reach 3) against discharge at Bonneville Dam. The response variable $y = \ln(\text{travel time})$. R^2=0.0.2556.

Coefficient	Estimate	S.E.	t	P-value
Intercept	0.9768	0.0917		
Discharge at BON	-0.0050	0.0006	-8.887	<0.0001

Discussion

In-River treatment groups

For Reach 1 between Lower Granite Dam and the acoustic array at RKM 202, longer (and heavier) In-River acoustic-tagged fish held for the shortest amount of time prior to release below Lower Granite Dam had the highest probability of survival. Also, travel times decreased as fish length (and weight) increased. Travel time also decreased for those fish collected later in the season. For Reaches 2 and 3 between the Bonneville Dam tailrace at RKM 202 and the primary estuary array at RKM 8.3, the measured physical and environmental covariates did not explain differences in survival. The low number of in-river fish available for analysis in this region reduced the statistical power to detect any but the strongest survival effect. However, for the Reaches below Bonneville Dam (2 and 3), travel times decreased with increased discharge at Bonneville Dam as would be expected. In Reach 2, increased fish weight (and length) was correlated to shorter travel times, whereas in Reach 3 weight at tagging was no longer significantly related to travel time.

Barged treatment groups

For Reach 1, barged fish collected later in the season had a lower survival probability than those collected earlier. From the barge release-site at Skamania Landing to the end of Reach 2 at RKM 35.6 (a distance of 191.4 km), fish collected from the sample room at Lower Granite Dam

and those released from the barge during times of greatest discharge at Bonneville Dam had the shortest travel times. Interestingly, and in contrast to in-river fish, longer (and heavier) barged fish travelled slower from barge release at RKM 227 to RKM 202. For the last Reach between RKM 35.6 and RKM 8.3, only discharge from Bonneville Dam was significantly related to travel time, with increasing flow resulting in shorter travel times. For Reaches 2 and 3, only elevated discharge at Bonneville Dam explained the increased survival probability for this stretch of the river and estuary.

8.0 CONCLUSIONS

The research objectives of this study were to:

(1) Estimate survival and travel time for run-of-river yearling Chinook salmon during transit through the lower Columbia River and estuary;
(2) Produce information on fish health/pathology to help understand (i) the timing and trends of mortality in groups of fish with different outmigration histories as they migrate through the Columbia River and estuary and (ii) potential net pen effects that may influence the comparison of transported and in-river fish; and
(3) Integrate survival, travel time, and physical and environmental factors to estimate the extent and potential causes of differential mortality of transported and in-river run-of-river yearling Chinook salmon in the lower Columbia River and estuary.

JSATS acoustic tags and concomitant detection arrays in conjunction with estuary net pens were utilized to gain a better understanding of the extent and possible causes of differential mortality of transported and in-river run-of-river yearling Chinook salmon in the lower Columbia River and estuary (LRE). Run-of-river yearling Chinook salmon surgically implanted with acoustic tags were divided into four groups: a transported and in-river group actively migrating through the LRE to determine actual survival and travel time, and a transported and in-river group housed at two estuary net pen sites to assess various health related metrics. To identify possible causes for differences in mortality in the LRE, various metrics were obtained on the condition of fish during outmigration and holding; the environment in which they were transported, swam, or held; and the handling these fish were subjected to. This final chapter presents a synthesis of the main findings with respect to the three study objectives, and identifies the main limitations of this study.

Objective 1: Survival and Travel Time

In-river fish took from 10 ($\widehat{SE}$ = 0.14) to 19 ($\widehat{SE}$ = 0.20) days to transit Reach 1, which consisted of travel from Lower Granite Dam to just below Bonneville Dam (RKM 202). Overall survival of in-river fish within this Reach was 53% ($\widehat{SE}$ = 0.01). Barged fish transited the majority of Reach 1 in a barge hold over roughly a 36-hour period; the mean survival probability was 94.5% ($\widehat{SE}$ = 0.01).

The mean transit time of in-river fish in the subsequent two Reaches below Bonneville Dam to the mouth of the estuary at RKM 8.3, a distance of 194 RKM, was slightly over 2 days. Within the LRE, travel speeds were slowest for in-river fish in Reach 3, which encompasses the last 27 RKM prior to ocean entry. The mean probability of survival of in-river fish in the LRE was 86% ($\widehat{SE}$ = 0.02) for the entire outmigration season, with specific values during the early, middle, and late periods of the outmigration season of 83 ($\widehat{SE}$ = 0.03), 86 ($\widehat{SE}$ = 0.04), and 89% ($\widehat{SE}$ = 0.04), respectively. Travel time and survival of in-river fish through the LRE varied significantly over the outmigration season, with maximum travel time differences of 5 hours and survival differences of 6% between early, middle, and late periods.

For barged fish, travel times between release site and the mouth of the estuary (Reach 2 and 3) were longer than those of in-river fish, with mean values progressively decreasing from 8 ($\widehat{SE}$ = 0.19) to 3 ($\widehat{SE}$ = 0.03) days over the outmigration season. Within the LRE, travel speeds were slowest for barged fish in Reach 3. The mean probability of survival of barged fish in the LRE was 72% ($\widehat{SE}$ = 0.02) for the entire outmigration season, with specific values during the early, middle, and late periods of the outmigration season of 64 ($\widehat{SE}$ = 0.03), 70 ($\widehat{SE}$ = 0.03), and 83% ($\widehat{SE}$ = 0.02), respectively. Despite the lower survival probabilities of barged fish through Reaches 2 and 3, the overall survival from Lower Granite Dam to river kilometer 8.3 was higher (68%, $\widehat{SE}$ = 0.02) than for in-river fish (46%, $\widehat{SE}$ = 0.02).

The extent of differential mortality between barged and in-river fish was assessed in terms of the Barge to In-River Survival Ratio ($\widehat{BI}$ ratio), with values greater than 1 indicative of a higher survival of barged fish relative to in-river fish, and vice versa. Estimates of $\widehat{BI}$ for treatment groups pooled over the season were 1.78 ($\widehat{SE}$ = 0.05) for Reach 1 (spanning from Lower Granite Dam to just below Bonneville Dam (RKM 202) in which barged fish spent the majority of transit distance in a barge hold) and 0.84 ($\widehat{SE}$ = 0.03) for Reaches 2 and 3 (spanning from RKM 202 to 8.3 in which both treatment groups actively migrated), with an estimate of 1.50 ($\widehat{SE}$ = 0.07) for the entire study area (Lower Granite Dam to RKM 8.3). The pooled $\widehat{BI}$ ratio between RKM 202 and 8.3 (0.84) was statistically different than a ratio of 1. The non-pooled $\widehat{BI}$ ratios were 0.77 ($\widehat{SE}$ = 0.05), 0.81 ($\widehat{SE}$ = 0.05), and 0.94 ($\widehat{SE}$ = 0.05) for fish transiting RKM 202 to 8.3 during the early, middle, and late periods of the outmigration season, respectively. The non-pooled $\widehat{BI}$ ratio of 0.77 was statistically different than a ratio of 1. The values of the $\widehat{BI}$ ratios suggest differential mortality in the LRE, with a higher incidence of mortality in barged fish than in-river fish.

Survival and travel times established in this study for actively migrating in-river yearling Chinook salmon in the LRE were comparable to previously published values (McComas et al. 2007, 2008). Additionally, avian predation rates for the in-river study fish were also similar to previously published values (McComas et al. 2007, 2008). However, the avian predation rates of actively migrating barged fish in the LRE were almost double those observed for in-river fish. Differences in avian predation rates may result, in part, from stress associated with transportation and differences in smoltification status between barged and in-river fish. Stress associated with transportation has been shown to lead to saltwater avoidance and the preferential use of the upper layer of freshwater in juvenile spring Chinook salmon (Price et al. 2003, Congleton et al. 2000). Additionally, differences in smotification status of barged and in-river fish can vary considerably (Eder et al. 2009) and may lead to increased transit time of barged fish in the LRE. Both increased transit time and usage of freshwater closer to the surface in the LRE, may in turn elevate the risk of avian predation in the barged population.

Objective 2: Net Pen Mortality and Fish Health

The magnitude of cumulative net pen mortality was strongly impacted by the location of the net pens (net-pen-location-effect). All treatment groups held at both net pen locations, whether pooled or separated by passage or collection site, experienced significantly greater mortality during holding at Tongue Point relative to Sand Island, with the exception of the Reference

group. Mortality in the Reference group over 28 days of holding was extremely low (1.9%) and did not differ significantly between Sand Island and Tongue Point (0.5% peak difference). Very low mortality for reference fish at both net pen sites suggested that the net pens themselves were not significantly contributing to the observed incidence of net pen mortality in barged and in-river fish; however, it is important to note that the reference fish had different experiences prior to net pen holding than barged and in-river fish, including: laboratory rearing; no outmigration; and no implantation of either acoustic or PIT tags. The elevated incidence of mortality of both barged and in-river fish held at both net pen locations would suggest that fish arrive at Bonneville Dam in a compromised condition that decreases their probability of survival during extended freshwater transit time.

The Barged treatment group experienced significantly greater mortality in the beginning of the net pen holding period relative to fish with an in-river outmigration history (Bonneville Dam group) at Tongue Point, while during the last days of holding this trend reversed. Furthermore, mortality of barged fish was higher in the Early passage cohort than the Late cohort. Trends in net pen mortality, consistent with findings from previous studies (Arkoosh et al. 2006; Dietrich et al. 2007; Dietrich et al. 2008), would suggest that barged fish, as a population, are not as healthy as in-river fish entering the LRE, and that fish barged late in the season are healthier upon entry into the LRE than fish barged early in the season.

Mortality of barged fish actively migrating through the LRE was compared statistically to mortality of barged fish held in the net pens at Tongue Point. A similar comparison with in-river fish was not made: fish that actively migrated in-river through the FCRPS that were subsequently collected at Bonneville Dam were only held in net pens at Sand Island (saline-influenced site), and the majority of the LRE is freshwater (~86%). Survival of the Barged treatment group in the net pens at Tongue Point was approximately 10% higher than the survival of actively migrating barged fish between Skamania (RKM 227) and RKM 8.3. The 10% difference likely reflects, in part, piscivore and avian predation. Using reported values of piscivore and avian predation in the LRE with mortality observed in the estuary net pens, the overall mortality of barged fish actively migrating in the LRE can be subdivided as: 7-11.8% related to causes identified in morbid net pen fish which were largely associated with infectious diseases; 2.2-9.2% minimum related to avian predation; and 5% minimum related to piscivore predation.

The majority of mortalities during net pen holding were diagnosed with mycotic infections and ceratomyxosis. The Bonneville treatment group had a considerably higher prevalence of mycotic infections than In-River or barged fish. The prevalence of mycotic infections in morbid fish was higher at the freshwater net pen site (Tongue Point) than at the saline-influenced site (Sand Island). Morbid Bonneville and in-river fish (fish with an in-river outmigration history) had a significantly higher prevalence of ceratomyxosis than morbid barged fish, which suggests in-river outmigration increased the risk of contracting this parasite.

Severe metabolic lesions associated with infectious disease and other stressors were highly prevalent in morbid fish in the Barged treatment group. Typically, metabolic lesions are found in stressed, diseased, and/or anorexic fish. The extent to which these lesions were caused by or connect to mycotic infections or stressors such as collection, transport, and release is currently unknown and beyond the scope of this study. Regardless of the cause of metabolic lesions, the

prevalence of these lesions in morbid barged fish increased over the first 7 days of net pen holding; the prevalence rate then stabilized in morbid fish for the duration of net pen holding. The initial spike in prevalence of metabolic lesions in morbid fish may be due to the mortalities among severely anorexic or otherwise stressed fish arriving at the net pens after barge transport.

Population prevalence of infectious diseases contributing to mortality in the net pens was highest for mycotic infections (presumably Saprolegniaceae species) and ceratomyxosis. Mycotic infections and metabolic lesions were responsible for most of the mortalities in the Barged and Bonneville treatment groups, while a portion of the mortalities of the latter treatment was caused by ceratomyxosis. Absence of ceratomyxosis in barged fish may be explained by the temporally and spatially reduced exposure of these fish to the habitat which promotes the transmission of this disease as compared to the Bonneville fish. Bonneville fish may have arrived from different natal hatcheries and presumably spent more time outmigrating than did barged fish and hence had an elevated risk of contracting the disease. The analyses of mortalities among Bonneville fish over the whole course of holding indicates favorable conditions for contracting and spreading of ceratomyxosis.

Objective 3: Integration of Survival, Travel Time, and Physical and Environmental Factors

Both outmigration history and environmental factors were found to influence the survival and travel times of yearling spring Chinook salmon migrating through all study Reaches (Reaches 1, 2, and 3). In the In-River group, bigger fish had significantly shorter travel times and a higher survival probability than smaller fish between Lower Granite Dam and RKM 202 (Reach 1). Fish collected later tended to travel faster, but holding them longer (~3 days vs. 2 days) after tagging decreased their survival probability. From the tailrace of Bonneville Dam to the array at RKM 35.6 (Reach 2), increased fish weight (and length) was correlated to shorter travel times, whereas in Reach 3 (RKM 35.6 to 8.3) weight at tagging was no longer significantly related to travel time. For the combined Reaches below Bonneville Dam (Reaches 2 and 3), travel times decreased with increased discharge at Bonneville Dam. The survival of fish in the LRE with an in-river outmigration history did not seem to be connected to any specific condition in the LRE.

For barged fish, in the Reach downstream from Bonneville Dam to RKM 35.6 (Reach 2), fish that were held longer at Lower Granite Dam or arrived at times of higher discharge at Bonneville Dam tended to travel faster. In the last Reach (Reach 3; RKM 35.6 to 8.3), barged fish that were released at times of higher discharge at Bonneville Dam tended to travel faster than fish that experienced lower discharge. Discharge at Bonneville Dam was the main factor for increased survival probabilities of barged fish in the LRE.

Overall Implications

In conclusion, barging of yearling spring Chinook salmon enhances survival through the FCRPS. Larger fish actively migrating in the river have a better chance of survival than smaller fish. Travel times in the LRE are influenced by discharge at Bonneville Dam. However, barged fish move more slowly in the LRE and spend more time in freshwater than fish with an in-river outmigration history. The slower travel time of barged fish in the LRE may increase their risk of disease-induced mortality and predation. Slowest travel times coincide with the zone of salt

water incursion into the estuary, suggesting that barged fish may be physiologically or behaviorally less prepared to enter the ocean than in-river fish. The results of net pen holding experiments support the notion that increased freshwater transit time has a negative effect on both fish survival and health. Measured variables correlated with a higher survival probability included (a) large fish size, (b) short estuary residency, (c) high discharge at Bonneville Dam, and (d) late season migration.

9.0 ACKNOWLEDGEMENTS

Funding for this study was provided by the US Army Corps of Engineers, Walla Walla District. Project oversight was provided by Derek Fryer of the US Army Corps of Engineers Walla Walla, WA, office. Surgery training, tagging assistance at Lower Granite Dam, collection and analysis of JSATS data, and reporting assistance were provided by Geoffrey McMichael, Katherine Deters, and Jessica Carter of Battelle Pacific Northwest National Laboratory. Programming of the PIT-tag separation-by-code functions was provided by Dave Marvin, Pacific States Marine Fisheries Commission. Assistance in fish collection and facilities were provided, in part, by Kent Blevins (USACE), Mike Halter (USACE), Doug Marsh and Neil Paasch (NOAA), Fred Mensik and Sean Rapp (Smolt Monitoring Program), at Lower Granite Dam; Dean Ballinger (Pacific States Marine Fisheries Commission), Jonathan Rerecich and Timothy Darland (USACE), and Lyle Gilbreath (NOAA) at Bonneville Dam; and Greg Kovalchuk (PMFC) at John Day Dam. Tagging assistance at Bonneville Dam was kindly provided by Lynn McComas, Jason Everett and crew (NOAA). Pathogen prevalence analysis was completed by Ahna Van Gaest. Net pen set-up and fish care was provided by Jerry Pruiett, James Lathe, Ahna Van Gaest, Samantha Bund, Paul Peterson, Jessica Umberger, and Michael Haebler. Special thanks go to the Tug boat captains and crew for their assistance in transferring fish on and off barges.

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11.0 APPENDICES

APPENDIX A: ACRONYMS AND ABBREVIATIONS

AFEP (Anadromous Fish Evaluation Program)
AT (Acoustic Tag)
ATS (Advanced Telemetry Systems)
$\widehat{BI}$ (Barge to In-River survival ratio)
BKD (Bacterial Kidney Disease)
cDNA (complementary DNA)
rDNA (ribosomal DNA)
CTD (Conductivity, Temperature, and Depth – water quality multiprobe)
DF (Degrees of Freedom)
DNA (Deoxyribonucleic Acid)
DO (Dissolved Oxygen)
ELISA (Enzyme-Linked Immunosorbent Assay)
ESUs (Evolutionarily Significant Units)
ESI (East Sand Island)
FCRPS (Federal Columbia River Power System)
FDL (Fish Disease Laboratory)
IHNV (Infectious Hematopoietic Necrosis Virus)
IPNV (Infectious Pancreatic Necrosis Virus)
IR (In-River)
JFF (Juvenile Fish Facility)
JSATS (Juvenile Salmon Acoustic Telemetry System)
KCFS (Thousand Cubic Feet /Second)
LGR (Lower Granite Dam)
LRE (Lower River and Estuary)
NCSS (Number Cruncher Statistical Systems)
NOAA (National Oceanographic and Atmospheric Administration)
NRS (Newport Research Station)
ODFW (Department of Fish and Wildlife)
PCR (Polymerase Chain Reaction)
PIT (Passive Induced Transponder)
PSMFC (Pacific States Marine Fisheries Commission)
PST (Pacific Standard Time)
PTAGIS (Columbia Basin PIT Tag Information System)
RKM (River Kilometer)
RNA (Ribonucleic Acid)
SAR (Smolt-to-Adult Return)
SbyC (Sort-by-Code)
STS (Submerged Traveling Screens)
TAE (Tris Acetate)
USACE (United States Army Corps of Engineers)
VHSV (Viral Hemorrhagic Septicemia Virus)

APPENDIX B: DESCRIPTIONS OF HISTOPATHOLOGY DIAGNOSES

Infectious Conditions

Mycotic Dermatitis

The most common disease condition associated with mortality in these fish regardless of treatment group was a fungal infection of the integument or mycotic dermatitis and gill or mycotic branchitis, although both conditions were considered as mycotic dermatitis the purpose of analysis and to avoid confusion since the cutaneous and branchial mycotic infections have a similar etiopathogenesis. Specifically, affected fish presented with focal to multifocal, erosive to ulcerative lesions of the integument including the fins with a filamentous or rhizoid, white to tan, cotton-like appearance of the lesions or a brown to reddish-brown or green appearance dependent on the accumulation of intralesional debris. In contrast, the lesions had a gelatinous, viscous quality following removal of the affected fish from the water due to collapse of the fungal mycelia. Fish with branchial mycosis had a similar appearance of the branchial filamental tissue. Microscopic examination of cutaneous scrapings or sections of the integument and branchial arches primarily revealed broad, aseptate, nonpigmented, fungal hyphae of variable length and an approximate width of 7-30 mm. Cutaneous inflammation was generally mild or absent, except in occasional fish with a concurrent or secondary bacterial dermatitis. The histological features of the fungal infection were consistent with a typical water mold (class Oomycete) infection that is most commonly due to *Saprolegnia* sp.

Infections with *Saprolegnia* sp. are the most common cutaneous mycoses of freshwater fishes, whereas the most common isolates from fish are *Saprolegniaparasitica* and *S. diclina* (family Saprolegniaceae; Noga 1993), although the family Saprolegniaceae also includes other pathogenic oomycetes of fish such as *Achyla* sp. and *Aphanomyces* sp. (Roberts 2001). However, the taxonomic placement of the various oomycetes is not definitive. For example, the oomycetes have been referred to as pseudofungi (Cavalier-Smith 1987) and have been considered as fungal-like protists with similarities to the diatoms, brown algae (heterokonts) within the Stramenopiles, and xanophytes, rather than the filamentous fungi (Kamoun 2003). Regardless of the taxonomic placement and the specific etiological agent, the disease condition is generally referred to as saprolegniasis.

Briefly, the oomycetes are ubiquitous, saprophytic organisms that are generally considered secondary or opportunistic pathogens that rarely infect healthy fish. However, saprolegniasis has been reported in fish as a primary condition due to the apparent absence of predisposing factors (Tiffney 1939; Hoshima and Ookubo 1956; Hoshina et al. 1960). Regardless, fish with saprolegniasis are generally debilitated or stressed due to handling, transport, poor husbandry or water quality, a primary disease condition, or pre-existent lesions of the integument (Noga 1996; Roberts 2001). Mortality following infection can occur in less than 36 hours especially with branchial infection (Roberts 2001). For *Saprolegnia* sp., dispersal and infection is primarily achieved by the motile zoospores that possess recurved attachment hairs to facilitate attachment to the host. Some species may occur in brackish environments but salinity greater than 2.8% is generally restrictive (Testrake 1959; Willoughby 1994).

Various intrinsic and extrinsic factors may influence the development of saprolegniasis including temperature since clinical manifestations of the disease have been reported to occur more frequently at lower temperatures (Hoshima and Ookubo 1956; Hoshina et al. 1960; MacMillan 1985). However, the role of temperature may be more complicated since higher temperatures within the normal temperature range of the host generally results in a more robust immune response, whereas lower temperatures may suppress the immune response (Roberts 1975; Bly et al. 1993). Saprolegniasis has been reported in precocious juvenile salmonids, whereas immature (or non-precocious) salmonids maintained in identical conditions did not develop the disease condition (Willoughby 1969). This latter phenomenon may be due to the maturation and consequent influence of the endocrine system in precocious fish, since saprolegniasis has been reproduced in fish following the administration of a variety of hormones (Robertson et al 1963; Roth 1972). However, the effect of precocious maturation on the development of mycotic dermatitis in fish from this survey could not be definitively determined.

Therefore, the mycotic dermatitis in these fish was generally considered a mixed fungal infection that primarily involved *Saprolegnia* sp., but also included other fungal species, although a definitive identification of the various fungal species requires culture and isolation of the fungal organisms and/or ancillary molecular diagnostic techniques that will be developed and performed in future surveys. These results are also consistent with the results of a previous survey of fungal pathogens isolated from salmonids in the Columbia River that primarily involved *Saprolegnia* sp., but also included other fungal species (Mueller and Whisler 1994).

Systemic Mycosis

Internal or systemic fungal infections or systemic mycosis in these fish was generally considered a consequence or sequela of the mycotic dermatitis in these fish, although systemic infections without a concurrent external fungal infection cannot be dismissed. Systemic infections involved various organs with localization of fungal elements within the vasculature and colonization of the heart, liver, kidney, spleen, swim bladder, intestine and coelomic membranes. As with cutaneous fungal infections, a definitive identification of the fungal elements involved in systemic mycosis could not be definitively determined and also requires culture and isolation and/or ancillary molecular diagnostic techniques. In this context, although *Saprolegnia* sp. was assumed to contribute to the systemic mycotic infections in these fish, the characteristic features of *Saprolegnia* sp. as observed in histological sections of cutaneous lesions were absent or not obvious in the histological sections of internal organs from fish with systemic mycosis. Therefore, systemic mycosis was also considered to be a mixed fungal infection that involved various fungal species.

In this context, saprolegniasis is generally an external infection with only rare reports of systemic involvement. Examples of a systemic or internal saprolegniasis include an intestinal infection in fingerling brook trout (Agersborg 1933), coelomic saprolegniasis in salmon and trout fry (Roberts 2001), and a vascular infection with thrombosis in guppies (Nolard-Tintigner 1973). An intestinal infection in fingerling brook trout due to the related oomycete *Aphanomyces* sp. has also been reported (Shanor and Saslow 1944). Fungi not included in the class Oomycete that have also been reported to cause systemic infection or internal infections in fishes include *Aspergillus* sp., *Fusarium* sp., *Phialophora* sp., and *Exophiala* sp. among others (Roberts 2001). *Phomaherbarum* has also been isolated from systemic mycoses in salmonids including Chinook

salmon from the Pacific Northwest (Wood 1968; Ross et al. 1975). As previously mentioned, the findings from this survey were also consistent with the findings of Mueller and Whisler (1994) that reported mixed systemic fungal infections in salmonids from the Columbia River.

The variability in growth rates of *Saprolegnia* sp. isolates obtained from Columbia River salmonids (Mueller and Whisler 1994) may also at least partially explain the apparent variation in the prevalence of systemic mycosis among the various treatment groups in the present study. Specifically, infection with fungal variants that exhibit less rapid growth may result in confinement of the pathogen to the external tissues especially if temperature and a mature immune response of the host are also factors that influence the development of systemic or internal mycosis. Finally, the development of systemic mycosis in fish with or without a concurrent mycotic dermatitis may have been a terminal event in severely debilitated fish.

Bacterial Kidney Disease

In comparison to fish included in the 2007 study, Bacterial Kidney Disease (BKD) due to the bacterium *Renibacteriun salmoninarum* was a relatively rare histopathological finding that was corroborated by the investigation of pathogen prevalence. This may have been related to the absence of fish from Dworshak National Fish hatchery in the 2008 study.

R. salmoninarum is an obligate, intracellular pathogen that does not survive for prolonged periods in the environment (Evelyn 1993). Horizontal transmission can occur in freshwater and marine environments via cohabitation with infected fish, ingestion, or exposure to contaminated water. Localization of the bacterium within the ova also results in vertical transmission that may not prevented with surface disinfection of the ova (Evelyn et al. 1984; Evelyn et al. 1986a). All species of freshwater and marine salmonids are susceptible to infection (Evelyn 1993). The disease has a cosmopolitan distribution and is endemic to the Columbia River basin (Evelyn 1993). Adult and subadult fish greater than six months of age are generally most affected by the disease, although younger fish are also susceptible to infection and clinical disease. Clinical disease is generally associated with stress such as spawning and transfer of smolts to seawater (Fryer and Sanders 1981). Transfer of infected fish may result in clinical disease immediately following movement and relocation, although clinical disease generally occurs during the winter and spring following transfer (Evelyn et al. 1998). Manifestation of clinical disease may be related to changes in temperature (Fryer and Sanders 1981), especially in cultured salmonids where manifestation of the disease is most prevalent in spring during elevation of water temperatures with a subsequent increased rate of mortality in the early summer (Roberts 2001).

It should also be noted that Renibacterium infections further result in an immunosuppressive condition in affected fish that can predispose fish to additional infectious disease conditions or exacerbate extant disease conditions.

Infection often results in significant morbidity and mortality within an affected population. The granulomatous inflammation is generally discrete and well-encapsulated in species that are more resistant to infection and clinical disease, such as Atlantic salmon, whereas the inflammatory foci are less discrete in more susceptible species, such as Coho and Chinook salmon similar to the lesions in the Chinook salmon examined in this survey. Furthermore, a diffuse inflammation

without discrete, nodular lesions that is typical of chronic disease may occur in fish with acute disease following stress that was also consistent with the findings in the fish from this survey.

Control and prevention of BKD is difficult especially in wild fish due to the chronic, insidious nature of the disease. Avoidance of BKD is achieved by elimination of infected fish and the use of specific pathogen-free (SPF) broodstock (Elliott et al. 1989; Evelyn 1993). Ideally, female broodstock are administered intramuscular (IM) or intracoelomic (IC) injections of 10-20 mg/kg erythromycin at 9-57 days prior to spawning to reduce or eliminate infection and prevent vertical transmission (Evelyn et al. 1986b; Lee and Evelyn 1994). Best results have been obtained with injections at 12-20 (Armstrong et al. 1989) or 15-40 (Moffitt 1991) days prior to spawning. This strategy not only reduces or eliminates infection in the adult but also prevents infection of the ova that persists to the alevin stage of development. Regardless, ovarian fluid from female broodstock should also be tested using immunological or molecular techniques to confirm the pathogen-free status of broodstock. Regardless, surface disinfection of eggs using 100 ppm iodophore for 15 minutes should be a routine procedure in salmonid operations. Progeny should not be exposed to potential carriers and should not be raised in water that may be contaminated with the pathogen if possible.

Enteric Ceratomyxosis

Ceratomyxosis was a common finding in fish collected from the latter portion of May and June that was consistent with the influence of temperature on *Ceratomyxa* infection as discussed below. The lesions were characterized by a prominent cellularity of the mucosa and submucosa of the anterior intestine and pyloric caeca, whereas the enlarged and multinucleated cells were consistent with the prespore developmental stages of the myxosporean *Ceratomyxa shasta*. However, the rare occurrence of mature spores in histological sections was indicative of an early Ceratomyxa infection in these fish. Regardless, C. shasta is an endemic parasite of the Columbia River, although differences in susceptibility among salmonid species and among strains of the same species have been reported (Zinn et al. 1977; Bartholomew et al. 1989). Chinook salmon are among the most susceptible species, although endemic strains from the Columbia River are relatively resistant whereas non-native strains are more susceptible to infection (Hoffmaster et al. 1985; Bartholomew et al 1989).

Fish are readily infected following exposure to habitat that is endemic for the parasite (Johnson et al 1979). The infection can result in increased mortality within populations of hatchery and wild salmonids with mortality approaching 100% in juvenile fish (Sanders et al. 1970; Ratliff 1983). Initial lesions or localization of the parasite has been reported to primarily involve the posterior intestine, although the anterior intestine and pyloric caeca was the primary site of infection in the fish examined in this survey. In the fish from this survey, rare parasites were also found in the kidney, spleen and coelomic membranes that was consistent with previous reports of the tissue localization of the parasite.

Renal Myxosporidiosis

A common finding in these fish regardless of treatment group was the generally mild myxosporean infection of the renal tubules and renal glomeruli. Specifically, developmental stages of a myxosporean parasite were associated with the surfaces of the renal tubular epithelial cells and present within the lumens of the renal tubules and glomerular capillaries. The infection was not considered significant relative to the health status of the affected fish, although the

infection was often associated with a membranous glomerulopathy in these fish (see below). The myxosporean parasites could not be further identified by routine histological examination which requires additional techniques for a more definitive identification. However, the parasites may have represented a *Sphaerospora* sp. (Ferguson 1989), although other renal myxosporeans of salmonids that have been associated with renal lesions have previously been described. For example, *Myxidium minteri* has been associated with renal tubular degeneration in salmonids and *Chloromyxum majori* has been associated with glomerulosclerosis in rainbow trout and Chinook salmon (Yasutake and Wood 1957). The myxosprean *Parvicapsula sp*. has also been associated with nephritis and renal tubular necrosis in Pacific salmon (Hoffman 1981; Johnstone 1985). Definitive identification of the myxosporen parasite in these fish will be attempted in future investigations.

Membranous Glomerulopathy

A common and remarkable finding in these Chinook salmon was the renal lesion referred to as membranous glomerulopathy. Specifically, the lesions were characterized by a segmental to diffuse, mild to severe, eosinophilic, often hyalinized thickening of the glomerular capillaries. The lesion was similar to membranous glomerulonephritis in higher vertebrates including mammals, although the more general diagnosis of a glomerulopathy was used for these fish since it could not be determined if the pathogenesis of the lesion was a consequence of an inflammatory process. The lesion in higher vertebrates is generally the result of an immune-mediated process that results in the subendothelial deposition of antigen, antibody or antigen-antibody complexes of the glomerular capillaries. Therefore, the condition is a consequence of an inflammatory process or a host immune response to an antigenic stimulus with the subsequent production of antibodies. Since the lesion is a result of an inflammatory process or an immune-mediated reaction in higher vertebrates, it was included within this discussion of infectious diseases for this report.

Electron-dense subendothelial deposits have previously been demonstrated in fish with a membranous glomerulopathy (Ferguson 1989), although the composition of these deposits have not been determined and the etiopathogenesis of the glomerulopathies in fishes requires further investigation. However, Ferguson (2006) further states that similar lesions in rainbow trout with a *Renibacterium salmoninarum* infection (or BKD) may be the result of an inmmune-complex mediated process. In this context, the glomerular lesion was present in these fish with and without the presence of definitive microscopic lesions that were consistent with BKD. In addition and as previously mentioned, the presence of renal myxosporeans in these fish was an interesting finding, although the presence of myxosporean parasites does not infer that the parasites were the etiological agents of the glomerular lesions in these fish. Regardless, membranous glomerulopathy can result in the loss of normal glomerular filtration and the consequent loss of the blood constituents but especially protein in the urine, but may also affect water balance and osmoregulation of the organism. Regardless of the etiopathogenesis of the lesions in these Chinook salmon, the lesions were considered significant lesions that can affect the health status of the affected fish.

Miscellaneous Infections

Various additional infectious conditions were found in these Chinook salmon, but were considered anecdotal findings that did not result in a disease condition in the affected fish, or

were not important findings relative to the health of the population. These included a generally mild trematode parasite infection within the intestinal tract that is not an unexpected finding in wild fishes and occasional encapsulated trematodes within the heart, kidney, liver and gill; branchial parasitic infections in occasional fish that included rare infections with protozoan and monogenean parasites; and occasional bacterial infections of the gill, integument and heart. In this context, the absence of significant protozoan and monogenean parasitic infections of the gill was an unexpected finding, since branchial parasitic infections are common disease conditions in fishes, but especially fishes that are stressed.

In contrast, there was no definitive histological evidence of various common disease conditions of salmonids in these fish including disease conditions due to the bacterial agents *Flavobacterium columnare*, *Flavobacterium psychrophilum*, *Flavobacterium branchiophilum*, *Aeromonas salmonicida*, and *Yersinia ruckeri*; the microsporidian agent *Nucleospora salmonis*; and the viral agents infectious hematopoietic necrosis virus (IHNV), viral hemorrhagic septicemia virus (VHSV), infectious pancreatic necrosis virus (IPNV) or infectious salmon anemia virus (ISAV). Finally, there was no histological evidence of an intracoelomic inflammatory response to the placement of PIT-tags in any of these fish.

Non-Infectious Conditions

Two related but nonspecific lesions of variable severity among these fish that are included for completeness were atrophic steatopathy and lipid hepatopathy. Atrophic steatopathy is characterized by the condensation and loss -or atrophy- of the coelomic adipose tissue, whereas lipid hepatopathy refers to the intracytoplasmic localization of lipid vacuoles within the hepatocytes. The latter lesion is generally a consequence of the catabolism of the extrahepatic adipose tissue that is subsequently transported to the liver for utilization as an energy source. The lesions are nonspecific lesions that can occur with stress or any moribund condition including an infectious disease condition(s) that results in an increased energy demand that is exceeded by the normal intake of feed. The lesions may also be a manifestation of a terminal event in extremely moribund or anorexic fish that are unable to feed. Essentially, the adipose tissue is catabolized and transported to the liver in an attempt to meet this increased energy requirement. It should be noted that most teleost fishes including salmonids do not normally store lipid in the liver. For the purposes of this report, fish with atrophic steatopathy and/or lipid hepatopathy and a concurrent infectious disease condition were considered separately from fish with atrophic steatopathy and/or lipid hepatopathy without definitive histological evidence of a concurrent infectious disease condition.

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