#### AN ABSTRACT OF THE THESIS OF

<u>Jeremy D. Romer</u> for the degree of <u>Master of Science</u> in <u>Fisheries Science</u> presented on <u>July 26, 2010.</u>

Title: <u>Survival and Behavior of Juvenile Steelhead Trout (Oncorhynchus mykiss) in</u>
Two Small Estuaries in Oregon

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#### Carl B. Schreck

Steelhead (*Oncorhynchus mykiss*) are at risk throughout Oregon including five populations that are federally listed as threatened or endangered. Very little information on behavior and survival is available for the final phase of salmonid smolt migration between upriver smolt traps and the ocean. In this study I investigated whether survival in this area varies on a temporal scale within years, a spatial scale between zones within the estuary, between years, and between basins within the same distinct population segment. I determined behavior by comparing run timing, migration rate, and estuarine residence times between basins to assess their relationship with survival. Gill ATP-ase levels were used to determine degree of smoltification, and parasites in each basin were identified and enumerated to assess whether parasite load affects migration timing, life history strategy or age of smoltification. Results showed that 1) estimates for survival to the ocean were typically only 40-50% for wild steelhead smolts tagged at upstream smolt traps, 2) probability of survival was lower in the lower estuary near the mouth, 3) survival

estimates for smolts are highly variable between years, 4) wild steelhead smolts spent a median time of 0.72 and 0.83 days in the estuary in the Nehalem and Alsea basin, respectively, in 2009 5) gill ATP-ase level is elevated during the peak and late portions of the run, and is a poor indicator for estuarine survival when assessed in fish captured at upstream traps, and 6) parasite assemblage, density and smolt life history strategies can vary markedly between basins within the same management area. This research provides a baseline by which we can measure change in estuarine survival and behavior over time, information that could be useful for estuarine restoration and reiterates the importance of the lower river and estuary environment for smolt survival.

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# Survival and Behavior of Juvenile Steelhead Trout (*Oncorhynchus mykiss*) in Two Small Estuaries in Oregon

by Jeremy D. Romer

#### A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

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Master of Science thesis of Jeremy D. Romer presented on July 26, 2010.
APPROVED:
Major Professor, representing Fisheries Science
Head of the Department of Fisheries and Wildlife
Dean of the Graduate School
I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.
Jeremy D. Romer, Author

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#### CONTRIBUTION OF AUTHORS

Carl Schreck and Shaun Clements contributed to development of experimental design, data analysis and editing of all chapters. David Noakes contributed to development of experimental design for chapter 2, and editing of all chapters. Camille Leblanc contributed to experimental design for chapter 2, ran the smolt trap, acoustic tagged smolts and collected trapping data in the Alsea basin. Michael Kent and Jayde Ferguson contributed to parasite portion of chapter 2 including analysis, general methods, identification, experimental design, and editing of all chapters.

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# SURVIVAL AND BEHAVIOR OF JUVENILE STEELHEAD TROUT (ONCORHYNCHUS MYKISS) IN TWO SMALL ESTUARIES IN OREGON

**CHAPTER 1: GENERAL INTRODUCTION** 

Five populations of steelhead (*Oncorhynchus mykiss*) are currently listed as endangered (1) or threatened (4) in Oregon by NOAA Fisheries (http://www.nwr.noaa.gov/ESA-Salmon-Listings). In addition, Oregon coastal steelhead are listed as a species of concern. The Oregon coastal steelhead distinct population segment (DPS) includes the populations in the Nehalem and Alsea basins. Native steelhead in both basins are also included in the Oregon Department of Fish and Wildlife vulnerable species list and regulations are currently in place to protect native winter run steelhead from harvest. Recreational anglers, commercial fishers, and fishery managers consider salmonids a precious commodity, with special efforts directed toward restoration and conservation of native stocks (Waples 1991; Oregon Native Fish Conservation Policy 2002).

Oncorhynchus mykiss have one of the most diverse ranges of life history strategies of any salmonid species (Barnhart 1986). To date, more than 30 different strategies have been documented (Thorpe 1998). It is possible that this life history diversity is paramount to recovery from current low population numbers. Life history diversity acts as a buffer from environmental stochasticity and anthropomorphic changes that have become commonplace throughout the range of *O. mykiss*. Steelhead display an ability to persist, and maintain anadromous migrations far inland that are rivaled by few other salmonids (Busby et al. 1996). Continually changing environmental variables (e.g. hydrology, temperature, land use, ocean conditions, and climate change) realized at different spatial scales continue to conserve *O. mykiss* life history plasticity and resilience. Examples of this diversity include adults that are

iteroperous while others exhibit semelparity, adults that are anadromous producing progeny that remain resident and vice-versa, and fish that appear to be resident that may remain in fresh water for up to seven years before migrating to the ocean (Lichatowich 1999; Peven 1994).

Diversity and variation are present in every phase of the *O.mykiss* life cycle and the process of smoltification is no exception. There is a large amount of variation in behavior, physical condition and survival expressed on both spatial and temporal scales during emigration (Stefansson et al. 2008). How existing variation in physical and behavioral smolt attributes affect survival, and how trends in survival between basins within the same management area relate to one another over time is not well understood.

Smoltification is a transformation triggered by environmental cues including photoperiod, water temperature, and flow that causes anadromous juvenile salmonids to begin their journey downstream toward the ocean. During smoltification, juveniles undergo changes in morphology, physiology, and behavior that prepare them for the upcoming marine portion of their life history. The transition from freshwater to marine environments is a crucial phase for salmonids exhibiting anadramous life history strategies for a variety of reasons. Smolts are energy deficient during their downstream migration (Stefansson et al. 2008) and in times of transition, environmental change, or increased stress there is a reduction in individual performance capacity (Schreck 1981; Schreck and Li 1991). These fish are more vulnerable to environmental hazards, particularly predation, during this developmental phase (Schreck et al. 2006). Smolts

experience changes in salinity, flow (tides), and temperature as they enter the estuary. They may also encounter unfamiliar predators and food items than those found in natal waters (Thorpe 1994). Combinations of the aforementioned factors create one of the most life-threatening events for anadromous salmonids (Levings 1994).

Very little is known regarding survival and behavior of salmonid smolts between the last count in the lower-river and their entry into the ocean, and little is understood about annual variation and interbasin variation. Chapter 2 of this thesis addresses these issues, and provides a baseline for further investigation. I investigated whether survival between smolt traps and the ocean varies on a temporal scale within years and between years, or on a spatial scale between zones within the estuary, and between basins within the same distinct population segment. I compared run timing, migration rate, and estuarine residence times between basins to assess their relationship with survival. I measured gill ATP-ase levels to determine the relative degree of smoltification. Furthermore, I identified and enumerated parasites in each basin to assess whether parasite load affects migration timing, life history strategy, or smoltification.

This thesis is presented in manuscript form. Chapter 2 investigates variation in survival and behavior on an annual and interbasin basis in the lower-river and estuary. Chapter 3 provides a general discussion and conclusion.

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# CHAPTER 2: SURVIVAL AND BEHAVIOR OF JUVENILE STEELHEAD TROUT (ONCORHYNCHUS MYKISS) IN TWO SMALL ESTUARIES IN OREGON

Jeremy D. Romer, Camille A. Leblanc, Shaun Clements, Jayde Ferguson, Michael Kent, David Noakes, and Carl B. Schreck

#### Abstract

A number of steelhead (Oncorhynchus mykiss) populations are at risk throughout Oregon, including five populations that are federally listed as threatened or endangered. However, little is known about behavior and survival of these fish as they transition from freshwater to seawater. We investigated whether estuarine survival varies on a temporal scale within or between years, on a spatial scale between zones within the estuary, and between basins within the same distinct population segment. We implanted acoustic transmitters on 69 wild steelhead smolts in the Nehalem basin and 70 in the Alsea basin and compared run timing, migration rate, and estuarine residence times between basins. We also measured gill ATP-ase levels and parasite loads in each basin to evaluate the relationship between these indices and migration timing, life history strategy or age of smoltification. In general, only 40-50% of the wild steelhead smolts tagged at upstream smolt traps were detected entering the ocean, although survival was highly variable among years. The majority of "mortality" occurred in the lower estuary near the mouth. Wild steelhead smolts spent a median of 0.72 and 0.83 d in the estuary in the Nehalem and Alsea basin, respectively, in 2009. This research provides a baseline by which we can monitor temporal changes in the survival and behavior of juvenile salmon in the estuary. Such information is vital for monitoring the impact of estuarine restoration and reiterates the importance of the lower river and estuary environment for smolt survival.

#### Introduction

In Oregon, five populations of steelhead (*Oncorhynchus mykiss*) are currently listed as endangered (1) or threatened (4) by NOAA Fisheries (http://www.nwr.noaa.gov/ESA-Salmon-Listings). In addition, Oregon coastal steelhead are listed as a species of concern. The Oregon coastal steelhead distinct population segment (DPS) includes the populations in the Nehalem and Alsea basins. Native steelhead in both basins are also included in the Oregon Department of Fish and Wildlife vulnerable species list and regulations are currently in place to protect native winter run steelhead from harvest.

Very little is known regarding survival and behavior of salmonid smolts between the last count in the lower-river and their entry into the ocean, and little is understood about annual variation and interbasin variation. There is a large variation in behavior, physical condition and survival expressed on both spatial and temporal scales during emigration (Stefansson et al. 2008). How existing variation in physical and behavioral smolt attributes affects survival, and how trends in survival between basins within the same management area relate to one another over time is not well understood. The purpose of this study was to address these issues, and provide a baseline for further investigation.

Previous studies completed in the Nehalem (Schreck et al. 2002; Clements and Schreck 2003) and Alsea basins (Johnson et al. 2010) suggest that estuarine mortality is high, and highly variable between years. Similar datasets collected from two basins within the distinct population presented a rare opportunity for comparison of survival

data from multiple years, and between basins within the same distinct population segment.

Specific objectives of this study were:

- 1) Compare survival estimates from the fish tagged in the Nehalem (2001, 2002, 2009) and Alsea river (2007, 2009) to establish the survival variability on a temporal scale within years, a spatial scale between zones within the estuary, between years, and between basins. We used acoustic telemetry to help determine survival to various downstream locations from where tagged fish were released (e.g., screw trap). Acoustic telemetry is a tool that has already been used successfully to identify estuarine mortality of smolts in many places throughout the world, including the Pacific Northwest (Stahl et al. 2000; Schreck et al. 2002; Clements et al. 2003; Clemens et al. 2009; Johnson et al. 2010; Moore et al. 2010), Canada (Voegeli and Lacroix 1999; Melnychuk et al. 2007) and Europe (Thorstad et al. 2002).
- 2) Document differences in run timing, migration rate, estuary residence time and age of smolting between basins, and between smolts migrating early and those migrating at the peak of the run. Smolt behavior and physical condition are two key factors contributing to their survival (or mortality) as they emigrate to the ocean (Mesa 1994; Olla et al. 1995; Schreck and Li 1991). Smolt size and basin of origin were addressesed as survival components in 2009 specifically because that was our only year data was collected in both basins simultaneously. Gill ATP-ase levels for migrants collected at smolt traps were analyzed to create an index by which we could

determine degree of smoltification over a temporal scale and analyze whether ATP-ase level is a relaible indicator for survival in the estuary.

3) Investigate additional factors that help to explain variation in smolt behavior and survival. Parasites and disease (Dobson 1988; Barber et al. 2000; Lafferty and Morris 1996; Schreck et al. 2006) could cause individual fish to express compromised physiological or behavioral differences. These are also factors that could help describe the wide range of variability in survival and behavior exhibited by smolts. We euthanized a subsample of smolts collected from smolt traps during the run, and fish of smolt size (≥120 mm) that remained in the river well after the run had diminished by hook and line sampling. We wanted to determine whether or not fish that were believed to exhibit partial outmigration toward the end of the run as documented by Clements and Schreck (2003), are of a distinct age class. We were also interested in determining whether or not older fish in the population have higher parasite loads due to accumulation throughout their residence in freshwater. In addition, we identified the parasite community assemblage in steelhead smolts from both basins, and assessed whether parasite load affected migration timing, life history strategy or ATP-ase level.

#### Methods

Study Sites

Estuary designation in both basins was based on channel morphology.

Receiver arrays were placed just upstream of where the channel becomes less constrained as fish move downstream and into the estuary to establish entry time to the

estuary. The Nehalem River is approximately 192 km in length, draining a watershed of approximately 2,210 km<sup>2</sup> (http://www.coastalatlas.net/index.php). The North Fork Nehalem represents 251 km<sup>2</sup> of the total watershed, and the smolt trap is located at river kilometer (rkm) 33. We defined the estuary as the area below the confluence of the North and South Forks of the Nehalem River. Subtracting the area of the islands in the estuary, the area that is accessible to fish at an average high tide is 7.6 km<sup>2</sup>.

The Alsea River, beginning at the confluence of the North and South Forks, is 78 km in length and drains a watershed area of 764 km<sup>2</sup> (http://www.coastalatlas.net/index.php). The smolt trap in Crooked Creek used in 2007 was located 83 km upstream of the mouth of the estuary, and in 2009 the trap in Fall Creek was 55 km from the mouth. The estuary is defined as the area below the Highway 34 bridge at Taylors Landing (Figure 2.1). Subtracting the area of the islands in the estuary, the area that is accessible to fish at an average high tide is 8.6 km<sup>2</sup>.

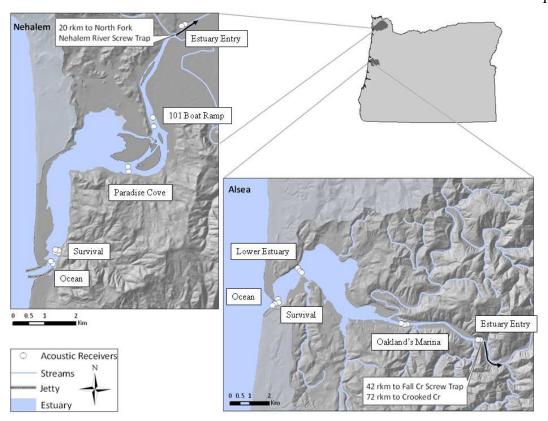


Figure 2.1. Map of receiver array locations in the Nehalem and Alsea estuaries. Upper right corner represents the outline of Oregon, located in the Northwest portion United States. Shaded areas highlight the two study basins. Enlarged sections illustrate the Nehalem and Alsea Estuaries and receiver array placement within each estuary. Receivers are represented by circles.

We apportioned the lower-river and estuary into distinct zones to determine where most mortality occurs on a spatially explicit level, and determined average residence time of steelhead smolts in Nehalem and Alsea River estuaries. A two basin study design enabled investigation of the possibility that mortality varies not only in a river system along a temporal scale but also between populations within the same DPS. Prior data from the North Fork Nehalem (Schreck et al. 2002; Clements and Schreck 2003) and Alsea rivers (Johnson et al. 2010) was utilized to provide three and

two years, respectively, of yearly spatial and temporal variation data that is critical in such studies (Schreck et al. 2002; Clements and Schreck 2003; Schreck et al. 2006).

#### Capture and Tagging

We collected wild steelhead smolts using 1.5 m diameter rotary screw traps, the standard method used to sample smolts in the Pacific Northwest. The traps were checked in the morning and wild steelhead smolts were transferred to separate holding containers. We tagged fish that were  $\geq 125$  mm fork length or > 20.0 g to ensure tag/body weight ratio did not exceed 8% consistent with recommendations by other researchers (Brown et al. 1999, La Croix et al. 2004, Chittenden et al. 2008). The length of fish that were tagged in 2009 is representative of nearly the entire size range of migrants that were captured and defined by ODFW as smolts (>120mm) (Figure 2.2). All the fish tagged in the North Fork Nehalem had a tag to body weight ratio of 5% or less. In the Alsea, 20% of the fish tagged had a tag to body weight ratio of 5-8% range, the remaining tagged fish had a tag to body weight ratio less than 5%. In 2007 Johnson et al. (2010) tagged wild steelhead smolts > 140 mm in Crooked Creek in the Alsea basin. All wild steelhead smolts from the North Fork Nehalem were tagged in the same location for all three years (2001, 2002, 2009), using the same methods as those used in 2009. Scales were collected from all O. mykiss captured in the trap, regardless of size.

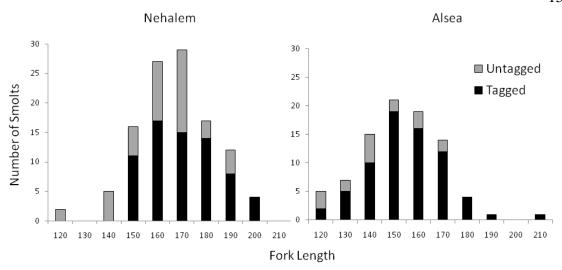


Figure 2.2. Bar graph showing number of steelhead smolts captured and available for tagging in the Nehalem and Alsea basins during 2009 field season. Smolts are apportioned to 10 mm bins by fork length.

To evaluate the temporal variability in survival throughout the outmigration, we tagged fish during two time periods (early and peak) in each river system (Figure 2.3). The groups were assigned to steelhead smolts according to when they were captured in screw traps. "Early" and "peak" groups are defined as fish tagged during the first portion of the run prior to the peak, and those tagged at the peak respectively. Peak is defined in this study as an approximately two week period when capture estimates are highest.

Within each period, we tagged fish on at least 3 consecutive days. On any given day we attempted to tag at least 10 smolts, though this was not always possible. All tags were checked for acoustic transmission before implantation. In the Nehalem, we tagged 35 smolts during the "early" period (April 7-9<sup>th</sup>) and 34 during the "peak" period (April 20-22<sup>nd</sup>). At Fall Creek, in the Alsea basin, we tagged 70 steelhead

smolts, 21 from the "early" group between April 9-17<sup>th</sup> and 49 from the "peak" group between April 18-27<sup>th</sup>.

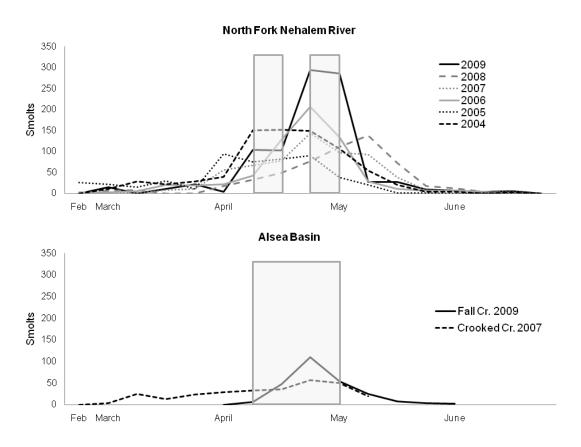


Figure 2.3. Line graph illustrating steelhead smolt outmigration timing from the North Fork Nehalem River 2004-2008 (ODFW Life Cyle Monitoring Project 2008), from Crooked Creek 2007 (Johnson et al. 2010), and Fall Creek 2009. Crooked Creek and Fall Creek are tributaries of the Alsea River. Gray boxes indicate weeks when tagging took place during the 2009 field season.

Prior to tagging each fish was anesthetized using tricaine methane sulfonate (MS-222) (50 mg/L MS-222 buffered with 125 mg/L NaHCO<sub>3</sub>). The fish was then placed ventral side up in a wet foam wedge. During the implantation procedure we perfused dilute anesthetic (50%) over the gills using a squeeze bottle. A 1-1.5 cm incision was made into the ventral body wall just anterior of the pelvic girdle using a

micro-scalpel blade with maximum penetration depth of 5.0 mm to avoid internal damage. The hydroacoustic transmitter was then inserted, and the incision was closed using 2 sutures (simple interrupted), tied with 2 square knots. We used Ethicon braided 4-0 VICRYL\*Plus Antibacterial coated nylon sutures and a 17 mm tapered needle. Following implantation, the fish were transferred to a recovery enclosure and placed in a slow moving pool downstream of the trap. The recovery enclosure consisted of a 2.5 cm tubular PVC frame encased with white fabric mesh (2.5 m long x 1.2 m wide x 1 m deep). We observed the behavior of the fish in the enclosure to ensure they recovered normal body posture, gill ventilation, and responsiveness to external stimuli. The fish were liberated at dusk of the same day to minimize predation and reduce holding stress. Survival during this period was 100%. Previous research suggests that mortality from this procedure is relatively low. Welch et al (2007) reported between 6-10% mortality after 12 weeks for salmonids of a similar size following implantation with acoustic transmitters. Research conducted by Schreck et al. (2002) and Clements and Schreck (2003) suggests that 0 - 5 % of fish perish within 1 month following implantation in the laboratory environment.

#### Non-migrant sampling

We used hook and line sampling to capture "non-migrants" of the same size range as the tagged smolts (>120mm). We collected 25 non-migrants (10 Alsea, 15 Nehalem) between May 19<sup>th</sup> and June 11<sup>th</sup> after the majority of steelhead smolts had migrated downstream of the smolt trap. These fish were processed in identical fashion

to the individuals captured in the smolt traps and used for gill ATP-ase and parasite sampling (not tagged).

#### Acoustic Tags

We used V7 acoustic tags (AMIRIX Systems Inc., VEMCO Division-Halifax, Nova Scotia) Each transmitter was programmed with a random pulse rate of 15-30 s to minimize the risk of not detecting fast moving fish that passed within range of a receiver. This pulse rate was based on analysis of data from Schreck et al. (2002) and Clements (2003). V7-2L tags are 20mm x 7mm weighing 1.6 g in air or 0.75g in water. This pulse rate allows a battery life of at least 41 days, which was sufficient time to encompass the entire outmigration season.

#### Acoustic Receivers and Deployment

We used VR2 acoustic receivers (VEMCO, Halifax, Nova Scotia, Canada). Each receiver was attached to 1.3 cm poly rope with the hydrophone facing downward in the water column and anchored to the substrate with a 18.14 kg kedge anchor (see Clements et al. 2005 for details).

Receivers were placed in arrays consistent with methods developed by Clements and Schreck (2003) and Clements et al. (2005). This method requires placement of receivers such that an uninterrupted vertical and horizontal zone is created where a passing acoustic tagged fish will be detected. Arrays were placed in the lower river between the screw trap and estuary, in the upper estuary, mid-estuary,

and in as close proximity to the ocean as was practical, allowing for interference created by wave action and tidal currents, and personnel safety.

Arrays were positioned prior to tagging and placement in both the Alsea and Nehalem basins was consistent among all years of sampling to simplify comparison. The only exception is that in 2009 the furthest downstream receiver array in each basin was not placed as far out in the ocean as in prior years (Figure 2.1).

In the Nehalem system, no receivers were deployed in the lower river between the trap and the estuary. Two receivers were placed to mark estuary entry; 8 were placed in the estuary and 2 to mark smolt entry into the ocean. One receiver was also placed approximately 1km upstream of the confluence with the South Fork Nehalem River to ensure that no fish were traveling back upstream at the confluence and heading away from the estuary (Figure 2.1).

In the Alsea River 5 receivers were placed in the lower river; 2 to mark estuary entry; 8 in the estuary and 2 at the entry into the ocean. There were two additional receivers placed in the Alsea system to ensure that we were not losing fish either upstream in the mainstem Alsea River or up Five Rivers, the only major tributary smolts would encounter before they entered the estuary (Figure 2.1).

Effective smolt emigration monitoring is dependent upon efficiency of acoustic detection arrays. Establishing receiver arrays with high efficiency allows the number of fish to be tagged to be kept at a minimum resulting in less time and money spent tagging, less handling of fish, provides yearly consistency for a long term project and reduces the complication of analyzing detection data.

Receivers were downloaded weekly to reduce the possibility of data loss or missed detections resulting from damaged, missing or otherwise fouled gear (filamentous algae accumulation, debris caught on buoy lines).

#### Survival Estimates

We used the Survival Under Proportional Hazards model to estimate survival and detection efficiency (SURPH 3.0

http://www.cbr.washington.edu/paramest/surph/). Survival estimates were calculated from the smolt trap to the array located at rkm 1.2 in the Nehalem estuary and to rkm 0.2 in the Alsea. These arrays are referred to as the "survival" arrays, and placement remained consistent for all years of study. Previous data supported acceptable detection efficiencies of approximately 90% or greater at these locations in the Nehalem (Schreck et al. 2002; Clements and Schreck 2003) and Alsea (Johnson et al. 2010) estuaries. The array closest in proximity to the ocean in each basin was used to calculate efficiency to the survival array for the SURPH estimates. These arrays are hereafter referred to as the "Ocean arrays", as smolts last detected at this array were assumed to be successful ocean entrants. There were instances when fish were detected at the ocean array in 2009, and later detected upstream at the survival array then never detected again (0 fish in the Nehalem, 4 in the Alsea). Since the *last* detection for these fish was upstream of the ocean entry array these fish were not considered to be ocean entrants. In 2009, there was no array placed offshore in the Pacific Ocean. Therefore, we determined the minimum number of tagged wild

steelhead smolts entering the Pacific Ocean using detections at the ocean array (array closest in proximity to the ocean). There are no standard errors associated with these numbers because there was no way to determine efficiency on the last array.

The detection data were quality checked using the following procedures. Tags that were only detected one time at any given array were validated on an individual basis as part of the quality control process. These detections were checked against available tag numbers and for legitimate time and date stamps within the context of the rest of the case history before they were considered viable detections.

#### Gill ATP-ase activity levels

To determine the degree of smoltification in juvenile steelhead we collected gill filament samples from all *O. mykiss* captured in the trap on the days that we tagged fish, including all acoustic tagged fish during the 2009 field season. We also collected gill tissue from fish captured via hook and line sampling within 3 km upstream or downstream of the smolt trap after the outmigration had ceased, as judged by lack of fish captured in the trap. Gill samples were removed using methods for non-lethal gill biopsy developed by McCormick (1993). Approximately 4-6 gill filaments from the first left arch were removed half way between cartilaginous tissue and end of filament. Excised filaments were placed in 0.1 mL SEI buffer (250 mM sucrose, 10 mM EDTA, 50 mM Imidazole, pH 7.3) in a 1.5 mL vial and frozen within 0.5 h of sampling. Samples taken in the field were kept on dry ice until they could be stored at

-80 °C freezer. All samples were processed consistent with methods detailed in McCormick (1993).

#### Parasites

To determine parasite prevalence and infection severity of *Nanophyetus* salmincola, *Apophallus* sp. and *Myxobolus* sp. that are common in other Oregon salmonid smolts (Ferguson et al. 2010), some fish that were evaluated for gill ATP-ase activity were lethally sampled. A fillet of muscle and portion of the posterior kidney were removed and stored at -4 °C until processing. The remaining portion of the body was fixed in 10% buffered formalin for histological evaluation.

Muscle and kidney samples were later thawed and the entire amount of tissue weighed. If the muscle sample was < 5 g, then the entire sample was evaluated, otherwise a sub-sample of 5 g was removed by cutting small portions off the fillet until the total weight was equal to 5 g. Muscle or kidney tissue was then placed between two pieces of Plexiglas with a small amount of water, and pressure was applied to create a wet mount (see Ferguson et al. 2010), which was examined under a compound microscope to identify and enumerate parasites. Samples were then standardized to parasites per gram of tissue. Parasite density is defined as the average number of parasites per gram of tissue from only infected fish and prevalence is the number of fish infected divided by number sampled.

The remaining, fixed portion of the carcass was dissected for histology with sections of the following tissues removed: spleen, liver, ovary (if present), lower

portion of intestine, heart, pyloric cecae, kidney, muscle, brain, and 3 gill arches (decalcified in 15% formic acid for 1 hour before embedding). Slides were stained with hematoxylin and eosin using standard techniques. Parasite identification for wet mount and histology were verified by Jayde Ferguson and/or Dr. Michael Kent.

#### Age Composition

We collected scale samples from each fish that was captured in the trap. Scales were removed from the area between the dorsal and adipose fin immediately dorsal of the lateral line and on the left side of all *O. mykiss* sampled, including acoustic tagged fish to determine age by counting the number of annuli. Scales were examined by trained professionals at ODFW Fish Research Lab in Corvallis, Oregon.

#### Data Analysis

Steelhead smolt estuary survival

We used logistic regression for binary response variables to assess the relationship between fork length, estuarine survival, and river origin in 2009. The dataset for 2009 was used exclusively for this analysis because this was the only year we acquired data from both basins concurrently.

In the Nehalem basin 53 acoustic tagged smolts reached the estuary and in the Alsea 62 tagged fish reached the estuary. These 115 smolts were used for estuary survival analysis. Survival was determined based on whether or not the fish was detected at the "survival array" at the mouth of the estuary. Fish that entered the

estuary but were not detected at the survival array were considered casualties. The full model tested was: logit(survival) = Intercept + fork length + basin + (fork length:basin). The interaction term between basin and fork length was tested to incorporate the possibility that the relationship between length and survival differed in the two river basins. There was no need to check for equal variance or normality of the residuals for a Bernoulli distribution because once a mean has been specified, the variance is fixed. The drop in deviance F-test was used several times to compare competing models to test the importance of individual parameters within the model. We used a pooled variance, two sample t-test to compare differences in fork length of smolts tagged at the smolt trap to tagged fish entering the estuary between the two basins. We used S+ 8.0 (Insightful Corp., Seattle, Washington, USA) for all statistical analyses. A P-value  $\leq$  0.05 was considered significant. Statistical comparisons were made using  $\alpha$  = 0.05.

#### Behavior

Nonparametric tests were used due to non-normality and unequal variance of variables of interest. We evaluated the relationship between condition factor, *Nanophyetus salmincola* densities in the posterior kidney, estuary residence time and migration timing between basins and between tagging groups Early and Peak using Wilcoxon rank-sum tests. Estuarine residence time was calculated using the time elapsed between the last detection at the estuary entry array and the last detection at the survival array at the mouth of the estuary. Medians and associated ranges are

reported as they are more informative and robust when dealing with outliers because they are not based on averages. Fulton's condition factor was calculated for all fish >120 mm using the formula K=(weight)10<sup>5</sup>/(length)<sup>3</sup> (Carlander 1977) where weight is reported in grams and length corresponds to fork length of each smolt measured in millimeters.

# Gill ATP-ase activity levels

One-way analysis of variance (ANOVA) was used to compare mean differences in ATP-ase activity levels between experimental groups (Early, Peak, Late, Nonmigrant). The response variable ATP-ase activity was log transformed because the variance of the residuals increased with increasing group means. Confidence intervals of 95% were calculated for specified linear combinations using the Tukey method.

Logistic regression for binary response variables was used to investigate whether elevated ATP-ase activity levels sampled at the smolt traps were a good indicator for survival through the estuarine environment. The full model tested was: logit(survival) = Intercept + ATP-ase + basin + (ATP-ase:basin). Variables that were not significant were removed in sequential order, the remaining variables remained in the model and the model was re-tested. This analysis only includes fish for which ATP-ase data were available. All tagged fish are not represented (n=57).

## **Parasites**

Nanophyetus salmincola density in the posterior kidney was compared between fish of different age groups. An Exact Wilcoxon sign-rank test was used in the Alsea for the two age groups (1,2), and a Kruskal-Wallis rank sum test in the Nehalem for the 3 groups (1,2,3). Nanophyetus salmincola densities were high and prevalence was 100%, making these parasites the most appropriate for analysis (larger sample size). We compared whether older fish had a higher parasite load, which could suggest that parasites are accumulated in fish as they age, potentially hindering growth or delaying age of smoltification.

Linear regression was used to test the possibility of a relationship between density of N. salmincola in the posterior kidney and condition factor in both basins. Model tested: Condition factor = Intercept + Nano density.

The relationship between ATP-ase activity level in the gills and the density of gill parasites was tested using general linear regression for all three parasites that were encountered in the gills. Specific parasites tested were *Echinochasmus milvi* (Nehalem only), *Sanguinicola* sp., *N. salmincola* and total (summed density of all 3 parasites) gill parasite density. Full models tested were: ATP-ase = intercept + Echino + age + (Echino\*age), ATP-ase = intercept + S.cola + age + (S.cola\*age), ATP-ase = Intercept + Nano + age + (Nano\*age), ATP-ase = Intercept + Total parasites + age + (Total parasites\*age). ATP-ase activity was the response variable and density of parasites found in the gill was the explanatory variable. The parasite and age interaction term was included to account for any interaction between age and density. Non-significant

explanatory variables were removed in stepwise fashion until all variables had been tested. Values reported in the results section are for full models.

# Results

The estimates of survival ranged between 49 - 78% in the Nehalem for the three years (2001, 2002, 2009), and from 41 - 59% in the Alsea for the two years (2007, 2009) (Table 2.1). Overall survival estimates in 2007 differ from values reported by Johnson et al. (2010) because we used the total number of smolts tagged as the sample size instead of fish that were detected at the first array located downstream of the tagging site.

Table 2.1 Number of smolts tagged each year and survival probability estimates between arrays based on SURPH 3.0 results. Estimates are calculated from point of tagging to the survival arrays with associated standard error in parentheses. Standard errors are not presented for ocean arrays because there is no array behind them with which we could estimate efficiency. "NA" indicates that no receiver array was present at this location for the corresponding year, therefore no survival probability was estimated. Minimum survival is based on the number of fish detected at the ocean array.

Nehalem River	SE)	Rkm		
	2001	2002	2009	-
Number Tagged	56	45	69	
Trap – Estuary Entry	0.63 (0.06)	0.84(0.05)	0.77 (0.05)	33.2 - 13.5
Upper Estuary	NA	1.00(0.00)	0.85 (0.05)	13.5-9.7
Mid Estuary	0.94(0.04)	0.98(0.03)	0.98(0.04)	9.7 - 6.8
Lower Estuary	0.85 (0.06)	0.95 (0.04)	0.77 (0.07)	6.8 - 1.2
Ocean	0.59	0.77	0.64	1.2 - 1.0
Overall Survival	0.50(0.07)	0.78 (0.06)	0.49 (0.06)	
Minimum Survival	0.29	0.60	0.32	

Alsea River	Survival Pro	bability (SE)	Rkm
	2007	2009	
Number Tagged	72	70	
North Fork Alsea – Fall Creek Confluence	0.79 (0.05)		83.4 -50.4
Fall Creek Trap – Fall Creek Confluence		0.97 (0.02)	55.0 - 50.4
Fall Creek - 5-Rivers	0.98 (0.02)	0.96(0.03)	50.4-39.8
5-Rivers – Head of Tide	0.95 (0.03)	0.98(0.02)	39.8 - 23.7
Head of Tide – Estuary Entry	1.00(0.00)	0.97 (0.02)	23.7 - 13.0
Upper Estuary	0.93(0.04)	0.97 (0.02)	13.0 - 9.0
Mid Estuary	0.83 (0.07)	0.90(0.04)	9.0 - 2.6
Lower Estuary	0.73 (0.09)	0.77 (0.08)	2.6 - 0.2
Ocean	0.39	0.58	0.2 - 0.1
Overall Survival	0.41(0.07)	0.59 (0.07)	
Minimum Survival	0.15	0.31	

Survival probability was estimated for wild steelhead smolts tagged in different groups (Early, Peak, Late) throughout the run to try and account for any temporal variation that may exist. Smolts were not tagged in every group for every year.

Table 2.2 Survival probability for wild steelhead smolts tagged in different groups (Early, Peak, Late) throughout the run. 0 indicates no fish were tagged in this group during the corresponding field season.

	Early	Probability of	Peak	Probability of	Late	Probability of
	(n)	survival (SE)	(n)	survival (SE)	(n)	survival (SE)
Nehalem						
2001	19	0.58(0.11)	19	0.63 (0.11)	18	0.28 (0.11)
2002	4	0.67(0.14)	28	0.76(0.10)	13	0.92 (0.07)
2009	35	0.37 (0.08)	34	0.62 (0.08)		
Alsea						
2007	72	0.41(0.07)				
2009	21	0.51(0.13)	49	0.63 (0.09)		

We apportioned each estuary into 3 zones (Upper, Mid, Lower) and estimated the mortality for each zone using SURPH 3.0. Percentage mortality for each zone was calculated independently. Smolt survival probability decreased as the smolts neared the ocean (Figure 2.4).

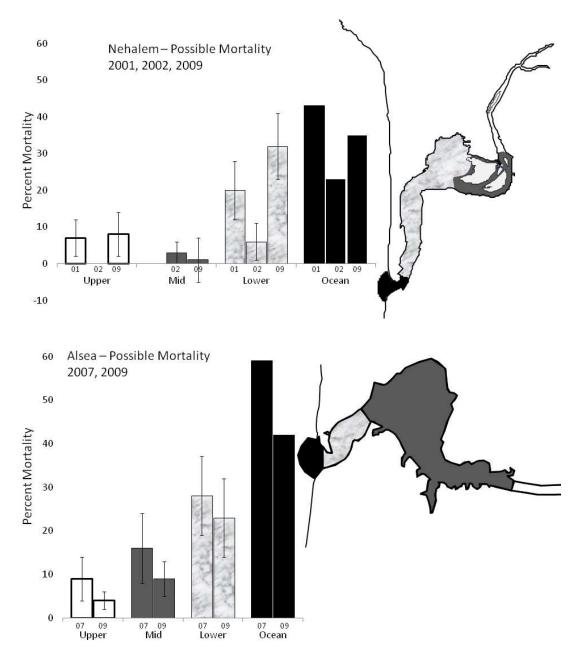


Figure 2.4 Percent mortality accrued in each estuary zone (Upper, Mid, Lower, Ocean) in the Nehalem (2001, 2002, 2009) and Alsea (2007, 2009) estuaries, respectively. Percent calculated using SURPH 3.0 model. Bars represent percent undetected within each zone and standard error. The ocean zone has no standard error bars because estimates for efficiency of the ocean array were not possible. Estuary outlines are not drawn to scale, and are for representation of zones only

During the 2009 field season two fish were detected at the survival array in the Nehalem estuary, but remained stationary for 35 and 36 days. These tags did not exhibit movement consistent with the rest of the tags in either basin, and were likely shed tags, tags excreted by predators, or mortalities that remained near that location until the battery expired. Even though these smolts reached the survival array they were not considered "survivors" in the survival analysis. There were no instances of this in the Alsea in 2009. However, Johnson et al. (2010) noted similar occurrences in the Alsea from their data in 2007 and these fish were not included as survivors. In the Nehalem during the 2009 season, one tag was detected at the mouth of the estuary and subsequently detected passing every array heading upstream until the final detection was recorded at the South Fork Nehalem receiver. This fish was not included as a survivor.

Array efficiencies for all years in all locations were approximately 90% or higher (Table 2.3) with the exceptions of the survival array in the Alsea estuary in 2009 and the lower estuary array in 2007, which had efficiencies of 82% and 86% respectively.

Table 2.3 Receiver efficiencies and associated standard errors reported using SURPH 3.0 survival model. NA indicates that no receiver array was present at this location for the corresponding year.

Nellalelli Kivei	er necesser / in a principle (32)					
Location	River Kilometer	2001	2002	2009		
Estuary Entry	13.5	1.00(0.00)	1.00(0.00)	1.00(0.00)		
Hwy 101 Boat Ramp	9.7	NA	1.00(0.00)	0.93 (0.04)		
Paradise Cove	6.8	1.00(0.00)	0.94(0.04)	0.88(0.06)		
Mouth	1.2	1.00(0.00)	0.96(0.04)	1.00(0.00)		
Alasa Bissan		Bassiner Arre	ay Efficiency (SI	E.\		
Alsea River		neceiver Arra	ay cinciency (Si	<u>- )</u>		
Alsea River Location	River Kilometer	2007	2009	-)		
	River Kilometer 50.4			<u> </u>		
Location		2007	2009			
Location Fall Creek Confluence	50.4	2007 1.00 (0.00)	2009 0.98 (0.02)			
Location Fall Creek Confluence Head of Tide	50.4 23.7	2007 1.00 (0.00) 1.00 (0.00)	2009 0.98 (0.02) 1.00 (0.00)			
Location Fall Creek Confluence Head of Tide Estuary Entry	50.4 23.7 13.0	2007 1.00 (0.00) 1.00 (0.00) 1.00 (0.00)	2009 0.98 (0.02) 1.00 (0.00) 1.00 (0.00)			

Receiver Array Efficiency (SE)

## Survival

Nehalem River

2009 is the only year for which we have data available to compare survival between basins within the same year. The probability of survival for steelhead smolts in the estuary during 2009 was not affected by the interaction of the variables fork length and river  $[\Pr(\chi^2_1>0.438)=0.508]$ . After removing the interaction term from the model, the probability of survival was not related to fork length  $[\Pr(\chi^2_1>1.777)=0.183]$  or river basin  $[\Pr(\chi^2_1>0.668)=0.414)$ . The simplest model containing only the observed survival rate, or the intercept  $(\beta_0)$ , was found to be the best fit model. All other variables were found to be non-significant, or not different from zero, and were removed from the model. The final model was logit(survival) =  $\beta_0$ , with  $\beta_0 = 0.51$ (SE 0.19  $Z_{114}$ =2.67, p=0.008). Thus, the survival probability constant for all tagged smolts

entering either estuary in 2009 was 62.6% (95% CI: 53.4-71.0) without accounting for the efficiency of the receivers. Smolts tagged in the North Fork Nehalem (mean length: 175 mm, 95% CI: 171-178 mm) were larger than those tagged in the Alsea (mean length: 160 mm, 95% CI: 156-164 mm) ( $t_{134}$ =-5.60, p<0.0001). Similarly, the size of the tagged smolts that entered the estuary from the Nehalem were larger than those from the Alsea basin ( $t_{113}$ =-4.58, p<0.0001). Mean fork length of tagged fish entering the estuary was 175 mm (95% CI: 168-182 mm) in the Nehalem, and 162 mm (95% CI: 143-181 mm) in the Alsea. The condition factor for fish >120 mm in the Nehalem basin was lower than in the Alsea basin (Z=8.38, p<0.0001). Median condition factor in the Nehalem basin was 0.93 (n=144, range 0.80, 1.1), and 1.03 (n=124, range 0.84, 1.3) in the Alsea.

### **Behavior**

The median travel time from the smolt trap to the first detection at the survival array in the Nehalem basin (33 km) was 10.0 d for the Early group (n=13, range 4.4-24.6) and 7.8 d for the Peak group (n=21, range 4.7-12.6). In the Alsea basin the median migration time from smolt trap to survival array (55 Km) was 20.4 d for the Early group (n=10, range 15.0-42.9) and 13.0 d for the Peak group (n=28, 6.1-33.2).

Steelhead smolt migration rate was calculated for Peak and Early groups in both the Nehalem and Alsea Basins from date of tagging to the first detection at the survival array. Results were suggestive but inconclusive that the Peak group moved faster than the Early group (W=197, p=0.066). Median migration rate of Early smolts

was 3.2 km/d (n=13, range 1.1-7.6 km/day), and median migration of Peak smolts was 4.2 Km/d (n=21, range 2.6-7.0). In the Alsea basin smolts migrating at the peak moved down to the survival array faster than Early smolts (W=117, P=0.0086). Median migration rate for smolts in the Alsea basin in the Early group was 2.7 Km/day (n=10, range 1.3-3.7 km/d), and median migration rate for smolts in the Peak group was 4.2 km/d for (n=28, range 1.7-9.1 km/d). There was no difference in migration rate between basins (Peak and Early groups pooled W=1364, p=0.649).

Median residence time for smolts in the Nehalem estuary was 0.72 d (n=34, range 0.14, 7.9) and 0.83 d (n=38, range 0.14, 6.3) in the Alsea Estuary. Residence time between Early (n=13) and Peak (n=21) groups in the Nehalem basin did not differ (W=266, p=0.181), nor did residence time differ between Early (n=10) and Peak (n=28) groups in the Alsea basin (W=160, p=0.257). Additionally, residence time between basins did not differ (W=1327 Alsea n=38 Nehalem n=34 p=0.504).

### Gill ATP-ase activity levels

Gill ATP-ase activity levels differed significantly among the four groups tested [(Early Peak, Late, and Nonmigrant),  $F_{(3,124)}$ =11.51, p<0.0001] but did not differ between rivers [(Nehalem, Alsea)  $F_{(3,1)}$ =0.003, p=0.96] (Figure 2.5). Tukey post-hoc comparisons of groups indicate that median ATP-ase activity levels in the Early group were 0.67 times (95%CI: 0.47, 0.95) that of the Peak group, the median of the Early group was 0.61 times (95%CI: 0.39, 0.98) that of the Late group, the median of the Early group was 1.71 times (95%CI: 1.03, 2.86) that of the Nonmigrant group, the

median of the Peak group was 2.56 times (95%CI: 1.60, 4.10) that of the Nonmigrant group, the median of the Late group was 2.77 times (95%CI: 1.59, 4.85) that of the Nonmigrant group. The exponentiated confidence intervals for the medians of the Peak and Late groups includes 1.0, therefore they are not different (median= 0.92 95%CI: 0.61, 1.41). Figure 2.6 illustrates the variation in gill ATP-ase levels between individuals throughout the run.

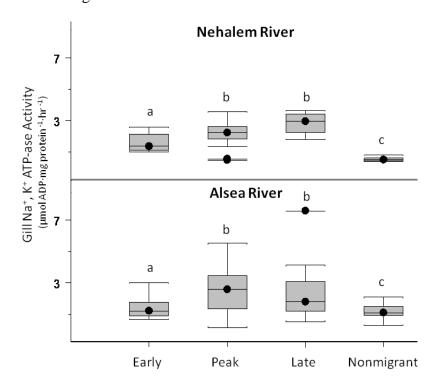


Figure 2.5 Box and whisker plots of ATP-ase activity levels for all tagging groups (Early, Peak, Late, Nonmigrant) in the Nehalem and Alsea basins. Each letter represents a different level of ATP-ase activity. Shaded boxes represent the interquartile range, or the 25<sup>th</sup>-75<sup>th</sup> percent of data. Horizontal line with a dot within the box is the median. The whisker ends represent data points within 1.5 times the interquartile range. A line with a dot located outside the whiskers represents an outlier.

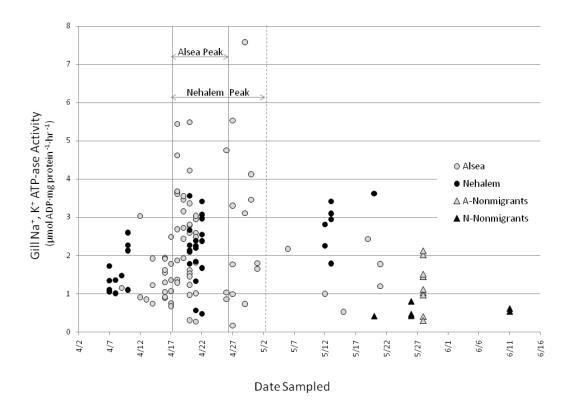


Figure 2.6 Gill ATP-ase activity levels for fish >120mm that were tagged in each group (Early, Peak, Late, Nonmigrant) throughout the emigration season. Circles represent smolts captured in smolt trap. Triangles represent nonmigrant juveniles captured by hook and line sampling.

Gill ATP-ase activity levels were not indicative of survival through the estuary, nor were any of the other variables included in the model (All p-values > 0.38); ATP-ase:Basin  $Z_{53}$ =0.15, p=0.88, Basin  $Z_{54}$ =0.88, p= 0.38, ATP-ase  $Z_{55}$ =0.25, p=0.80.

# Parasites

We identified several parasites in tissue from fish captured in Fall Creek (Alsea basin). These included: *Nanophyetus salmincola* in the brain, heart, gills,

muscle, anterior kidney, posterior kidney, pyloric cecae and intestines; *Sanguinicola* sp. in the gills; *Chloromyxum majori* in the anterior kidney; *Myxidium salvelini* in the anterior kidney; *Myxobolus* sp. in the muscle and brain tissue; adult digenean trematode in the pyloric cecae and intestine; and *Salminicola* sp. were found externally behind the pectoral fins and on the gills. All parasite species found in Fall Creek were also present in fish from the North Fork Nehalem in the corresponding tissue. In addition, we also found *Echinochasmus milvi* in the gill; *Ceratomyxa shasta* in the intestine; *Apophallus* sp. in the muscle; and *Philonema* sp. in the ceolomic cavity in the North Fork Nehalem smolts.

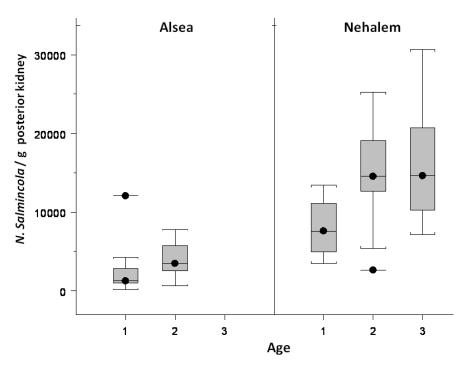


Figure 2.7 Box and whisker plots of *N. salmincola* per gram of posterior kidney tissue for smolts in each age group from the Alsea and Nehalem basins. Shaded boxes represent the interquartile range, or the 25<sup>th</sup>-75<sup>th</sup> percent of data. Horizontal line with a dot within the box is the median. The whisker ends represent data points within 1.5 times the interquartile range. A line with a dot located outside the whiskers represents an outlier.

The density of *N. salmincola* in the posterior kidney was higher (W=239, p<0.0001) in fish from the North Fork Nehalem (n=24; median= 13,454; range 2,614 – 30,673) than in Fall Creek (n=20; median=2,792; range 150 - 12,050). There was no difference in the density of *N. salmincola* in the posterior kidney among the 3 age groups in the Nehalem basin (Kruskal-Wallis  $\chi^2_2$ =4.297, p=0.12), nor did *N. salmincola* density differ between 1 and 2 year old juveniles in the Alsea basin (Wilcoxon rank-sum W=82, p=0.09) (Figure 2.7). There was no relationship between *N. salmincola* density in the posterior kidney and condition factor in either the Nehalem basin (F<sub>(1,22)</sub>=0.12, p=0.73) or Alsea basin (F<sub>(1,18)</sub>=0.05, p=0.82). Average sample weight for posterior kidney samples was 0.10 g (n=44, range 0.02, 0.24).

Selected parasites, their prevalence and density are illustrated in Table 2.4. These parasites are presented for each age class within each basin. There was no relationship between gill parasite density and ATP-ase activity levels in any of the combinations for the models involving the selected three gill parasites, or the total of those three species combined (all p-values >0.61); *Sanguinicola* sp.  $F_{(3,28)}$ =0.60, p=0.62, *N. salmincola*  $F_{(3,28)}$ =0.76, p=0.53, *E. milvi*  $F_{(3,28)}$ =1.10, p=0.37, all gill parasites combined  $F_{(3,28)}$ =0.57, p=0.64.

Table 2.4 Parasite prevalence and density of select parasites in select tissues from the Nehalem and Alsea basins organized by smolt age class. <sup>a</sup> Data from muscle and kidney were derived by wet mount, which were standardized to number of parasites per gram of tissue. Data from all other tissues are from histology and are presented in terms of parasites per histological sections. 3 gill arches were included on each gill slide. <sup>b</sup> For these Alsea age 2 fish n=9, and no muscle sample was available for one fish in this group. *Nano* = *Nanophyetus salmincola*, *Apo* = *Apophallus* sp., *Echino* = *Echinochasmus milvi*, *S.cola* = *Sanguinicola* sp., *myxo* = *Myxobolus* sp.

	Kidneya	Mu	scleª		Gill		В	rain	Heart
	Nano	Nano	Apo	Nano	Echino	S. cola	тухо	Nano	Nano
				Pr	evalence	;			
Nehalem									
Age 1	100%	100%	100%	100%	100%	75%	25%	0%	50%
Age 2	100%	100%	92%	100%	46%	85%	46%	0%	92%
Age 3	100%	100%	71%	100%	86%	100%	71%	14%	100%
Alsea									
Age 1	100%	100%	0%	80%	0%	80%	50%	0%	60%
Age 2	100%	100%	0%	80%	0%	90%	20%	20%	60%
				Dens	sity (Rang	ge)			
Nehalem									
Age 1 (N=4)	8,020 (3,450-13,450)	41 (4-72)	3 (1-6)	14 (1-41)	5 (1-12)	34 (5-57)	8 (8)	0	6 (3-8)
Age 2 (N=13)	14,710 (2,614-25,215)	49 (2-87)	4 (1-18)	20 (1-76)	7 (2- <b>1</b> 7)	86 (3-346)	12 (1-47)	0	10 (1-35)
Age 3 (N=7)	16,692 (7,163-30,673)	34 (10-66)	6 (1-18)	32 (7-56)	13 (1-34)	136 (7-374)	9 (2-21)	1(1)	9 ( <b>4-1</b> 9)
Alsea									
Age 1 (N=10)	2,736 (150-12,050)	11 (2-25)	0	4 (1-12)	0	53 (2-2 <b>1</b> 2)	24 (3-69)	0	2 (1-5)
Age 2 (N=10)	3,918 (636-7,807)	9 <sup>b</sup> (5- <b>1</b> 7)	$\mathbf{O}_{\mathrm{P}}$	5 (2- <b>11</b> )	0	20 (2-73)	5 (4-6)	1 (1)	2 (1-5)

# Age composition

In 2009, the outmigrants from the Nehalem basin consisted of 1 (18%), 2 (74%), and 3 (8%) year old smolts (n=119). In contrast, 46% of the outmigrants in Fall Creek (Alsea basin) were 1 year olds, and 54% were 2 year olds (n=98). There were no three year olds captured from Fall Creek. Confidence intervals for age composition

are not provided because true age validation is rarely attainable for wild fish reared in their natal stream.

### Discussion

## Survival and Behavior

The majority of outmigrating wild steelhead smolts migrated successfully to the estuary following release (range 63-89%) in both the Nehalem and Alsea basins. However, the probability of survival decreased as the smolts neared the ocean (Figure 2.4). A large proportion of smolts appear to be lost in the lower estuary within 1-1.5 km of the ocean (mean loss: 59.4% over the 5 years). This decrease in number of tags detected between the survival and ocean arrays occurs within a distance ~300 m. Decreased detections can partially be attributed to lower receiver efficiency in close proximity to the ocean (i.e. wave action, strong tidal current, boat traffic). However, in previous years when receivers were placed in the ocean just offshore of the Nehalem estuary, the ocean array efficiency was estimated to be 83% (Clements and Schreck 2003). Johnson et al. (2010) noted possible breaks in adequate coverage of offshore arrays in the Alsea in 2007. The area just offshore is difficult to cover adequately and each estuary presents its own challenges. However, large numbers of predators are also known to congregate near the mouth of each of these rivers (Clements and Schreck 2003; Wright et al. 2007). Therefore, actual numbers for smolts entering the ocean probably lie somewhere between smolt survival probability estimates calculated from the survival array and the number of smolts detected by the ocean arrays. We consider the number of smolts detected by the ocean array as the minimum, or lower confidence limit for survival estimation.

Survival estimates for steelhead smolts in the two small basins in our study are higher than estimates from researchers working in much larger estuarine environments. Melnychuk et al. (2007) reported conservative survival estimates for steelhead smolts leaving the Cheakamus River, British Columbia of 27% during 2004 and 2005, and Moore et al. (2010) reported combined survival from populations in four separate rivers through Puget Sound and into the Strait of Juan de Fuca as 28.3%. This could be a result of the distance traveled within the estuarine environment, as Melnychuk et al. (2007) observed significant correlation between distance traveled through the Strait of Georgia and mortality. In both of the referenced studies, smolts were travelling 155-230 km, depending on release site and route travelled. Regardless of estuary size, estuarine survival is low for most years in both small and large estuaries. Low survival is somewhat expected, as the transition from fresh water to marine environments is one of the most life-threatening events for anadromous salmonids (Levings 1994).

If larger fish have higher survival, as other researchers have suggested, we might have expected higher survival in the Nehalem estuary compared to the Alsea. However, there was no effect of fork length on estuarine survival in either basin, or between basins, consistent with other studies (Moore et al. 2010; Johnson 2010). However, Ward and Slaney (1988) studying wild steelhead in the Keogh River in

British Columbia, and Wagner (1967) who studied hatchery steelhead in the Alsea River both found that mortality was highest for smaller smolts when information was collected subsequently from returning adults. While using a 17 year dataset for coho salmon, Holtby et al. (1990) noted that smolt size in coho salmon was not consistently related to smolt to adult survival, but that large smolts did survive better in the ocean when marine survival was relatively poor. Taken together, these observations suggest that size selective mortality of smaller smolts occurs primarily in the marine environment.

We tagged smolts within nearly the entire size range of fish captured at the smolt traps (Figure 2.2). There does not appear to be any reason for concern that there was a deleterious tagging effect on smaller fish. In our study, a high percentage of tagged fish in each basin survived the journey through the lower river below the capture site and into the estuary (77% in the Nehalem, 89% in the Alsea in 2009). Of the 7 fish tagged in Fall Cr. with fork length < 140 mm, 4 entered the estuary and 3 were detected at the survival array. Welch et al. (2007) conducted an acoustic tag retention study on *O. mykiss* using dummy tags (8mm x 24 mm, 1.4 g in air) similar to the V7 tags that we used. Welch's 7 month study reported combined losses from mortality and tag shedding for fish between 120-130 mm was between 30-40%, but dropped to <15% for fish over 140 mm. We acknowledge the possibility of underestimating survival probabilities due to a number of factors including residualization of tagged fish in the river, tag shedding and mortality due to surgery complications.

Estuarine residence time has been shown to be inversely proportional to survival probability in several studies (Handeland et al. 1996; Clements et al. 2003; Schreck et al. 2006; Truelove 2006; Kennedy et al. 2007). In our study, wild steelhead smolts spent little time in the estuary, and movement was primarily unidirectional toward the ocean with few instances of smolts being detected at an upstream receiver array once they had been detected downstream. In all five datasets used in this study, wild steelhead smolts that were detected at the survival array spent ~1 d in the estuaries.

The probability of survival for steelhead smolts in the Nehalem and Alsea basins between the upriver smolt traps and the ocean varies substantially between years (Table 2.1). It should also be noted that survival varies greatly within groups tagged on consecutive days (data not shown). Salmonid survival in the ocean varies from year to year and productivity is often linked to Pacific Decadal Oscillation and El Nino cycles (Beamish and Bouillon 1993, Mantua et al.1997). Understanding how estuary and ocean survival are related to one another leads us to question whether increased survival in the estuary can aid in buffering less productive ocean conditions while smolts are at sea, or whether low estuarine smolt survival is necessary to reduce competition for limited ocean resources on lower ocean production years.

Smolt survival to the ocean has previously been estimated from smolt traps located well upstream of the estuary. Thus, any mortality incurred in the zone between these traps and the ocean is included as ocean mortality in survival models (Jepsen et al. 2006). Our data suggest that 50% or more of the mortality previously considered to

occur in the ocean actually occurs in the estuary. In estuaries, efforts could be made to improve survival using methods such as habitat restoration or predator control. Our results combined with results from other researchers (Melnychuk et al. 2007; Johnson et al. 2010; Moore et al. 2010) show that estuarine survival estimates for steelhead smolts appear low. However, we can not say whether these mortality rates are outside of the historic range because there is little or no historic data available.

Our results suggest that smolts migrating during the peak period of the run had survival probabilities greater than 60%. Possible reasoning for this consistent, relatively high survival rate at the peak is that fish have evolved to emigrate, triggered by environmental factors at times optimal to their survival, in what McCormick et al. (1998) termed an "ecological smolt window". In contrast, late migrating groups had both the highest (2002) and the lowest (2001) overall probability of survival, exhibiting the greatest variation in survival probability of any of the tagging groups.

Temporal differences in survival, or the difference in shape of survival probability curves are likely a result of a culmination of factors that change throughout the run in any given year as well as between years. Predation has already been identified as one of the primary factors for smolt mortality in estuaries (Stahl et al. 2000; Schreck et al. 2002, 2006; Clements and Schreck 2003). Additional reasons for differences in estuarine survival between temporally dispersed migrants could be flow (Schreck et al. 2006), turbidity (Emmett 2006), differences in preparedness for salt water transition (Schreck et al. 2006; Kennedy et al 2007), or assemblage and abundance of predators. Predators could become alerted to the food source by the first

migrants and predator numbers would increase as more smolts become available (Clements and Schreck 2003). In this case mortality would be especially high for smolts migrating during the peak and late portion of the run as the number of predators reaches an apex, and the number of smolts begins to decrease. Johnson et al. (2010) found no evidence of this in 2007 in the Alsea basin.

Another scenario of smolts emigrating in or around the peak of the run could relate to survival advantages associated with larger aggregations. It has been demonstrated that schooling fish are more efficient at capturing food and are less susceptible to predation than those that remain solitary (Pitcher 1986). Smolts migrating at the peak of the run could potentially realize higher survival as they would already be traveling in a large group beginning to exhibit schooling behavior prior to ocean entry. There may also be a dilution in predation pressure provided by release of hatchery smolts that are less well adapted to natural ecological factors.

Considerable historical hatchery legacy exists in both the Alsea and Nehalem basins with the first hatcheries constructed in the early 1900's. Both basins support large winter steelhead fisheries utilizing supplemental hatchery smolts. Volitional release of 70,000 smolts in the North Fork Nehalem River (ODFW North Nehalem operations plan 2009) and 120,000 smolts in the North Fork Alsea River (ODFW North Fork Alsea River Hatchery operations plan 2009) begins in early April in correspondence with the observed wild smolt timing. Even though our tagged fish were reared in their natal streams it is likely that there has been genetic influence from hatchery fish (Jepsen et al. 2006; Johnson et al. 2010). We recognize the possibility

that contributions from hatchery reared parents and hatchery smolt releases could be variables that influence behavior and survival within and among years but were not the focus of this study.

# Gill ATP-ase activity levels

Na<sup>+</sup>, K<sup>+</sup> ATP-ase activity was higher in smolts that were tagged in the Peak and Late groups. Elevated levels of gill ATP-ase activity suggests that the fish could be better prepared for transition into salt water (McCormick et al. 1987, 1993; Schrock et al. 1994; Kennedy et al. 2007) and are able to travel quickly through the estuary. However, we found no evidence that elevated levels of gill ATP-ase activity were indicative of survival through the estuary when gills were sampled at capture sites well upstream of the estuary. Early migrants and fish captured by hook and line sampling late in the season (probable non-migrants) had lower ATP-ase activity than those fish captured at the peak, or late in the run (Figure 2.5). These fish may not have been as well smolted as fish sampled during the majority of the run.

### **Parasites**

There is a stark contrast in parasite densities and parasite community assemblage between these two basins located within the same distinct population segment (Table 2.4). This could have management implications for restoration projects and land use management. From our data, it seems probable that in the Nehalem basin many of the smolts successfully entering the ocean, are doing so with a

high parasite load. *N. salmincola* infections have been linked to parasite associated mortality during early ocean residence of juvenile coho salmon (Jacobson et al. 2008), and metacercariae of *N. salmincola* have been documented to remain viable in coho salmon for at least 33.5 months, including their stay in the ocean (Farrell et al. 1964). How parasite loads are affecting fish in the ocean phase of their life history is still poorly understood.

Histology is not the most accurate method for parasite enumeration, but has been used for presence/absence and comparison of infection density (Rodnick et al 2008; Ferguson et al. 2010). Wet mount methods are preferred for proper enumeration. This suggests that it is likely that gill parasite density may be higher than what we have seen here in our subsample of 3 gill arches per fish. Although we did not see a noticeable decrease in ATP-ase activity levels with high density of gill parasites when sampled at our smolt traps, it still seems plausible that parasites could affect the ability of individual smolts to osmoregulate effectively during transition into saline waters. None of the parasites that we encountered in the gills are shed in brackish water, as they are encysted within the gill tissue.

We observed nematodes, *Philonema* sp. in the body cavity of 5% (5/100) of the fish sampled in the North Fork Nehalem, consistent with a 5% infection of coho salmon from the same system found by Ferguson in 2007 (pers. comm 2010). This parasite was not encountered in fish sampled from Fall Creek. Freshwater copepods, *Salminicola* sp. were observed in both basins behind the pectoral fins and in the gills in low prevelance.

High parasite loads could be a contributing factor to lower condition factors observed in North Fork Nehalem smolts. Juvenile *O.mykiss* that are heavily parasitized may not be able to reach the energy storage threshold necessary for successful metamorphosis. However, the smolting process is also known to decrease condition factor (Wagner 1967) and fish from the two basins might be in different stages of the smoltification. Lower condition factor could also be a result of environmental conditions less conducive to rapid growth. *N. salmincola* are transmitted by infected freshwater snails *Juga plicifera*, which are more abundant on gravel and cobble substrates than on sand or silt (Diamond 1976). The North Fork Nehalem River may contain better habitat for this intermediate host.

## Age Composition

Average age of outmigration in steelhead smolts in these systems is primarily 2 years old, but we observed 3 year old smolts present in the North Fork Nehalem.

Peven et al. (1994) suggested that juveniles rearing in colder, upstream habitats take longer to reach smolting size. Three year old smolts have previously been documented in the Alsea basin (Chapman 1958, Wagner et al. 1963). The stratification of age composition for smolts in the two basins may look the same overall, but smolts from different tributaries within the same basins appear to exhibit different migration strategies, which is consistent with what others have found (Peven et al. 1994).

Wagner (1963) analyzed scales from 1,542 steelhead smolts from the Alsea basin between 1956-1959 and observed that only 5% were age 1, 82% were age 2 and

13% were age 3. Chapman (1958) estimated that 90% of wild adult steelhead returning to the Alsea River had spent at least 2 years in fresh water before emigration to the ocean. In contrast, nearly 50% of the total number of smolts that we captured in the Fall Creek smolt trap were 1 year olds. In addition, 21 (30%) of our *tagged* smolts in the Alsea were 1 year old fish. Of those 21 fish, 16 survived to the estuary showing that they were indeed migrating to the ocean and not residualizing in the stream. Does this imply that migrant age composition is changing over time, or that age 1 fish had a higher probability of being captured in the trap during the 2009 field season?

In another study, older steelhead smolts from Waddell Creek in California had higher marine survival than younger smolts, with 3 year olds exhibiting smolt to adult survival three times higher than 2 year olds (Shapovalov and Taft 1954). If increased age of smolting increases smolt to adult survival, then we would expect greater reproductive fitness for the three year old smolts from the North Fork Nehalem River than those smolts emigrating from Fall Creek.

Of the 25 non-migrants that we captured that were >120 mm (15 Nehalem, 10 Alsea), only 2 (1 Nehalem, 1 Alsea) were 2 year olds that would have been expected to migrate. It is probable that the remaining 23 nonmigrant 1 year old fish were waiting an additional year prior to smolting, as is typical of a majority of smolts from these two systems. We did not find increased parasite loads in the 2 year old nonmigrants compared to the 2 year old migrants, but the sample size was very small and there is also a possibility that "nonmigrant" fish were merely late migrants. Although there was no increase detectable between age classes with our current

dataset, there is still a possibility that older fish have higher parasite densities. Again, small sample size and large amounts of variation limited our analysis, with n=4 for the 1 year old age class in the Nehalem where the densities were much higher than in the Alsea basin. *N. salmincola* has been shown to persist in overwintering coho salmon (Ferguson et al. 2010) and higher infection found in older juveniles would suggest continued infections accumulating the freshwater portion of their life history (Table 2.2). This is encouraging for further investigation. If freshwater parasites are accumulated over time this would be an additional factor in the cost vs. benefit analysis that determines if and when a juvenile fish is going to smolt. More research needs to be conducted to determine effects of these parasites on the smolting process.

Inconsistency in age of smolting complicates estimation of smolt to adult survival, and prediction of adult returns for management purposes. It also promotes the need for multiple monitoring sites within a management area, providing sufficient spatial representation for distinct population segments.

Estimates of smolt to adult survival should incorporate freshwater, estuarine and marine survival. Results from this study highlight the importance of including an estuarine survival component to survival models for increased spatial resolution regarding salmonid survival. Investigating smolt survival in the estuary using acoustic telemetry enabled us to pinpoint an area where approximately 50% of the mortality occurred that has previously been included in survival models as ocean mortality. Information collected from this study should be incorporated into restoration strategies. Our results also provide baseline data on additional variables that are

known to affect smolt survival. Information regarding smolt behavior, age composition and parasite prevelance and assemblage between basins within the same distinct population segment contribute to the understanding of the complexities involved in the management of anadromous salmonids, and could be used to direct future research.

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# **CHAPTER 3: GENERAL CONCLUSIONS**

Chapter 2 focused on behavior and survival of steelhead smolts between smolt traps and the ocean. There is a large amount of variation in behavior, physical condition and survival expressed on both spatial and temporal scales during emigration (Stefansson et al. 2008). How existing variation in physical and behavioral smolt attributes affects survival, and how trends in survival between basins within the same management area relate to one another over time is not well understood.

We found that typically only 40-50% of the wild steelhead smolts reaching the estuary actually enter the ocean, and survival proabability between years is highly variable, ranging from 41-78% (Table 2.1) for the 5 years of data. Successful smolts spent a median time of 0.72 and 0.83 days in the estuary in the Nehalem and Alsea basin in 2009, respectively, and travelled toward the ocean at a rate between 2.7 and 4.2 Km/day. Survival between smolt traps and the estuary was relatively high (63-89%) in all years studied, however, survival probability decreases as smolts get closer to the ocean (Figure 2.4) with the lowest survival probabilities recorded near the mouth of the estuary. Smolts that were tagged during the peak of the run had higher survival probability than those tagged during the early portion of the run for every year of study. Smolts tagged at the peak had an overall probability of survival consistently greater than 60% for each of the 4 years that the Peak group was monitored.

There was no relationship between fork length and survival in either basin for 2009 field season. There was an increase in gill ATP-ase activity levels during the Peak and Late portions of the run, but there was no relationship between gill ATP-ase

activity level and survival when gill samples were collected at smolt traps located well upstream from the estuary in either basin.

Parasite assemblage, density and smolt life history strategies can vary markedly between basins within the same management area. The North Fork Nehalem River smolts exhibited higher density of parasites, and were host to several additional species of parasite that were not present in Fall Creek (Alsea basin). Nehalem smolts captured in the trap were larger overall, and were generally older with 8% smolting at age 3, where there were no 3 year old smolts in Fall Creek. One year old smolts from the North Fork Nehalem had nearly twice the *Nanophyetus salmincola* parasite density observed in Fall Creek 1 year old. Although the sample size for 1 year old smolts in the North Fork Nehalem is small (n=4), this could suggest that juvenile *O.mykiss* that are heavily parasitized may not be able to reach the energy storage threshold necessary for successful smoltification. *N. salmincola* is thought play a significant role in early marine survival of juvenile coho salmon (Jacobson et al 2008).

This research provides a baseline by which we can measure change in estuarine survival and behavior over time, information that could be useful for estuarine restoration and reiterates the importance of the lower river and estuary environment for smolt survival. Monitoring smolt survival in the lower river and estuary provides important data that accounts for 50% percent or more of mortality that was previously considered ocean mortality in survival models. Several studies have found that substantial smolt mortality occurs in the estuary (Clements and Schreck 2003; Johnson et al. 2010; Moore et al. 2010) and have likely lead to artificial

inflation of ocean mortality (which managers have little control over). If adult return trends reflect a strong relationship with estuarine survival, then not only does that enable better adult return estimation but would also be encouraging for the importance of looking for methods to decrease smolt mortality in the estuary.

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# **4: APPENDICES**

Appendix I. Nehalem and Alsea estuary area approximation, estuarine smolt survival by fork length, and receiver locations from 2009 field season (April-June).

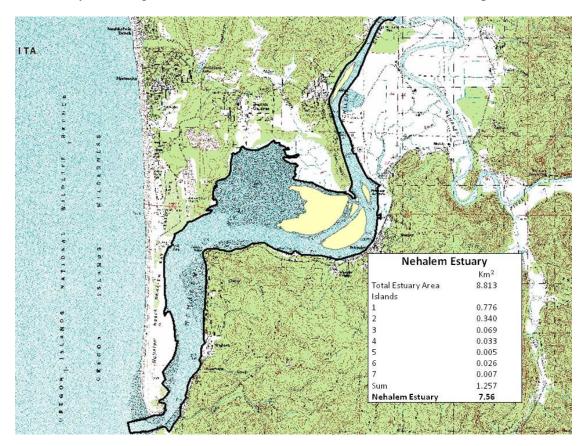


Figure 4.1 Map illustrating method for figuring area of Nehalem estuary ( $\rm Km^2$ ). Polygon area estimated using ESRI, ArcGIS 9.2.

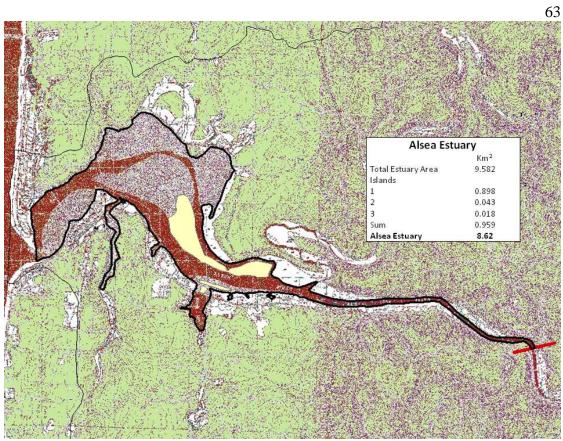


Figure 4.2 Map illustrating method for figuring area of Alsea estuary (Km²). Polygon area estimated using ESRI, ArcGIS 9.2.

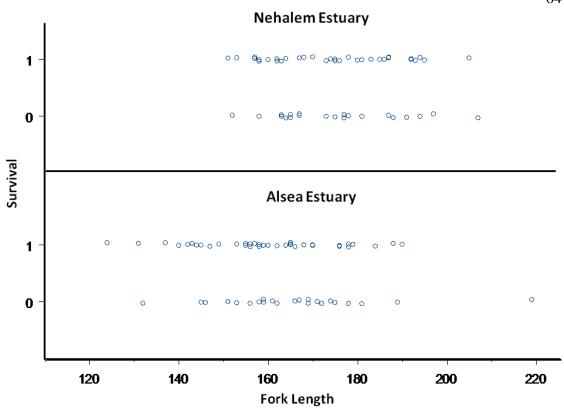


Figure 4.3 Estuarine survival plotted by fork length for tagged, wild steelhead smolts in the Nehalem and Alsea basins (2009). (Survival = 1, Mortality = 0).

Table 4.1 Receiver locations in the Alsea and Nehalem river basins for the 2009 field season. Location defines the placement of each individual receiver. Multiple receivers are combined to form an array. Side defines where in the channel or estuary each individual receiver was placed, directions orientated as if you were facing downstream.

River	Location	Array	Side	Receiver Number	Depth (ft)	NAD83UTM Northing	NAD83UTM Easting	River Km
Alsea	Fall Creek Screw Trap	SmoltTrap			( ')	4917266	440227	55
Alsea	UpperAlsea	FallCreek	Middle	6086	6	4913857	441786	50.4
Alsea	MouthFallCr	FallCreek	Middle	6083	6	4913904	441587	50.4
Alsea	Upstream of 5 Rivers	Upstream 5Rivers	Right	6090	5	4912300	433974	39.8
Alsea	FiveRivers	FiveRivers	Middle	6079	10	4912006	434653	
Alsea	HeadofTide Upper	HeadofTide	Middle	6082	20	4917080	428785	23.7
Alsea	HeadofTide Middle	HeadofTide	Middle	6080	20	4916967	428720	23.7
Alsea	HeadofTide Lower	HeadofTide	Middle	5535	8	4916867	428735	23.7
Alsea	Taylor Upper	Taylors	Middle	6085	28	4917488	424746	13
Alsea	Taylor Lower	Taylors	Middle	6097	12	4917481	424614	13
Alsea	Oakland Upper	Oaklands	Middle	6084	7	4918260	420995	9
Alsea	Oakland Middle	Oaklands	Middle	6081	8	4918245	420847	9
Alsea	Oakland Lower	Oaklands	Middle	6089	6	4918311	420698	9
Alsea	LowerEstuary North	LowerEstuary	Right	2897	15	4921146	415399	2.6
Alsea	LowerEstuary Middle	LowerEstuary	Middle	2397	11	4920990	415522	2.6
Alsea	LowerEstuary South	LowerEstuary	Left	2895	13	4920906	415602	2.6
Alsea	JawsInner North	Survival	Right	1579	13	4919542	414376	0.2
Alsea	JawsInner South	Survival	Left	2894	21	4919383	414435	0.2

Table 4.1 (Continued)

				Receiver	Depth	NAD83UTM	NAD83UTM	River
River	Location	Array	Side	Number	(ft)	Northing	<b>Easting</b>	Km
Alsea	JawsOuter North	Ocean	Right	3111	16	4919303	414213	0.1
Alsea	JawsOuter South	Ocean	Left	3110	20	4919233	414115	0.1
Nehalem	LowerScrewTrap	SmoltTrap				5073011	441233	33.2
Nehalem	Confluence Upper	Confluence	Middle	1727	6	5065021	432041	13
Nehalem	Confluence Lower	Confluence	Middle	2248	5	5064953	431935	13
Nehalem	Mainstem	NehalemMainstem	Middle	1995	12	5064617	432458	
Nehalem	101BoatLaunch Right	101BoatLaunch	Right	1733	5	5061431	430947	9.7
Nehalem	101BoatLaunch Left	101BoatLaunch	Left	2773	7	5061745	430919	9.7
Nehalem	ParadiseCove	ParadiseCove	Left	2013	14	5059852	430071	6.8
Nehalem	ParadiseCove	ParadiseCove	Right	2032	10.5	5060009	430047	6.8
Nehalem	JettyFisheryUpSouth	Survival	Left	3106	20	5056968	427598	1.4
Nehalem	JettyFisheryUpNorth	Survival	Right	4344	15	5057010	427478	1.4
Nehalem	JettyFisheryLowSouth	Survival	Left	6098	17	5057072	427608	1.4
Nehalem	JettyFisheryLowNorth	Survival	Right	4342	12.5	5057104	427493	1.4
Nehalem	Jaws South	Ocean	Left	2893	15	5056610	427410	1
Nehalem	Jaws North	Ocean	Right	3107	15	5056697	427333	1