# THE INFLUENCE OF ESTUARINE HABITATS ON EXPRESSION OF LIFE HISTORY CHARACTERISTICS OF COHO SALMON SMOLTS IN SOUTH-CENTRAL ALASKA

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## Abstract

Expression of traits that lead to life history diversity in salmonids may provide population-level resilience and stability in dynamic environments. I examined habitat use and variability in life history trait expression in juvenile coho salmon *Oncorhynchus kisutch* occupying two contrasting estuary environments in south-central Alaska. My goal was two-fold: first, to determine if salmon were using estuaries as rearing environments and were therefore potentially vulnerable to selection pressures within; and second, to compare traits of salmon that reared in contrasting estuary environments to explore the potential for differential trait expression related to estuary size and habitat complexity differences. Juvenile coho salmon reared in estuaries for extended periods of time and patterns of use corresponded to environmental conditions within the estuaries. Populations using adjacent but contrasting estuary environments exhibited differential trait expression and were genetically distinct. My work highlights how pristine, functioning estuary habitats contribute to resilience of salmon populations to environmental changes in two ways: first, by providing habitats for individuals to increase in size and condition prior to ocean entry; and second, by providing for alternative life history tactics (providing quality habitat to delay marine entry times and increase body size). Management approaches for resilient salmon runs must therefore maintain both watershed and estuary function.

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## Dissertation Introduction

The overall goal of this dissertation is to relate environmental variability in estuarine environments to the ecology and life history of juvenile coho salmon. To adequately address this goal, it is critical to understand the basic processes that contribute to life history diversity, ecology, and trait expression of salmonids and how these processes contribute to population- and species-level resilience in the dynamic and sometimes unpredictable environments of the Pacific Northwest. This introduction provides a review of the current literature on primary drivers of life history diversity in salmonids, how this diversity is expressed, and how the ecology of individuals and populations can lead to overall resilience in the face of environmental change. I place particular emphasis on the role of estuarine and marine environments in driving these changes, as estuaries are the focus of this study.

### *The physical template: setting the stage for life history diversity*

Northeast Pacific environments are influenced by cyclic climate regimes, resulting in dynamic physical changes that affect coastal ecosystems (Beamish et al. 1999a; Hare and Mantua 2000; Mantua and Hare 2002). The regime shifts of the El Niño Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO), in particular, may drive shifts in productivity in northeast Pacific Ocean fisheries as well as alterations in trophic structure of marine ecosystems (Beamish and Bouillon 1993; Francis et al. 1998; Beamish et al. 1999a; Mantua and Hare 2002; Thomson et al.

2012). Temporal and spatial shifts in physical conditions, such as sea surface temperature and wind patterns, change mixing zone depths and ocean currents, thereby affecting the availability of nutrients and altering trophic structure and dynamics (Francis et al. 1998; Hare and Mantua 2000; Hollowed et al. 2001). Interactions between physical conditions in biological systems will have a temporal frame where conditions are optimal to meet the needs of an individual organism. In phytoplankton, for example, we can anticipate intermediate water column stability may be optimal for plankton production because strong stability can be nutrient limiting, whereas weak stability limits access to ambient light. This period has been termed the optimal environmental window (Cury and Roy 1989) or, in direct reference to water column stability, the optimal stability window (Gargett 1997). The optimal environmental window for primary production in the marine environment will vary spatially and temporally both seasonally and annually creating dynamic environmental conditions for selective processes.

Estuaries are the points where the freshwater river systems meet the oceans and are known to be some of the most dynamic and productive places in the world (Kaiser et al. 2005). Numerous physical processes are acting in estuarine environments as freshwater rivers enter and mix into the salt water environments, and these processes are constantly changing with seasonal tidal and river discharge fluctuations, mediated by geomorphology and latitude (Mann and Lazier 2006). These dynamic environments create complex habitats that vary in their seasonal suitability for animals within (Mann and Lazier 2006). As transitional habitats, estuaries play an important role for smolting salmon, providing mixing zones of fresh and saltwater environments that buffer against osmoregulatory and physiological stress (Healey 1982; McMahon and Holtby 1992; Miller and Sadro 2003; Beamish et al. 2004; Bottom et al. 2005). Estuaries also have potential as important salmon rearing habitats; Chinook salmon *Oncorhynchus tshawytscha*, in particular, have increased survival rates (Magnusson and Hillborn 2003) and life history variability (Bottom et al. 2005; Campbell 2010; Volk et al. 2010) with estuarine habitat use.

Estuaries fed by different freshwater hydrologic regimes may provide contrasting rearing environments for resident biota (Saltveit et al. 2001). Freshwater influx into northern estuaries is expected to be particularly high during snowmelt periods; however, within Alaska, many estuarine habitats are fed by glacial river systems. Glacially-fed estuaries are unique in that the peak freshwater discharge occurs in mid-summer rather than early spring, yielding cold water discharge with high sediment loads from glacial erosion during the warmest months. Differences in rearing conditions may contribute to variability in the timing and duration of estuarine use for juvenile salmon. An investigation into environmental drivers of life history variability informs our understanding of the bet-hedging evolutionary strategies employed by salmon that ensure a portion of offspring meet the optimal marine survival window of size and time that allows for successful feeding (Healey 2009).

### *Life history diversity in salmonids*

An organism's fitness is influenced by the suite of individual characteristics expressed, such as body size and age at maturation, fecundity, seasonal timing of maturation, and migratory patterns. A life history tactic is described as one of a suite of inherited traits that can contribute to positive fitness in the face of ecological problems, such as habitat disturbance and changes in availability of prey (Stearns 1976; Schaffer 2004). Life history tactics may vary both within a species and within a population across temporal and spatial scales (Olsen and Vøllestad 2001; Rikardsen et al. 2004). Environmental conditions can select for specific traits within a population, particularly during life stages when high mortality can occur (Stearns 1976). Dynamic environments, in particular, can select for a wide range of life history tactics within and among populations of a single species. Coined "the portfolio effect", intraspecific genetic and phenotypic diversity that exists between groups of populations buffers a species against environmental variability (Schindler et al. 2010). The portfolio metaphor is a tangible model of how evolutionary processes that lead to a range of life history tactics or types can increase the resilience of a given species, metapopulation, or population to environmental change (Beamish et al. 1997; Hillborn et al. 2003; Kaeriyama et al. 2004; Waples et al. 2009).

Diversity of life history expression in salmonid fishes has permitted occupation of a broad range of habitats and persistence in dynamic climates and variable topographies (Healey 1994; Parker et al. 2001; Hendry 2004; Waples et al. 2009). Pacific salmon show a diversity in phenotypic expression described as a form of bet-hedging (Healey 1994), where an organism will express a range of behaviors or tactics that will increase the probability of survival or persistence of a population in the face of environmental variability. Bet-hedgers will produce multiple phenotypes in progeny that range from those that are adapted to a more stable environment (e.g., resident) to those adapted to an environment subject to stochastic processes (e.g., migratory). Trait variation is strongly influenced by the natural selection pressures of the environment. Pacific salmon, therefore, are an excellent study organism for examining selective processes related to differing environments.

Salmon populations well demonstrate selection for both genetic and phenotypic variation in response to the frequency, magnitude, duration and predictability of environmental variability (Adkison et al. 1996; Smith et al. 2001; Quinn 2005; Wood et al. 2008). These aspects of the environment are drivers for selection of traits, with expression balancing between gene flow and phenotypic plasticity (Sultan and Spencer 2002; Waples et al. 2009). For example, sockeye salmon *Oncorhynchus nerka* populations in Bristol Bay with different spawning behaviors presented patterns of single population dominance over time periods that corresponded to regional climate conditions (Hilborn et al. 2003). Each individual population possessed a suite of traits that increased fitness in a particular climate regime, leading to an alternating pattern of dominance that buffered the population against major climatic changes over the past century. Wood et al. (2008) proposed the recurrent evolution hypothesis to describe and predict the patterns of trait dominance and genetic diversity and structuring that can be expected in response to specific life history tactics and predicted climate change. Likened to individual stocks in an investment portfolio, each ecotype has developed a role under differing environmental conditions that, when combined with different ecotypes, allows for the species, as a whole, to be more resilient to environmental variability (Schindler et al. 2010). Understanding the conditions that give rise to varying ecotypes within a population is, therefore, important to developing effective management and conservation plans to provide for regional resilience.

The life cycle of anadromous salmon encompasses a range of habitats, from the headwater streams used during spawning and early rearing, to the open ocean used during juvenile development and sexual maturation (Schaffer 2004). The duration each species occupies fresh and saltwater rearing habitats varies both among and within species. Generally, two major ontogenetic shifts take place in Pacific salmon: smolting (the transition between the fresh to the salt-water environment) and sexual maturation. How these shifts manifest is greatly influenced by environmental conditions experienced over ontogeny (Thorpe et al. 1998). Smolting, in particular, lends itself to high selection pressure because it occurs during the juvenile stage while individuals are undergoing a period of behavioral, physiological, morphological change (Williams 1996; Thorpe et al. 1998; Beamish et al. 2004). Ocean conditions, particularly those encountered during smolting and early marine rearing, can have a profound effect on survival of salmon to the adult phase (Beamish and Mahnken 2001; Beamish et al. 2012). Specific traits, such as timing of outmigration, size, and condition of smolts at marine entry are related to survival through the ocean rearing period (Holtby et al. 1990; Hobday and Boehlert 2001).

### *Life history variability and estuary use in coho salmon*

North Pacific coho salmon *Oncorhynchus kisutch* show great variation in life history tactics. The mechanisms leading to expressed adaptations of juvenile coho salmon are not well understood, particularly aspects of bet-hedging (Stearns and Hendry 2004) and developmental traits that have profound fitness consequences (Thorpe et al. 1998). This species is found in greatest densities in the coastal waters of British Columbia and ranges from northern California to the northwestern coast of Alaska (Pearcy 1992). Coho salmon generally have both a long freshwater rearing phase (1-2 years) and ocean rearing phase (1-3 years), although the duration of each phase is variable both among local drainages and across the species' distribution (Beamish et al. 1999b; Hobday and Boehlert 2001). In addition, coho salmon exhibit strong population structuring similar to sockeye salmon (Smith et al. 2001; Wood et al. 2008) that has the potential to be reflected in fine scale phenotypic and behavioral differences. Therefore, differences in life history characteristics may be expected between populations that occupy habitats that differ greatly in the environmental conditions during periods of the life cycle, such as smolting, when strong selection pressures are present.

Estuary residence is thought to be a relatively brief, but important life history period for coho salmon (McMahon and Holtby 1992; Miller and Sadro 2003). Smolting salmon undergo both physiological and behavioral changes during estuary occupancy that are hypothesized to affect survival in open ocean environments (McMahon and Holtby 1992). The timing, age, and size of fish at the point of estuary and early marine entry are related factors that are expected to influence individual survival (Healey 1982, Bohlin et al. 1993; Beamish et al. 2004). Large estuaries are considered high quality rearing habitat for juvenile coho salmon (Koski 2009), and fish using these habitats survive better over time than those using small estuaries or bypassing them altogether, moving directly to the open ocean (Beamish et al. 1997). Distribution of coho juveniles within estuaries relates to availability of cover and salinity gradients, and estuaries that provide greater cover could improve ocean survival by increasing growth and providing gradual salinity gradients that allow for optimal osmoregulatory adaptation (McMahon and Holtby 1992). The wide diversity of pristine estuarine habitats in south-central Alaska combined with the reproductive traits of salmon, offer a template upon which several predictions regarding the influence of estuarine habitats on trait development may be tested.

### *Management and conservation implications*

Direct relationships between stock strength and physical ocean conditions are difficult to ascertain due to a time-scale lag in trophic structure, variability within spatial scales, and variability within dynamics of the populations of interest (Francis et al. 1998; Hollowed et al. 2001). Management approaches developed to maximize the diversity expressed within a species are critical for effective resource use (Ford 2004; Waples et al. 2009). Management at a scale finer than that of the stock or population level is a recent development, practiced primarily with populations already determined to be at risk or under heavy exploitation. For example, the concept of Ecologically Significant Units (ESU) was introduced as one solution to describing and defining diversity expressed within a species into conservation units that warrant distinct management attention (Ryder 1986). The ESU was defined as a unit below the species level that still expressed significant diversity and an evolutionary legacy within the species (Williams 1996). Understanding factors that may lead to population divergence and the scale upon which distinct stocks that contribute diversity in trait expression exist is critical for conserving trait diversity leading to population resilience (Schindler et al. 2010).

### *Dissertation focus*

In this dissertation, I use a three-part approach to investigate the links between estuary habitats and expression of life history traits in juvenile coho salmon. I begin with a site-scale approach, comparing life history traits such as size, age structure, and body condition of juvenile coho salmon found within a single estuary habitat type along the intertidal zone of a glacial estuary. Second, I apply a comparative approach, examining patterns of estuarine occupancy, condition, size and age structure of fish rearing within glacial and snow-melt, spring-fed estuaries. Finally, I use microsatellite genetic analysis to examine two populations of fish and investigate reproductive isolation and genetic differentiation of coho salmon captured within the two estuaries. This approach allows examination of the ecology of coho salmon within a single estuary and comparison of two systems with sharply contrasting environments. This work demonstrates that these two kinds of estuary environments contribute to life history diversity in coho salmon by providing rearing habitats in which variable tactics may emerge, therefore providing for resilience in salmon populations to environmental changes. The dissertation is written in three chapters structured as manuscripts, bookended with this introduction and an overall dissertation conclusion. The work and content of each individual chapter reflects guidance and assistance of a group of coauthors and is written under this context. Because each chapter is intended as a stand-alone manuscript, readers of this dissertation should expect some repetition in introductory material.

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# Chapter 1. Use of Glacial River-fed Estuary Channels by Juvenile Coho Salmon: Transitional or Rearing Habitats?[1]

## *Abstract*

Estuaries are among the most productive ecosystems in the world and provide important rearing environments for a variety of fish species. We illustrate how juvenile coho salmon *Oncorhynchus kisutch* use a glacial river-fed estuary through examination of spatial and seasonal variability in patterns of abundance, fish size, age structure, condition, and local habitat use. Fish abundance was greater in deeper channels with cooler and less variable temperatures, and these habitats were consistently occupied throughout the season. Variability in channel depth and water temperature was negatively associated with fish abundance; this was also the case for salinity, though weakly. Fish size was negatively related to site distance to the high tide line, while fish condition did not relate to channel location within the estuary ecotone. Body size within each age class of coho salmon increased as the growing season progressed. Our work demonstrates that this glacially fed estuary potentially serves as both transitional and rearing habitat for juvenile coho salmon during smolt outmigration and that patterns of fish distribution within the estuary correspond to environmental conditions.

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[1] Hoem Neher, T., A. Rosenberger, C. Zimmerman, C. Walker, and S. Baird. 2012. Use of Glacier River-fed Estuary Channels by Juvenile Coho Salmon: Transitional or Rearing Habitats? Prepared for submission in Environmental Biology of Fishes.

## *Introduction*

Anadromous salmon exhibit a bet-hedging approach to survival, producing large numbers of offspring that incur high mortality, while expressing a range of life history traits (Holtby and Healey 1990; Healey 1994). Because salmon use dynamic habitats that vary in selection pressures over their life cycle, bet-hedging ensures that a few individuals will possess the appropriate suite of traits to survive to maturity, permitting positive fitness for a subset of an individual's progeny and persistence at the population level in temporally stochastic environments. The range of life history traits that any one population contains is a combination of genetic composition and a plastic phenotypic response to environmental conditions encountered during development (Stearns 1976; Schaffer 2004). Coho salmon, in particular, can exhibit a wide range of life histories within a single population, including variability in age or size at which critical ontogenetic shifts take place (such as smolting), seasonal timing of these shifts, and duration of rearing in freshwater versus marine systems (Miller and Sadro 2003; Koski 2009). Spatial variability in trait expression among populations has been linked to inter-annual changes in both marine and freshwater environments (Gargett 1997; Beamish and Mahnken 2001; Ebersole and Colvin 2009) and linked to resilience and stability in yield of a aggregated population under climatic shifts and a dynamic environment (Hilborn et al. 2003; Schindler et al. 2010).

Marine entry is considered a crucial period for salmon smolt because conditions experienced during this transition can greatly affect survival (Gargett 1997; Beamish and Mahnken 2001; Beamish et al. 2004; Beamish et al. 2008). Prior to ocean entry, estuaries are thought to provide a gradual transition between fresh and saltwater during a stressful physiological shift (Healey 1982; McMahon and Holtby 1992; Beamish et al. 2004). Estuaries, through provision of staging and possible rearing habitats, have the potential to influence plasticity in life history traits such as the timing and size of marine entry. Large estuarine ecotones, such as those described in Koski (2009), provide quality forage, and fish within ecotones have higher survival rates over time than those abruptly transitioning to open ocean conditions (Beamish et al. 1997). Factors that are expected to affect individual marine survival include the duration of estuary occupancy and timing of early marine entry, environmental conditions, and body condition at outmigration (Healey 1982; Bohlin et al. 1993; Beamish et al. 2004).

Estuary ecosystems are complex and variable regarding the effects of anthropogenic changes and interaction with seasonal and regime-level climate shifts, constrained by the geomorphic structure of the system. Estuaries are spatially defined by: 1) an upper, primarily freshwater region; 2) a central, dynamic region of fresh and saltwater mixing; and 3) a lower mouth that is primarily saltwater (Kaiser et al. 2005). Seasonal changes in lotic discharge, interacting with tidal regimes, will introduce variability in freshwater and allochthonous material input that alter the stream hydrology, thereby influencing salinity gradients and thermal regimes within these zones, while providing for additional habitats and changing ecosystem dynamics (Mann and Lazier 2006). Anthropogenic influences on natural flow regimes can therefore have a profound affect on estuary ecosystems and the composition of species sensitive to changing thermal and salinity gradients. Regional shifts in temperature and precipitation levels also alter freshwater discharge regimes, particularly in temperate latitudes where climate-related shifts are occurring at an amplified rate (Hinzman et al. 2005). These changes interact with or are amplified by anthropogenic alteration of river flow for hydroelectric, flood, or irrigation purposes that alter sediment, nutrient content, and the total amount of freshwater inputs into estuaries.

In this study, we examine juvenile coho salmon use of estuarine environments and relate fish characteristics to habitat use. We determined whether variability in size, age, body condition, and patterns of abundance were linked to specific estuarine environments along the tidal inundation gradient. This work provides insight into the roles that functioning estuaries and the environmental conditions within, play in the early ontogeny of coho salmon. This is relevant to management of both commercial salmon stocks and populations of conservation concern in other parts of their range where estuary function may be compromised by anthropogenic disturbance.

### *Study area*

Work was conducted in tributary channels of the glacial melt-water fed Fox River estuary, located at the head of Kachemak Bay, approximately 27 km east from the Homer spit (Figure 1). The Fox River transitions through an approximately 6.0 km long large delta into Kachemak Bay, which provides a large, gradual and extended ecotone between the riverine and marine environments of Cook Inlet. Work conducted in a pilot study in 2009 determined that the Fox River estuary, particularly its tributary channels, provide migratory and possible nursery habitat for coho salmon (Hoem Neher 2009, unpublished data). Using these data as a basis for site selection, we chose four tributary channel habitats along the tidal inundation gradient within which we conducted a focused sampling effort. This habitat type is characterized by square channels with soft, muddy substrate, steep banks with overhanging sedges and grasses, standing water, and slow water velocities. Surface salinities in tributary channel habitats sampled in 2009 ranged from 0-7 ‰ (measured with a YSI™ model 30 hand held temperature and conductivity meter). Water temperatures ranged from 5.6 °C to 13.8 °C, and turbidity, though not measured consistently, was high, with visibility less than 3.0 cm below the water surface from mid-May through mid-September (rain/snow-melt and glacial discharge related). Fox River salmon escapement data have not been collected to date, and only limited data exist for juvenile coho salmon (Walker et al. 2009).

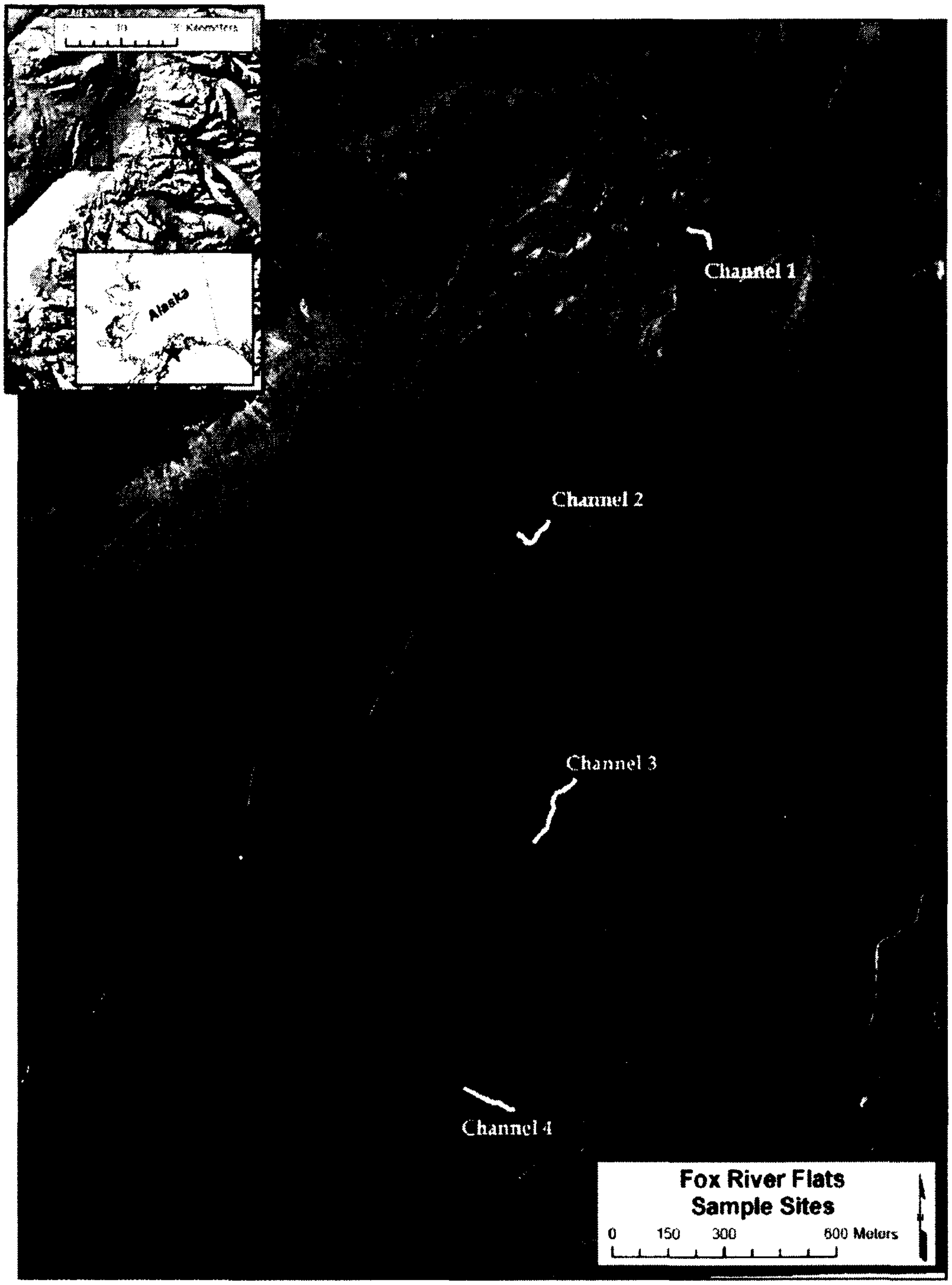

Figure 1. Study area map. The Fox River estuary sampling sites located on the southern Kenai Peninsula, Alaska.

## *Methods*

### *Habitat characteristics.—*

Environmental data were collected continuously for each site using temperature and depth loggers set at 15 minute recording intervals, 5 m upstream from the channel mouth in each of the four channels. In addition, point measurements were collected before each fish sampling event at a cross section downstream of the stationary loggers. Data included thalweg depth (m), conductivity (µS, standardized for temperature), salinity (‰) and temperature (°C), using a YSI model 30 probe held just below the surface, in mid-water column, and at the bottom.

### *Fish capture and processing*[2]*.—*

Sites were sampled in each of four channels over consecutive days, twice per month from early May to late September, 2011. Fish abundance for each site was estimated using multiple-pass depletion methods (Hayes et al. 2007), validated to determine if they reflected actual fish abundances (see below). A 20 m length of channel was measured from the stationary logger location parallel to the channel upstream. The start and end points of each sampling unit were then obstructed with block nets (2.2 m x 6.1 m, 0.31 cm mesh) secured along the sides and bottom with stakes to prevent fish escape. Pole seines (2.2 m x 6.1 m, 0.31 cm mesh) were used to sample the site, pulled three times in the downstream direction. Fish from

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[2] UAF Institutional Animal Care and Use Permit #149489-4

each haul were placed in separate, 19 L aerated tubs filled with water from the channel.

All fish captured were identified to species and counted. The first 50 juvenile salmon captured from each seine haul of each species were anesthetized in 70 mg/L methane tricane sulfonate, MS-222 (Bailey et al. 1998; Chittenden et al. 2008) for three minutes (until fish experienced loss of equilibrium) and measured for fork length to the nearest 1.0 mm. Up to three coho salmon (not to exceed 10% of the total catch), distributed among three size classes (small, medium, and large), were randomly selected and euthanized at each site using 140 mg/L MS-222 for five minutes following cessation of respiration (maximum 24 individuals each month). These fish were labeled and frozen for laboratory analysis to determine condition, weights, and age.

To validate depletion methods, we generated mark-recapture estimates for a subset of our sampling events. Fish were captured using the same methods described for depletion (three hauls of the seine net). They were then batch dyed in one of the channels each month with Bismarck brown mixed in concentrations of 21mg/L (Gaines and Martin 2004). All captured salmon were placed in containers of dye solution with portable aerators for 50 minutes. Water temperature was checked for increases that could cause thermal stress to the fish at 20-minute intervals during dying. Salmon were then released into the enclosed transect and allowed to acclimate and disperse randomly within the channel for 1 to 3 hours. After recovery, the channel was resampled using the same effort (multiple pass seining), noting recapture of marked individuals.

*Laboratory methods.—*

We used water weight, wet weight, and Fulton's condition factor ($K = (W*L^{-3})* 100{,}000$, where W= laboratory wet weight [g] and L= laboratory length[mm]) for metrics of condition (Jonas et al. 1996; Pope and Kruse 2007). Coho salmon specimens were measured for fork length (±1 mm), then weighed to determine wet weight (± 0.01 g). Samples were placed in a 70 °C drying oven for three days, weighed, and returned to the oven for 24 hours to be dried and re-weighed. Samples were considered dried when a minimal weight change (<0.001g) detected between consecutive daily weights (Jonas et al. 1996). Water weight was determined by subtracting the dried sample weight from the wet weight (Jonas et al. 1996; Sutton et al. 2000).

Sagittal otoliths were removed from fish in the laboratory, rinsed, and stored in labeled plastic vials. Otoliths were aged after preparation for microstructure and microchemistry analysis (see Chapter 2 in this dissertation) by counting the winter annuli characterized by large, translucent rings composed of numerous, relatively small incremental growth bands (Campana and Neilson 1985). Ages generated from otolith analysis were used to validate size-at-age inferred from length frequency histograms.

*Data analysis.—*

Stationary logger data were summarized as cumulative thermal units (CTU, daily average summed over sampling period), 7-day maximum temperature, 7-day temperature variance, 7-day average depth, 7-day maximum depth, and 7-day depth variance. We determined periods of exceedance of the Alaska Department of Environmental Conservation temperature criteria for salmonid rearing (maximum daily temperature 15 °C, ADEC 2011) and U.S. Environmental Protection Agency temperature criteria for migratory corridors (7-day average maximum <20 °C, USEPA 2012). Measurements of salinity collected at each sampling event were combined and expressed as average, minimum, and maximum recordings for each event. Spatial comparisons were made using channel locations along the intertidal zone from most upstream (channel 1) to most downstream sampling site (channel 4). We compared environmental conditions (temperature, depth, distance from low tide line, salinity) with patterns of coho salmon abundance body condition, and size for each channel to determine relationships.

Removal estimates of abundances with 95% confidence intervals were generated for each species using depletion techniques for a closed population (Hayes et al. 2007). Removal estimates may be negatively biased due to declining sampling efficiency among passes, and this bias can be affected by habitat conditions within sites (Rosenberger and Dunham 2005). To determine how well removal abundance estimates and total catch reflected actual fish numbers, we used mark-recapture sampling techniques as baseline measures of fish abundance once per sampling event within a single channel. Mark-recapture abundance estimates were calculated using single marking and single recapture estimates for a closed population following Hayes et al. (2007).

For fish retained for laboratory analyses, our protocol was to sample evenly across age classes; as a result, the composition of the laboratory fish sample did not correspond to catch composition. Age class composition of the total catch was inferred via length-frequency histograms, validated with otolith age for each sampling event. We examined the data for differences in means between the channels for fish size (fork length) and condition (Fulton's condition, dry weight, water weight) using one-way ANOVA. Abundance data were examined for relationships to environmental data using simple linear and multiple regression analyses. Catch data were tested for temporal autocorrelation using the Durbin-Watson test for autocorrelation, and based on those results, each sampling event was treated as an independent event (Durbin and Watson 1950; Durbin and Watson 1951). All environmental data were standardized (mean = 0.0, SD = 1.0) and abundance data were square-root transformed to meet homogeneity assumptions and assumptions of normality using R 2.14.1 statistical analysis software (R Development Team 2011).

## *Results*

### *Environmental data.—*

Seasonal thermal characteristics (CTU) were variable among sites, most likely related to water depth, surface run-off, and vertical stratification (Figure 2A, Table 1). Water depths were low and more variable in May and early June, consistently increasing in depth with the glacial melt water until the first fall freeze (Figure 3, Table 2). The most upstream and downstream channels exhibited the greatest ranges in water temperature and depth, with patterns and variances most similar to each other (Figures 2B and 3B, Table 1). These channels were also the shallowest of the four, lacking vertical stratification (variability from surface to bottom) in point measurements of salinity (Figure 4). These shallower channels exceeded daily maximum temperatures of 15.0 °C in 12 and 34 of the 149 days measured and 20.0°C in one and four of the 149 days measured in the most upstream and downstream channels, respectively (Table 1). The deeper and less variable channels (2 and 3) were less extreme in temperature and depth: channel 3 exceeded daily maximum temperatures of 15.0 °C in two of the 149 days measured, and neither channel exceeded maximum daily temperatures of 20.0°C (Table 1).

Salinity measurements corresponded to the preceding tidal levels: higher salinity measurements followed the wide-ranging spring tides, and lower salinity levels followed moderate or low neap tides. Salinity levels were highest in the bottom strata of the centrally located channels (2 and 3), where water depth was sufficient to provide vertical stratification (Figure 4). Channel 3 was consistently the most saline of the four channels, likely due to its depth, increased water retention allowing evaporative concentration of salts, and location in the intertidal zone.

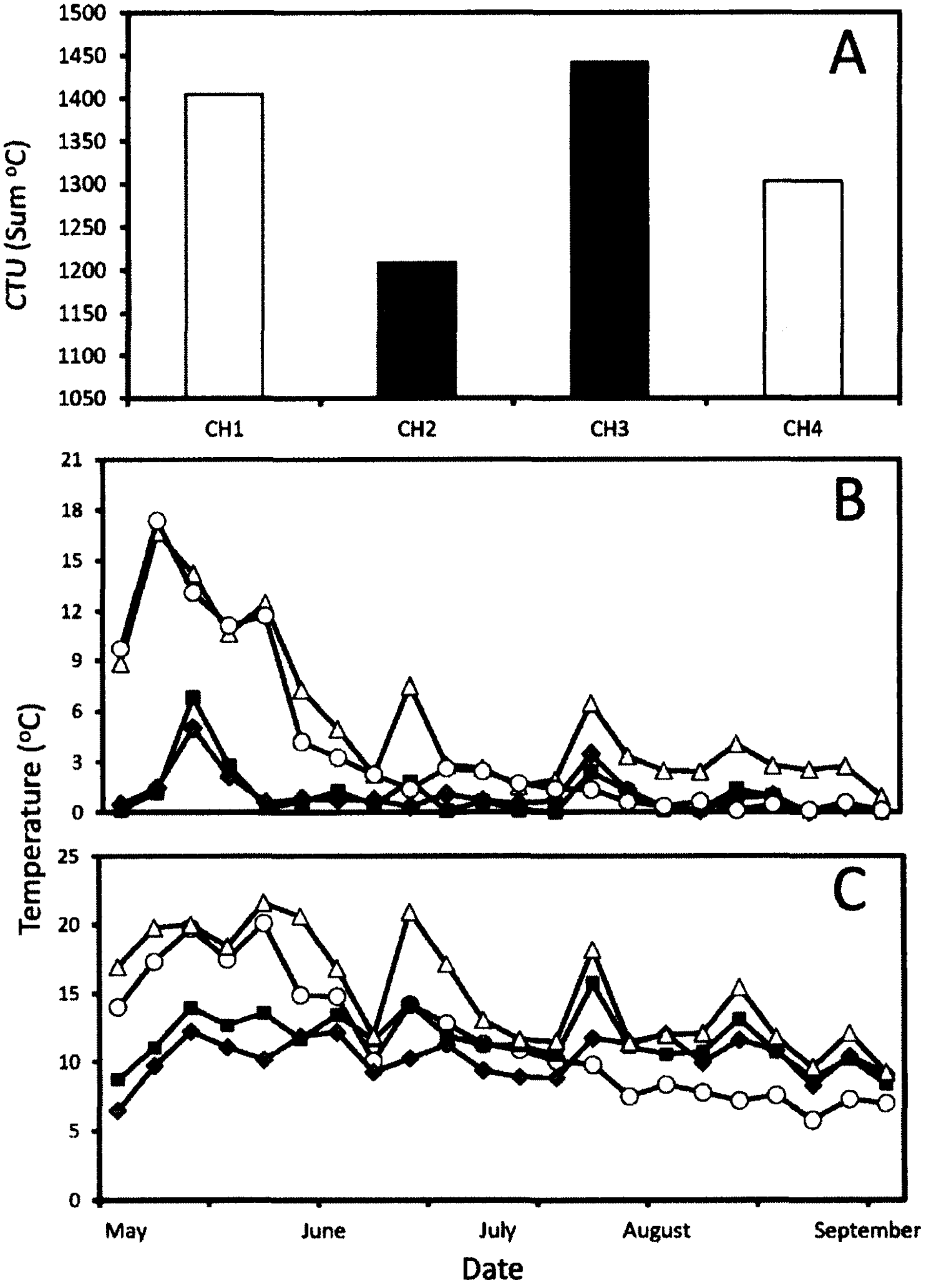

Figure 2. Water temperature data plots. Plots for side channels of the Fox River estuary, south-central Alaska: A) seasonal cumulative thermal units (sum of average daily temperatures through season); B) 7-day temperature variance (logger data); C) 7-day maximum temperature (logger data). Symbols and colors indicate most upstream Channel 1(O); Channel 2(◆); Channel 3 (■); to most downstream Channel 4 (Δ).

Table 1. Water temperature data summary. Seasonal metrics for water temperature (°C) from stationary logger data from the Fox River estuary, south-central Alaska. Data are from the most upstream channel 1 to most downstream channel 4.

| Temperature Metric | Channel 1 | Channel 2 | Channel 3 | Channel 4 |
|---|---|---|---|---|
| Average (variance) | 7.21 (6.19) | 8.13 (2.82) | 9.69 (2.39) | 8.77 (7.39) |
| Maximum | 20.14 | 12.21 | 15.72 | 21.61 |
| Minimum | -2.23 | 3.64 | 4.42 | 0.89 |
| Days maximum daily >15°C | 12 | 0 | 2 | 34 |
| Days maximum daily >20°C | 1 | 0 | 0 | 4 |
| Period 7 day maximum >15°C | 4 | 0 | 1 | 11 |
| Period 7 day maximum >20°C | 1 | 0 | 0 | 4 |

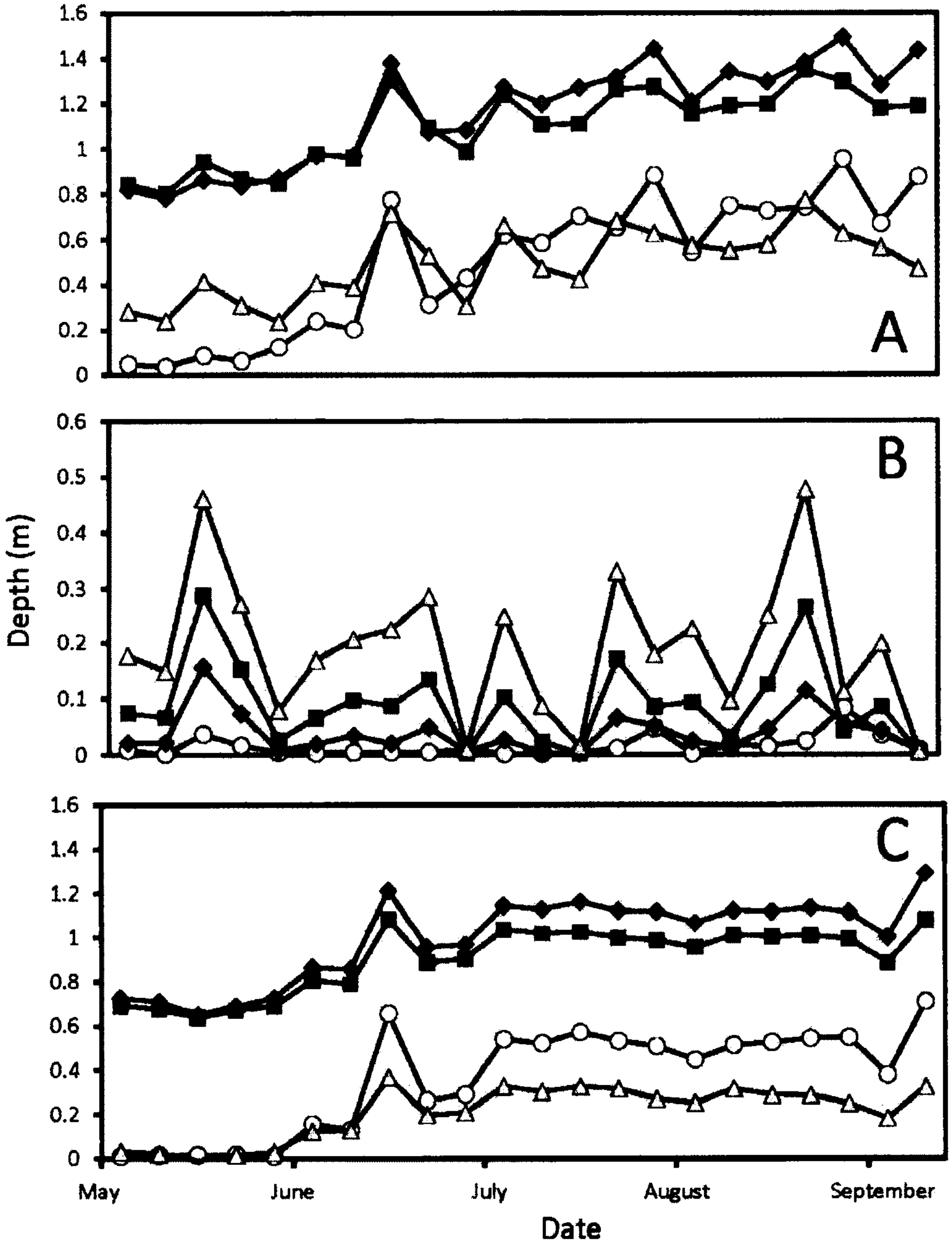

Figure 3. Water depth data plots. Plots for channels of the Fox River estuary, south-central Alaska: A) 7-day average depth; B) 7-day depth variance; C) 7-day minimum depth. Symbols indicate most upstream Channel 1(O); Channel 2(♦); Channel 3 (■); to most downstream Channel 4(Δ).

Table 2. Water depth data summary. Seasonal water depth metrics (m) from stationary loggers for most upstream channel 1 to most downstream channel 4, Fox River estuary 2011.

| Depth Metric | Channel 1 | Channel 2 | Channel 3 | Channel 4 |
|---|---|---|---|---|
| Average depth, m (var) | 0.49 (0.12) | 1.15 (0.09) | 1.09 (0.12) | 0.49 (0.22) |
| Maximum depth (m) | 1.39 | 2.77 | 3.18 | 2.94 |
| Minimum depth (m) | 0.01 | 0.65 | 0.64 | 0.01 |
| Minimum <0.4m (days) | 61 | 0 | 0 | 131 |

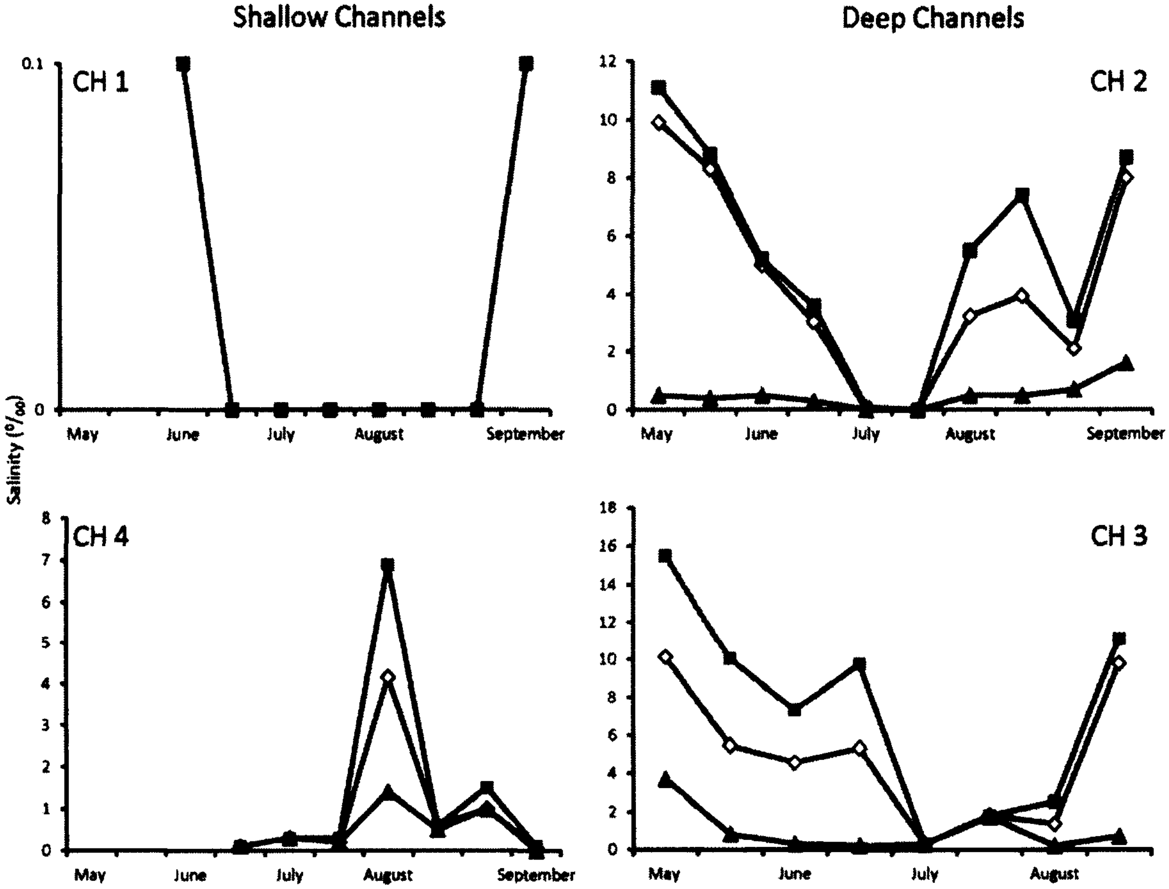

Figure 4. Salinity data plots. Point measurements of salinity from the Fox River estuary channels located in south-central Alaska, from most upstream channel (CH1) to most downstream channel (CH4) with sampling date. Line colors and markers delineate maximum (■); minimum (▲); and average (◇) of all salinity measurements taken within each channel for the sampling period.

*Fish data.—*

Fish abundance for all sites was quantified using total catch and multiple pass depletion estimates (removal). For a subset of sites, these numbers were compared to mark-recapture (m-r) estimates (used as a baseline measure with the exception of the July sampling event, during which one block net failed) to determine which technique (total catch or removal) most consistently corresponded to baseline measures of fish abundance (Table 3). Both of these metrics had a high degree of correspondence to m-r estimates ($R^2$ values = 0.73, 0.76 for total catch and removal estimates respectively). Both estimates were lower than the baseline m-r value (78% and 66% of m-r estimate on average for total catch and removal estimates, respectively), but were consistently so. We did not have sufficient sample sizes to examine correlates of bias (such as differences in channel size, depth, individual sampling technique). We therefore used the uncorrected total catch for relative fish numbers with standardized effort for description and analysis.

We captured 4,099 juvenile coho salmon, 1,586 of which we measured, composed of three age classes (0, 1, and 2). Peak capture dates occurred in late July; however, the timing of peak capture differed for each age class, with most age-2 fish captured in May and June and most age-0 and age-1 fish captured in July and August (Figure 5). Few fish were captured during the May sampling events, and higher numbers were captured in July, corresponding to a large proportion of age-0 fish in the July total catch (Figure 5).

Table 3. Coho salmon abundance metrics. Bias estimates and correlation coefficients from the Fox River estuary in south-central Alaska. Values assume mark-recapture estimates accurately reflect actual fish abundances (Rosenberger and Dunham 2005).

| Date | Total catch | Removal estimate | Mark-recapture estimate (baseline) |
|---|---|---|---|
| 5-May | 89 | 78 | 89 |
| 24-May | 70 | 64 | 83 |
| 21-Jun | 306 | 280 | 341 |
| 27-Jul | 110 | 110 | 278 |
| 24-Aug | 222 | 197 | 332 |
| 27-Sep | 77 | 69 | 80 |
| Average bias (% m-r) | 79% | 86% | |
| Correlation | $R^2$= 0.73 | $R^2$= 0.76 | |

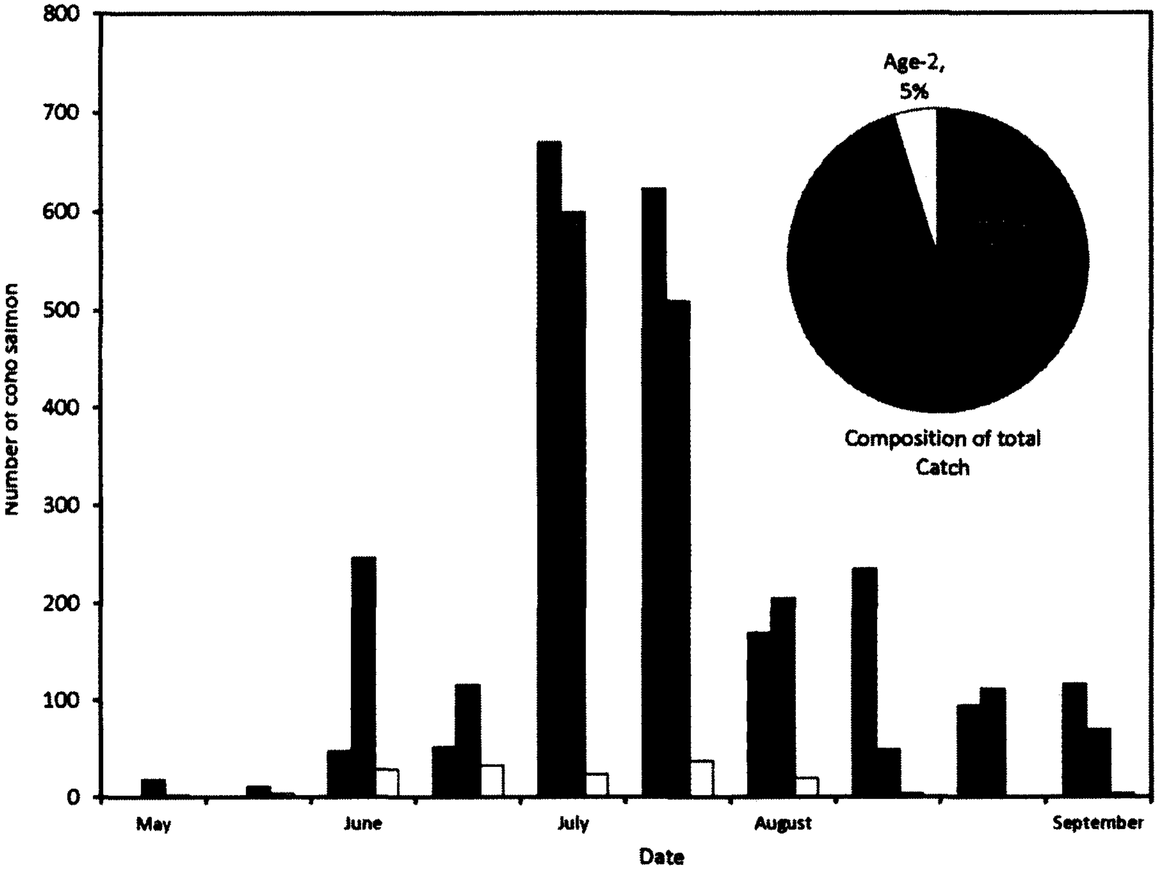

Figure 5. Coho salmon age composition and timing of catch. Coho salmon catch data are for all channels combined for the Fox River estuary channels, south-central Alaska. .

We noted spatial variability in coho salmon total catch among channels with significant, though weak, relationships to variability in channel depth and temperature ($F$= 6.57, $P$=0.01, adj. $R^2$=0.13; $F$=6.163, $P$=0.02, adj. $R^2$=0.13, depth and temperature respectively, 34 DF). Abundance was negatively correlated with minimum salinity and variance (of stationary logger readings) in channel depth and water temperature, and positively correlated with average channel depth (Table 4). The centrally located, deeper channels (2 and 3) had highest total catch throughout the season, with most salmon captured in the more upstream of these two sites (channel 2, Figure 6). The shallow, most upstream and most downstream channels (1 and 4) were seasonally available to salmon from mid-June to late August. Increases in glacial water inputs connected these shallow estuary channels from the main stem river consistently during mid-summer and for a brief period in early spring during Kachemak Bay's large (> 8 m) spring tides. When it was accessible to fish, abundance was high for coho salmon in the most upstream channel, but coho salmon abundance in the most downstream channel was consistently low throughout the season, despite apparent accessibility.

We euthanized 69 coho salmon to examine body condition. Body condition, when compared by cohort between channels, was not significantly different. The age-2 cohort was composed of a limited small sample size (n=5) that were missing entirely from one channel, and therefore we could not test for differences for this cohort (Table 5).

Table 4. Correlation coefficients (*R*) of environmental variables to fish catch. Data is shown for Fox River estuary coho salmon sampling in year 2011. Significance values (*P*) follow correlation coefficients.

| Variable | Correlation to Catch, *R* (*P*) |
|---|---|
| Average salinity ($^0/_{00}$) | -0.15 (0.38) |
| Maximum salinity ($^0/_{00}$) | -0.11 (0.53) |
| Minimum salinity ($^0/_{00}$) | -0.26 (0.12) |
| Temperature (CTU) (°C) | -0.11 (0.54) |
| Temperature variance (°C) | -0.26 (0.12) |
| Average depth (m) | 0.26 (0.13) |
| Depth variance (m) | -0.34 (0.04) |

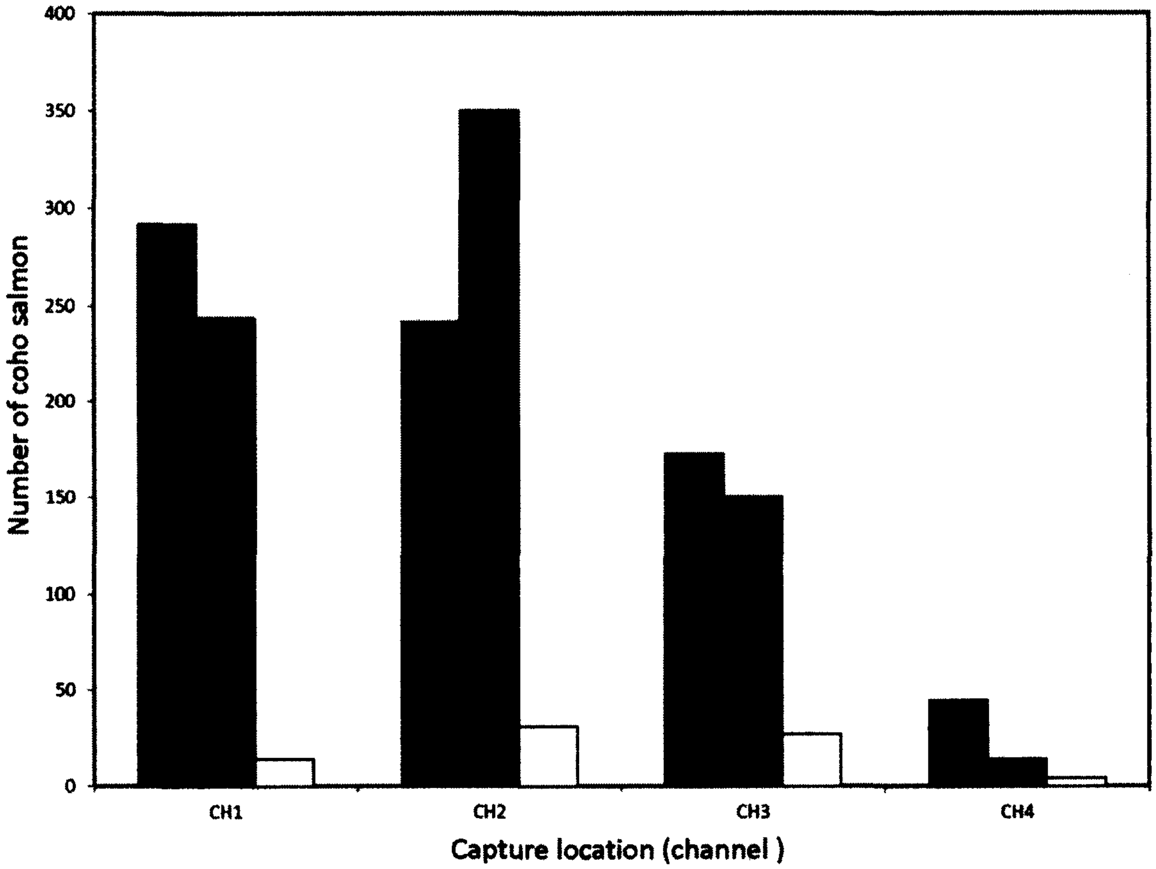

Figure 6. Coho salmon age composition by channel. Fox River estuary channels, south-central Alaska. Age classes correspond to colors: 0(■); 1(■); and 2( □); CH# indicates channel, numbered from most upstream (channel 1) to most downstream (channel 4).

Table 5. Statistical results for spatial differences in condition. Sample size and ANOVA test results for differences in Fulton's condition factor of fish captured in estuary channels, Fox River estuary, south-central Alaska.

| Age | Channel 1 | Channel 2 | Channel 3 | Channel 4 | *F* (*P*>*F*) |
|---|---|---|---|---|---|
| All | 14 | 29 | 20 | 6 | 1.19 (0.32) |
| 0 | 5 | 12 | 4 | 2 | 2.98 (0.06) |
| 1 | 9 | 15 | 14 | 3 | 0.63 (0.60) |
| 2* | 0 | 2 | 2 | 1 | * |

*Note sample size insufficient for statistical tests

## *Discussion*

Both spatial and temporal variability in coho salmon abundance were related to temperature and water depth, constrained by accessibility of channel habitats to fish. These environmental conditions varied seasonally, prior to and following the glacial melt-water runoff, and spatially, particularly in the channels most influenced by connectivity to the river. Deeper, cooler channels with less variability in both environmental measures were associated with consistent catches of coho salmon of older age classes (ages 1 and 2). Conversely, the abundances of coho salmon were lowest in shallow warm channels and/or channels with highly variable depth and water temperatures. Accessibility was also a factor contributing to seasonal patterns in catch; when water depth permitted access to the shallow upstream channel during the high glacial runoff period, abundances of salmon were high, particularly of young-of-year salmon potentially attracted to forage and/or warmer temperatures for growth. However, they declined dramatically when water levels decreased in September.

Water depth was significantly related to fish catch, suggesting it is related to habitat features (such as refuge from predation or thermal strata) particularly important for juvenile salmon in estuaries. Water depth is often altered or manipulated in watersheds for use by municipalities and agriculture through river flow alterations that provide power generation or crop production (Montgomery 2003; Mann and Lazier 2006). These changes affect estuarine physical processes by altering the freshwater flow regime (Mann and Lazier 2006), including presence and distribution of deep water and thermally suitable habitat for juvenile salmon. Low abundances associated with shallow water depths support findings of Hering et al. (2010) that showed that little movement occurred in Chinook salmon using estuary channels when water depths were <0.4 m and that these shallow, strongly tidally influenced channels were often used intermittently through the tidal cycle (abandoned at low tides). Fish abundance was also more related to water depth than salinity; we observed the highest, most variable levels of salinity in the deep, centrally located downstream channel (channel 3) with intermediate seasonal patterns of fish abundance. These data also agree with the findings of Webster et al. (2001), that water depth, rather than salinity, was more strongly related to the presence of Chinook salmon smolt. Salinity stratification in deeper channels may permit juvenile coho salmon to select microhabitats with optimal or consistent salinities.

Patterns of abundance, seasonal persistence, and distribution of cohorts among the channels within the estuary ecotone suggest that this glacial estuary serves as both rearing and transitional habitat. We observed a reverse relationship between the distance to low tide mark and the composition of fish captured; a larger proportion of age-2 fish were captured in the downstream deep channel, implying both staging and rearing, and a greater proportion of age-0 fish were captured in the most upstream channel closest to freshwater habitats, implying a stronger rearing function for this age class. The high abundances of age-0 fish pose some curiosities regarding behavior patterns in this glacial estuary. Miller and Sadro (2003) found patterns of young of year coho salmon using the upper estuary ecotone for prolonged periods (up to eight months) before returning to side channels and ponds within the lower river to overwinter. It is possible that young of year coho salmon in the Fox River estuary also exhibit this pattern, taking advantage of the warmer water temperatures, abundant prey (Walker et al. 2012, unpublished data), and turbid water to optimize growth and reduce predation risk before returning to freshwater. Individuals in these habitats may represent a unique early life history tactic, or they may be using estuaries as supplemental or complementary habitats when freshwater habitats upstream are saturated or unavailable. We did not examine movement patterns between the estuary, lower-river, or marine environments during this study, which is an important topic for future study.

We observed patterns of increasing size within age cohorts throughout the season; fish are either using these estuary channels as rearing habitats, or freshwater growth continues throughout the summer as fish enter the estuary. Although we do not present direct evidence of estuary rearing, partner studies illustrate summer estuarine residency in the less variable environments for up to 82 days (Chapter 2 of this dissertation) and specific channel use of the estuary by coho salmon for periods up to 68 days (Walker et al. 2012, unpublished data). Partner studies also demonstrate evidence of feeding in the estuary channels, with differential diet composition corresponding to available prey surrounding each channel (Simenstad et al. 2012, unpublished data). In concert, these studies illustrate that the Fox River glacial estuary is an important rearing habitat for juvenile coho salmon; individuals may use these environments to not only make the physiological transition from freshwater to saltwater, but also attain body condition conducive to marine survival.

The Fox River watershed and estuary are located at the head of Kachemak Bay. Anthropogenic alterations in this area are limited to all terrain vehicle use and cattle grazing, with little influence from chemical pollutants, and no anthropogenic flow alterations. The relatively undisturbed and undeveloped upriver habitats above the estuary are a sharp contrast to those of many of the Northern Pacific watersheds that have lost habitat quality and complexity (Bottom et al. 2005; Shaffer et al. 2009). This study argues for a more detailed observation of habitat use by juvenile salmon outside of core areas of abundance and the importance of functioning estuary habitats to healthy salmon populations. Estuaries, though largely ignored in most juvenile salmon studies, may provide key rearing environments within which to explore alternative life history tactics such as movement timing and size during ontogenetic shifts, or they may provide conditions that supplement against loss or saturation of rearing habitats in the upper watershed. We therefore argue that maintaining and restoring estuary habitats could facilitate resilience in salmon populations to both environmental changes and loss of upstream rearing habitat elsewhere.

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# Chapter 2. The Role of Contrasting Estuarine Environments as Rearing Habitats for Juvenile Coho Salmon[3]

## *Abstract*

For anadromous juvenile salmon, estuaries are typically considered transitional staging habitats prior to out-migration, and the role that estuaries play for rearing and growing is poorly understood. We examined the use of contrasting estuaries by juvenile coho salmon *Oncorhynchus kisutch* using microchemistry and microstructure analyses of sagittal otoliths to investigate the fitness consequences of estuary residence via comparisons of behavior patterns and traits. Our objectives were two-fold: 1) to determine if juvenile coho salmon were rearing in estuarine habitats; and 2) to characterize and compare patterns of expression of life history traits in juvenile coho salmon (size, age, condition, duration, and timing of estuarine occupancy) captured in two estuary environments that contrasted in size and habitat complexity. Traits significantly differed between coho salmon using estuaries and those that did not: estuary residents were larger with higher body condition and greater weights than non-residents. Coho salmon averaged 24 days of estuarine habitat use during the summer season in a snow-melt, spring-fed estuary and 39 days in a glacial-fed estuary, with fish in both estuaries showing

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[3] Hoem Neher, T. D., A. E. Rosenberger, C. E. Zimmerman, C. M. Walker, and S. J. Baird. The Role of Contrasting Estuarine Environments as Rearing Habitats for Juvenile Coho Salmon. Prepared for submission to Transactions of the American Fishery Society.

definite patterns of overwintering in estuarine and/or near shore environments. Coho salmon using the glacial estuary were composed of younger age classes with generally smaller but more variable sizes, weights, and condition in each age class. Differences in patterns of use were also observed; fish in the glacial estuary entered later and resided longer during the summer, whereas a larger proportion of older fish were captured exhibiting overwintering patterns in the snow-melt, spring fed estuary. Our findings highlight the potential of estuaries as important alternative rearing and overwintering habitats, providing supplemental habitats for those individuals that move out of upstream freshwater rearing areas due to habitat loss and/or density dependent processes depending on characteristics of the particular estuary environment and upstream habitat conditions.

## *Introduction*

Pacific salmon exhibit multiple life histories in response to variability in selection pressures and habitat conditions (Healey 1994). A 'bet-hedging' life history strategy can be linked to numerous factors, including inter-annual fluctuations in marine and freshwater environmental conditions (Gargett 1997; Beamish and Mahnken 2001; Ebersole and Colvin 2009). Further, ontogenetic shifts in the life cycle of salmon, such as smolting, hold the potential for hard selection for specific traits or behaviors, depending on environmental conditions (Quinn 2005). For example, early marine entry and pre-smolt growth just prior to entry is a time of severe selective pressure due to the physiological and environmental changes experienced by smolts. This life stage has been linked to an optimal outmigration survival 'window' that corresponds to a time period when ocean conditions provide suitable temperatures and abundant resources for growing and feeding juvenile salmon (Gargett 1997; Johnsson et al. 1997; Beamish et al. 2008). The time period and duration of the optimal window may change from year to year depending on precipitation levels, wind patterns, and solar energy inputs (Gargett 1997; Beamish et al. 2008). Fish size, body condition, and timing of marine entry are instrumental for meeting this window and ensuring coincidence with both the quantity and quality of available prey and the ability of the individual to use it (Beamish and Mahnken 2001; Hobday and Boehlert 2001). A bet-hedging strategy among and within stocks provides for a range of times and sizes of marine entry of smolt, thereby permitting persistence of populations over fluctuating ocean conditions, a central requirement for resilience to a changing climate (Hobday and Boehlert 2001; Healey 2009).

Estuaries are some of the most dynamic and productive habitats in the world, providing important nursery habitats for a wide range of marine and diadromous fish species (Kaiser et al. 2005). These areas provide ideal nursery habitats through quality foraging opportunities accompanied with lowered predation risk, warmer water temperatures, and protection from adverse weather conditions (Abookire et al. 2000). Estuaries are often defined by the magnitude and extent of the upper, middle and lower zones delineated by physical processes such as tides, river outflow, oceanic currents, and thermal stratification therein (Kaiser et al. 2005). Biota take advantage of the incredible diversity of estuarine habitats by segregating their habitat use along physical gradients of temperature, salinity, substrate composition, and tidal influence (Abookire et al. 2000; Mann and Lazier 2006). These patterns change with inter-annual variability in weather conditions (e.g., precipitation, stream discharge, and wind mixing), mediated by geomorphology and climate (Mann and Lazier 2006; Love et al. 2009).

As transitional habitats, estuaries play an important role for smolting salmon; the mixing zone of fresh and saltwater environments buffers against osmoregulatory and physiological stress (Healey 1982; McMahon and Holtby 1992; Miller and Sadro

2003; Beamish et al. 2004; Bottom et al. 2005a). Estuaries also have potential as important salmon rearing habitats; Chinook salmon *Oncorhynchus tshawytscha*, in particular, have increased survival rates (Magnusson and Hillborn 2003) and life history variability (Bottom et al. 2005a; Campbell 2010; Volk et al. 2010) with estuarine habitat use. Factors expected to influence individual smolt survival include the duration of estuary occupancy, timing of early marine entry and environmental conditions interacting with body condition (Healey 1982; Bohlin et al. 1993; Beamish et al. 2004). Strong spatial and temporal variability within estuaries may play a key role in how juvenile salmon use these habitats.

Estuaries fed by different freshwater hydrologic regimes may provide contrasting rearing environments for resident biota (Saltveit et al. 2001). Freshwater influx into northern estuaries is expected to be particularly high during snowmelt periods; however, within Alaska, many estuarine habitats are fed by glacial river systems. Glacially-fed estuaries are unique in that the peak freshwater discharge occurs in mid-summer rather than early spring, yielding cold water discharge with high sediment loads from glacial erosion during the warmest months. Differences in rearing conditions may contribute to variability in the timing and duration of estuarine use for juvenile salmon. An investigation into environmental drivers of life history variability, including contrasting estuarine environments, informs our understanding of evolutionary strategies employed by salmon (Healey 2009).

Coho salmon are ideal species with which to examine variability in life history expression related to habitat conditions because they are thought to be phenotypically plastic in life history tactics, particularly in the juvenile stage (Small et al. 1998; Smith et al. 2001; Waples et al. 2009). Coho salmon range from northern California to the northwestern coast of Alaska and are found in the greatest densities in the coastal waters of British Columbia (Pearcy 1992). This species generally has long freshwater (1-2 years) and ocean rearing phases (1-3 years), though this can vary among and within drainages and across the species range (Beamish et al. 1999; Hobday and Boehlert 2001). Coho salmon also exhibit population structuring and local adaptation in phenotypes and behaviors, similar to what has been observed for other *Oncorhynchus* species (Smith et al. 2001; Wood et al. 2008). Estuary use in coho salmon can differ by age class or life stage (McMahon and Holtby 1992); young-of-year, for example, undertake seasonal migrations between the upper estuarine ecotone and freshwater river channels and sloughs (Miller and Sadro 2003, Koski 2009). Fingerling (age-1 and -2) coho salmon are present in estuaries for relatively short periods of time (up to two months; McMahon and Holtby 1992) and, prior to this study, have had short documented residence times (up to 17 days; Chittenden et al. 2008).

Direct and unbiased documentation of estuarine habitat use by juvenile salmon is difficult, given a limited suite of tracking and marking techniques for small fish. However, our understanding of estuary residence can be enhanced by employing microanalyses of fish otoliths. Otoliths, or ear stones, develop by deposition of calcium carbonate in patterns that represent growth; they also contain several trace elements proportional to chemical concentrations from the surrounding aquatic environment (Campana 1999). The use of otolith microchemistry in combination with examination of microstructure (incremental growth layers) can therefore be used to determine patterns of habitat occupancy over ontogeny where water chemistry contrasts strongly between habitats (Nielson et al. 1985; Campana 1999, Kennedy et al. 2002; Re´veillac et al. 2008). The salinity of the surrounding environment, in particular, has been linked to ratios of strontium to calcium (Sr:Ca) deposited in otoliths, a useful feature for measuring life history patterns in diadromous fishes (Zimmerman 2005; Brown and Severin 2009). In tandem with microchemical analysis, microstructural analysis of incremental growth patterns and age of fish can allow discernment of habitat transitions through time (Campana and Neilson 1985; Nielson et al. 1985; Volk et al. 2010). It can, however, be difficult to determine and validate daily incremental growth patterns, particularly during periods of low growth (Campana and Neilson 1985). In that case, seasonal growth patterns may provide sufficient resolution to determine the life history patterns, particularly in the case of estuarine or marine versus freshwater habitat use.

In this study, we investigated and compared the ecology and life history patterns of juvenile coho salmon captured within two contrasting estuary environments. Our first objective was to determine if juvenile coho salmon were feeding and growing within estuary systems. Using otolith microanalyses, we examined timing and duration of use and correspondence with fish size, body condition, and weight of different ages of coho salmon captured within estuary channels. We anticipated that fish using estuaries for rearing would benefit from the productivity of these environments, exhibiting larger sizes and greater body condition when compared to those that did not show evidence of estuary residence. The second objective of our work was to determine if juvenile coho salmon patterns of estuary use, including timing and duration of occupancy, differed between contrasting estuarine and freshwater environments. This would suggest estuarine physical processes are important drivers of ontogenetic variability in use of estuarine environments and therefore life history expression in juvenile salmon.

### *Study area*

The work described here is a key component of a broader study examining juvenile salmon rearing habitats within the Fox River Flats Fish and Wildlife Critical Habitat Area, located within Kachemak Bay at the southern end of the Kenai Peninsula in south-central Alaska (Figure 1). Juvenile coho salmon were captured in the Anchor and the Fox River estuaries (Figure 1), primarily within the middle transitional zone of each estuary, downstream of highest tide line to channels upstream of the lowest tide line, bordered by mud flats and vegetation. The wide tidal range of Kachemak Bay and Cook Inlet (NOAA 2012) can create large ecotonal regions with diverse habitat conditions, particularly in glacial rivers with heavy silt deposition zones.

The Anchor River delta is a bar-built estuary that abruptly transitions into the marine environment, with an approximate estuary length of 0.8 km (measured from mean high water tide line to its confluence with the Cook Inlet). The Fox River delta is an open glacial-fed estuary located at the head of Kachemak Bay, approximately 27 km east of Homer and 29 km south of the mouth of the Anchor River estuary. The Fox River transitions through a large, approximately 6 km delta into Kachemak Bay, which results in a more gradual and extended estuarine ecotone between the marine environments of Cook Inlet and freshwater environments of the Fox River (Figure 1). Adult coho salmon were counted by sonar in the Anchor River as they return to spawn, with a six-year range of 2,692-18,977 fish (years 2004-2009; Kirkvliet and Booz 2012). Juvenile outmigration has been estimated since 2010 using a rotary screw trap located approximately 1 km above the high tide line (Anderson and Stillwater Sciences 2011). Fox River salmon escapement data exist only as annual single-pass aerial counts in a small tributary of the upper river; full escapement data have not been collected to date. Some information is available regarding spawning migration and timing (Faurot and Palmer 1992), but limited data exist for juvenile coho salmon abundance and outmigration for the Fox River (Walker et al. 2009).

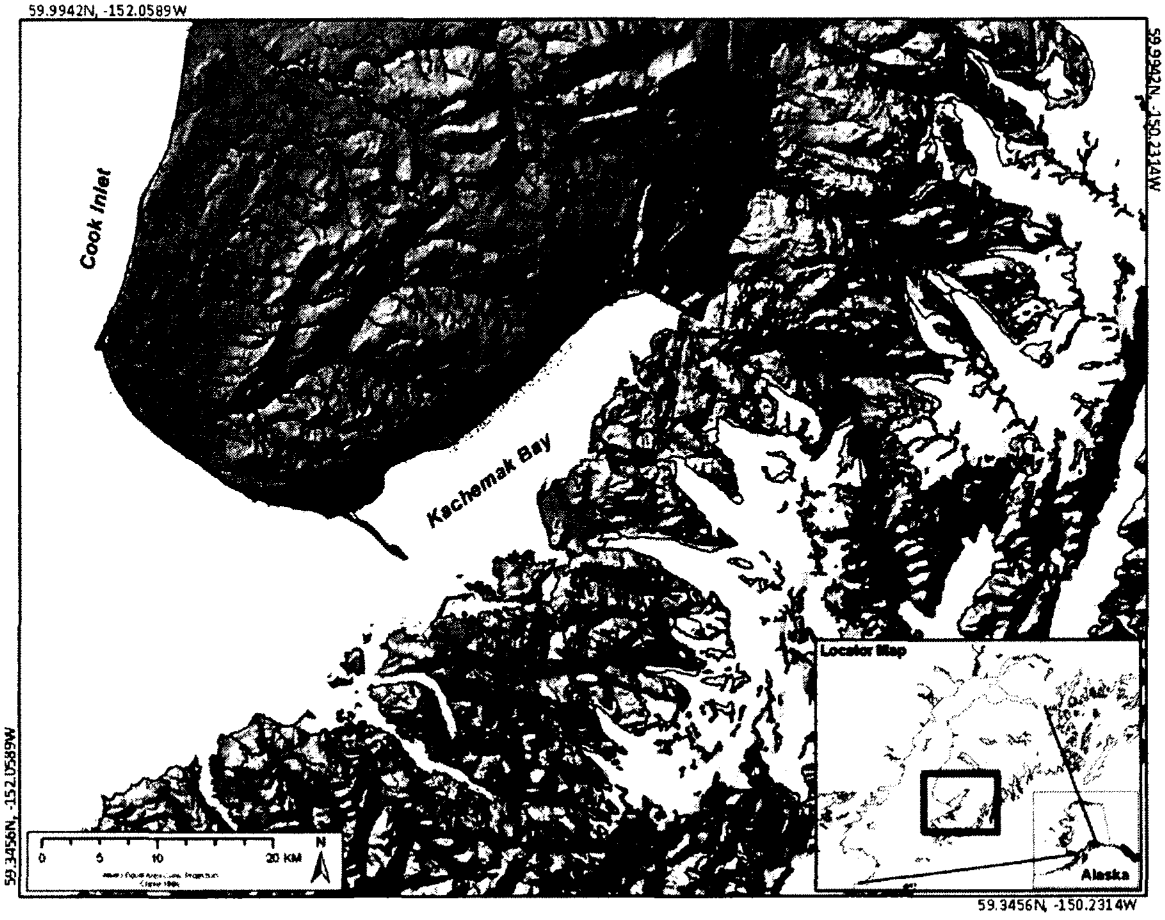

Figure 1. Study area map. The study area was located on the lower Kenai Peninsula, Alaska. The Anchor River (triangle) and Fox River (square) estuaries are outlined.

## *Methods*

### *Habitat characteristics.—*

We sampled fish and recorded environmental data in tidally influenced channels spaced between the high to low tide marks of the estuary ecotone. Temperature and depth were measured and recorded using Solinst TM 3001 level loggers (Solinst Canada Ltd., Ontario, Canada) calibrated with a Solinst TM 3000 barologger set onsite at the high tide line. Level loggers were set at 15 min recording intervals and placed in 5 cm wide by 25 cm long plastic PVC housings attached to steel fence posts driven approximately 25 cm into the substrate. Fence posts were located five meters upstream from the channel mouth in each of the six channels sampled, and one logger was placed along the margin of each river channel (Fox and Anchor Rivers). In addition, measurements were taken for each sampling event at a cross-section downstream of the fence posts for each sampling event. Thalweg depth, conductivity (direct and standardized for temperature), salinity (expressed as temperature compensatedparts per thousand, $^{0}/_{00}$), and temperature (°C with probe held just below the surface, in mid-water column, and at the channel bottom) were measured using a YSI Model 30.

*Fish capture*[4].—

Juvenile coho salmon were captured in tidally influenced side-channels of the Fox and Anchor River estuary ecotones within 25 m reaches using multiple depletion passes with a pole-seine (2.2m x 6.1m, 0.31 cm mesh) twice per month from late April through September, 2011. Prior to fish sampling, each unit was closed with block nets (2.2m x 6.1m, 0.31 cm mesh) secured along the sides and bottom with stakes to prevent fish escape. Fish from each pass were placed in separate, 19 L aerated tubs filled with water from the channel. All fish captured were identified to species and counted. Random samples of 50 juvenile coho salmon captured from each pass of the seine were anesthetized in 70 mg*$L^{-1}$ methane tricane sulfonate (MS-222) for three minutes (until fish experienced loss of equilibrium) (Bailey et al. 1998; Chittenden et al. 2008), and measured for fork length (mm). Age classes of coho were apparent by size; therefore, three juvenile coho (not exceeding 10% of the catch at each site representing small -age-0; medium -age-1; large -age-2 sizes) were randomly chosen and euthanized at each site via overdose of 140 mg*$L^{-1}$ MS-222 (held for five minutes following cessation of respiration), labeled, placed on ice, returned to the laboratory, and frozen.

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[4] UAF Institutional Animal Care and Use permit number 149489-4

*Fish condition.—*

We used water weight, wet weight, and Fulton's condition factor ($K = (W*L^{-3})*100{,}000$ where W = laboratory wet weight [g] and L = laboratory length [mm]) for metrics of condition (Jonas et al. 1996; Pope and Kruse 2007). Coho salmon were measured for fork length (±1 mm), then blotted and weighed to determine wet weight (± 0.01 g). Samples were placed in a 65-70 °C drying oven for three days, weighed, and returned to the oven for 24 hours, dried then re-weighed. Samples were considered dried when minimal change (<0.001g) was detected between consecutive daily weights (Jonas et al. 1996). Water weight was determined by subtracting the oven dried sample weight from the wet weight (Jonas et al. 1996; Sutton et al. 2000).

*Otolith microchemistry and microstructure.—*

Sagittal otoliths were removed from fish prior to condition analyses, rinsed, and stored in plastic vials. Otoliths were mounted in thermoplastic cement on sections of cover slips and glued to standard microscope slides (following Donohoe and Zimmerman 2010). Otoliths were mounted sulcus down, and the sagittal plane was ground with 2000-grit sand paper to expose a clean, flat surface. The sample was reheated, turned over to expose the sulcus, and ground to expose the nucleus (described in Zimmerman 2005 and Donahoe and Zimmerman 2010). The sample was labeled, age determined, and the cover slip cut to remove the mounted sample. The sample was then glued in a 2.54 cm diameter circle centered on a petrographic slide for analysis. Once the slide was filled, it was washed, rinsed with deionized water, and allowed to air dry prior to processing.

We used the Laser-ablation Inductively Couple Plasma Mass Spectrometer (Agilent mass spectrometer 7500ce fitted with a CS lens stack combined with a New Wave UP213 laser, La-ICPMS) housed at the Advanced Instrumentation Laboratory of the University of Alaska Fairbanks to complete the microchemical analyses (Brown and Severin 2009; Volk et al. 2010). Transects were ablated from the primordia perpendicular to the growth increments into the mounting medium beyond the distal edge of one otolith from each fish. Count data were collected for the elements strontium ($^{86}$Sr, $^{88}$Sr) and calcium ($^{42}$Ca, $^{43}$Ca). Calcium ($^{43}$Ca) was used as an internal standard and background-subtracted counts of Sr were adjusted to Ca and calibrated to glass standard reference material (NIST 610, National Institute of Standards and Testing). Calibration standards were run between ten samples or less depending on the number of samples on the slides and one sample duplicate (both sagittal otoliths from one fish) was run for the entire batch. Laser speed was set at 5µm/s with a 25 µm spot size on a single pass transect set to 80% power. The elemental count per second output of the La-ICPMS was then converted to concentration and sampling distance using the elemental weights for each constituent and the laser settings, respectively. Strontium-to-calcium ratios were then calculated for each of the distance measures. Otoliths were photographed under 4x, 10x, 20x, and 40x magnifications using a Leica DM1000 compound light microscope fitted with a Leica DFC425 digital camera housed at the Alaska Science Center (Anchorage, Alaska). Images were taken using a 1000 μm stage standard at all magnifications to calibrate otolith measurements and the images digitally processed to enhance clarity of incremental growth patterns. ImageJ software (version 1.46h, http://imagej.nih.gov) was used to process digital images and to overlay distance-ratio graphs on the image, calibrated to the laser distance.

Estuarine residence time was determined using incremental growth marks on otoliths from juvenile salmon captured in the estuary (Nielson et al. 1985, Miller and Simenstad 1997). Incremental growth is defined as a ring of alternating zones of translucent and opaque matrix material, measured from the visible point proximal to the nucleus where each translucent (or dark bands when viewed under light microscopy) begins, across the opaque band to that same point on the next band (Nielson et al. 1985). We defined daily growth within the estuary as the number of incremental growth bands following the point of estuarine entry determined by the Sr: Ca ratio inflection point. The inflection point, or estuary signature, was defined as an abrupt increase in the Sr: Ca ratio (visually determined as the consecutive ratio increase of >0.3 per reading with levels remaining >1.0 following the freshwater mean ratios, see Figure 2). Fish were categorized according to the presence/absence of an estuary signature, and incremental growth analysis was completed on those with estuary signatures to determine residence time. Residence time was determined by visually identifying and digitally marking the inflection points on the digital image of the laser transect overlaid by the distance-matched (µm) Sr: Ca ratio graph. Inflection points often correspond with dark banding, identified by some researchers as an estuary growth check (Lind-Null and Larsen 2011). These growth checks, though not always easily identifiable or consistent among individuals, corresponded to inflection points and provided additional support in identifying these points of estuary entry. Growth increments were counted along two different radii from the distal edge of the otolith to the inflection point to determine days of residence (Figure 2). If counts differed between readings, a third count was made, and the median of the three counts was used. Dates of estuarine entry were calculated from the date of capture less the number of growth increments (days). Comparisons were made using summer season residence times calculated for the overwintering fish group as the date of capture less the incremental growth count (days) to the first discernible increment. The growth increment to time relationship was validated by marking a small sample of fish (n=4) with Alizarin Complexone (Zimmerman 2005), holding them in a small net pen in an estuary channel for six days, euthanizing the fish, and counting the increments past the Alizarin mark on prepared otoliths.

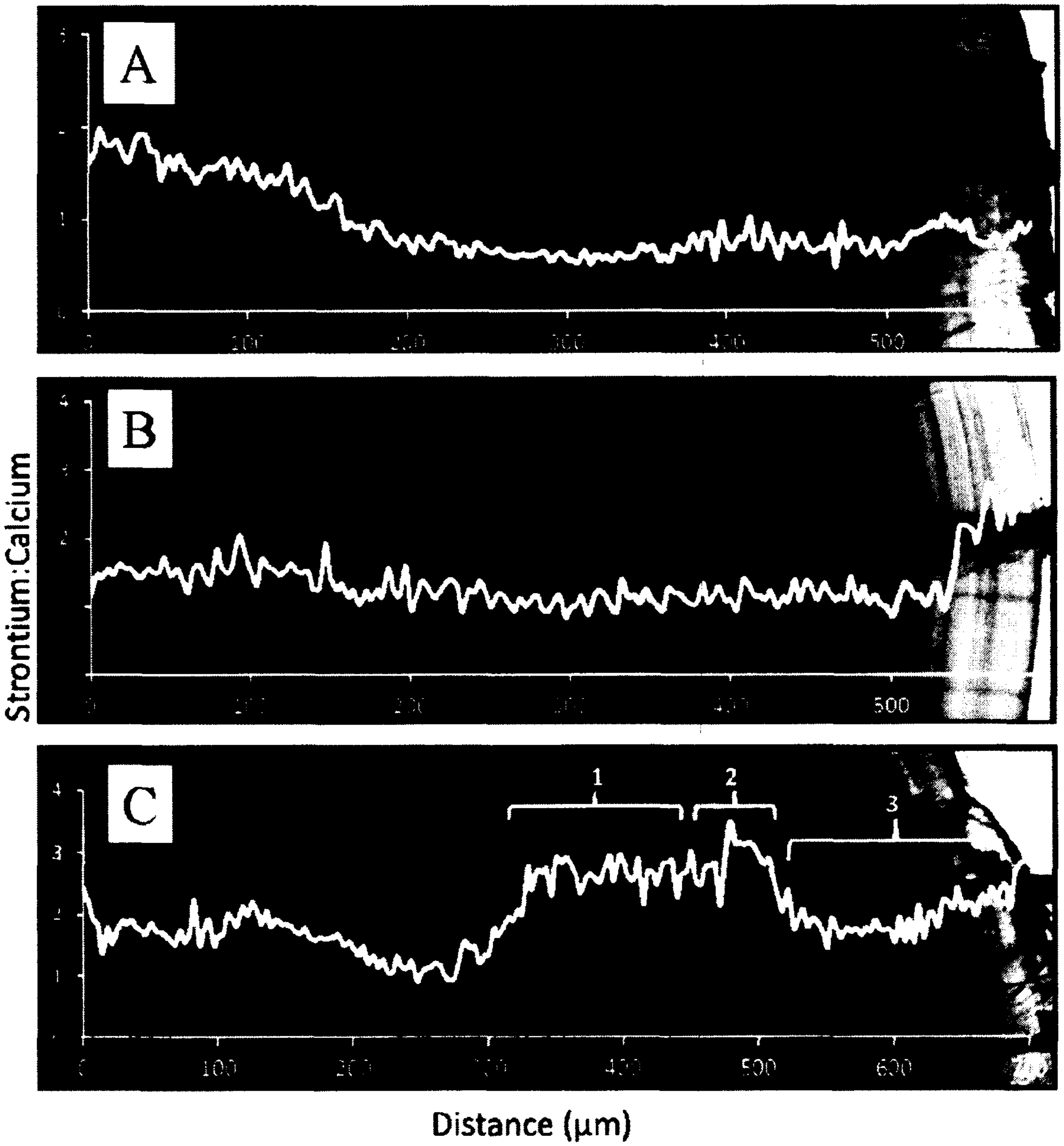

Figure 2. Patterns of Sr:Ca observed on otoliths. Images of otoliths of coho salmon with Sr: Ca ratio graphs overlaid with laser transect distances from the Fox and Anchor River estuaries, south-central Alaska. Different estuary use patterns are depicted: A) no estuary use; B) summer season estuary use signature; and C)Estuary use signature with overwintering and variable use of salinities, inset 1) summer estuary signature; 2) winter estuary signature; and 3) second summer estuary use signature with changing seasonal salinity patterns.

*Statistical analyses.—*

Continuous water level data were summarized as seven-day mean, minimum, and maximum depths for each estuary channel and the main stem river. Continuous temperature data were summarized as 7-day average and cumulative thermal units (daily average summed for the season). Point measurements of salinity collected at each sampling event were combined and expressed as monthly mean, minimum, and maximum recordings. Our protocol was to sample evenly across age classes for fish retained for laboratory analyses such that the composition of the laboratory fish sample did not correspond to catch composition. We therefore ran analyses to compare size, age class composition, and capture date based on two sub-samples of the total catch; those that were caught, measured, and released (hereafter measured group), and those euthanized and analyzed in the laboratory (hereafter laboratory group). Age class composition of the measured group was inferred via length-frequency histograms validated with otolith age for each sampling event. We compared data from laboratory fish between estuaries, separated by age class and presence or absence of an estuary growth signature (based on otolith analysis), to determine differences in use patterns (duration, time of entry) and general condition (Fulton's condition, dry weight, water weight). To test for differences in age class composition between estuaries and in collected samples versus catch compositions, we used chi-square goodness of fit tests. For residence times, entrance date, condition, size, dry weight, and water weight, comparisons were made between estuaries using student's unpaired two sample t-tests for each of the two groups (measured group and laboratory group) separated by age. Pooled comparisons between estuaries and signatures (estuary signature versus lacking a signature) were made using catch-composition weighted data. Data were tested using a general linear model for each comparison (between estuary or between signature) with each variable (size, weight, entry date, condition). We used a three-way catch-weighted analysis of variance to determine if age, estuary, or estuary signature contributed to variance of fish size, condition, entry date, and residence in the laboratory group. Fish size, condition, and weight data were square-root transformed to meet homogeneity assumptions. Equal variance was tested using *F*-tests for equal variance for single variable comparisons. If samples had unequal variances and could not be transformed to meet this assumption, a Welch two-sample, unpaired *t*-test was used for comparison.

## *Results*

### *Habitat features.—*

Temporal trends in habitat features followed trends and differences anticipated for snowmelt versus glacially-fed estuaries. Minimum salinities were higher and more variable in the snow-melt fed Anchor River estuary channels, particularly in midsummer ($t = 1.32$, $P < 0.001$, 18 DF, Figure 3). Data from the stationary loggers placed in the sampling sites showed expected patterns in trends associated with each watershed type: the glacial Fox River showed seasonal increases in water depth and decreases in temperature associated with the mid-summer glacial run-off, whereas the snow-melt and spring-fed Anchor River exhibited peak water depths and coolest temperatures in the early spring (Figure 4). The highest 7-day average water temperatures occurred in late May (13.3 °C) and late July (15.3 °C) for the Fox and Anchor River estuaries, respectively (Figure 4).

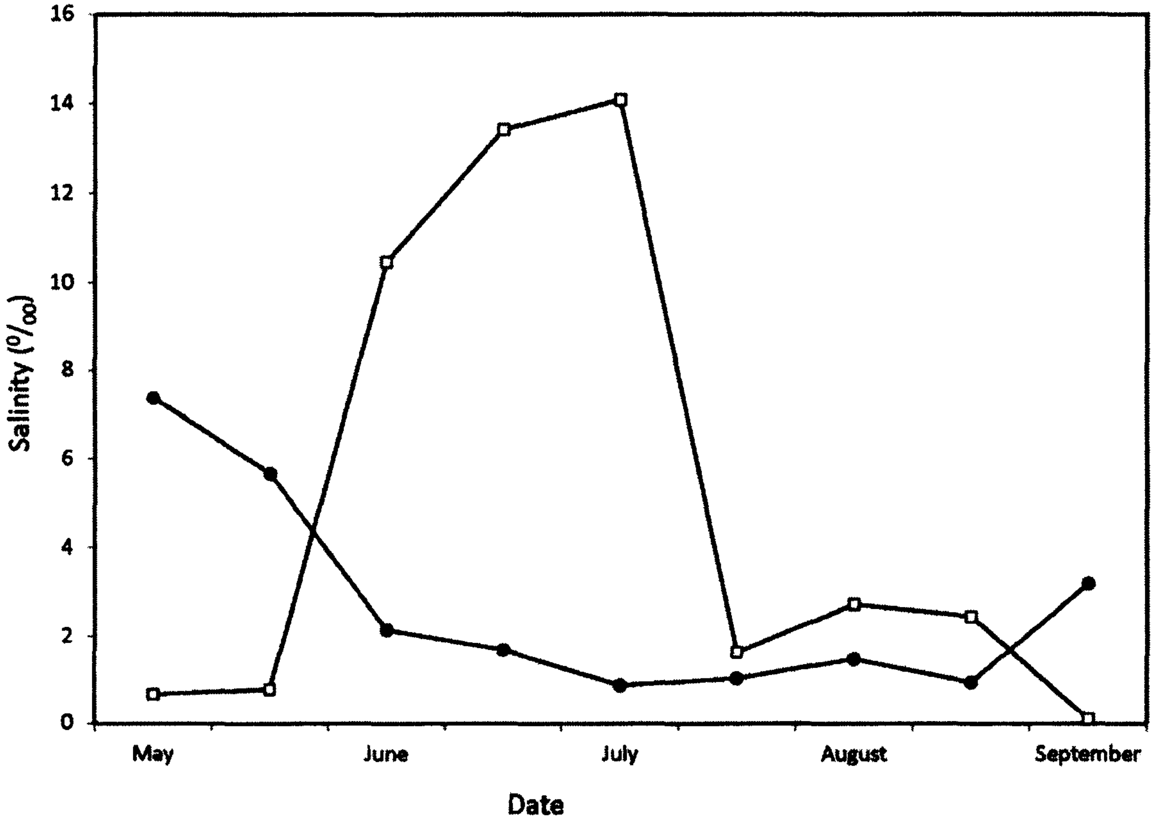

Figure 3. Salinity data plots. Point measurements of salinity (measured in parts per thousand ($^0/_{00}$) by seasonal time period of fish sampling in the Fox (dark) and Anchor River (light) estuaries.

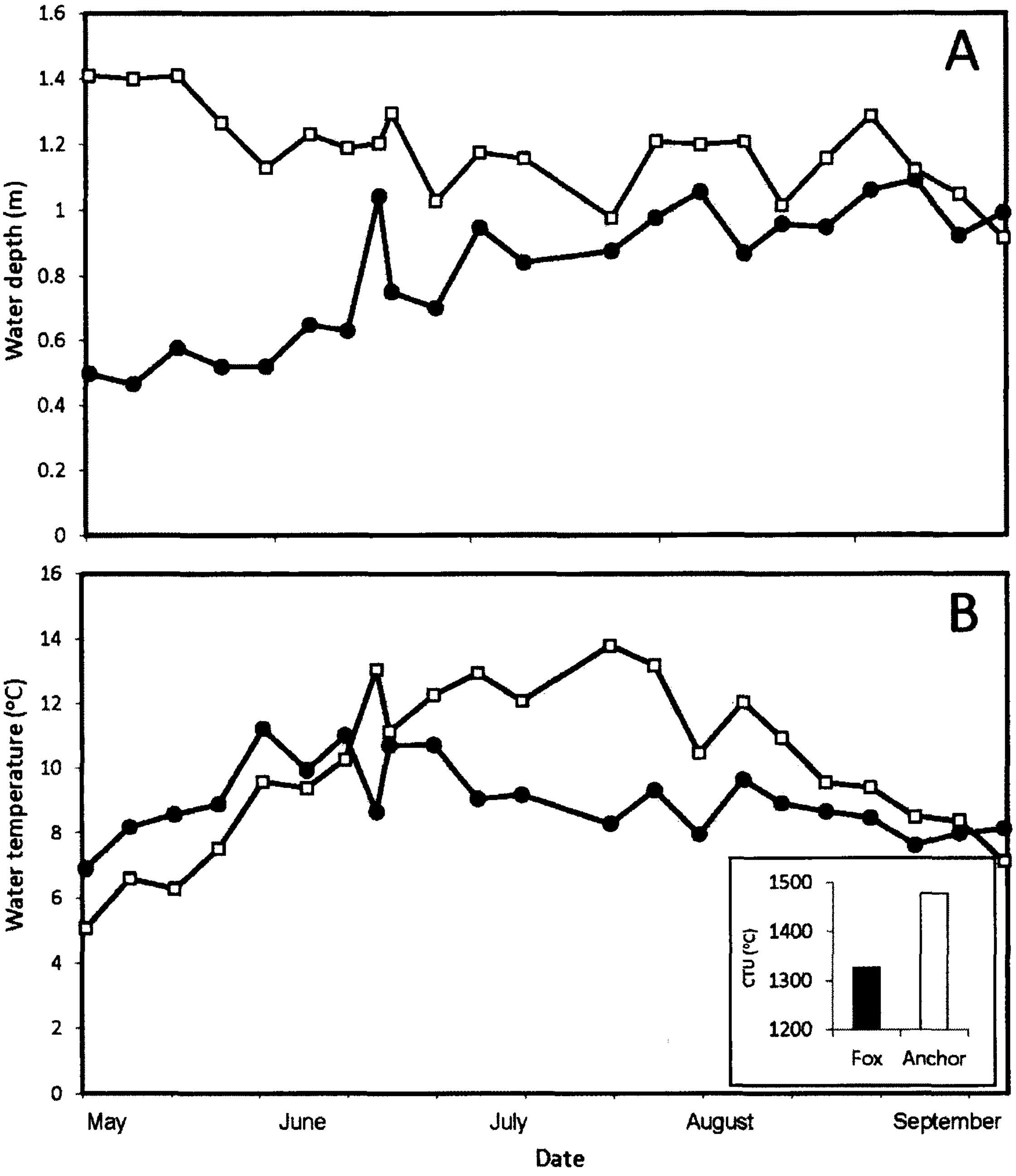

Figure 4. Water temperature data plots. Seven-day average water levels (A) and water temperatures (B) for all loggers deployed in the Fox River (dark) and Anchor River estuaries (light), Seasonal cumulative thermal units (sum of daily average) are for each river shown in B inset.

*Fish.—*

We captured a total of 1,743 and 4,232 coho salmon, measuring 532 and 1,621 individuals for fork length in the Anchor River and Fox River estuaries, respectively. We euthanized and retained 73 fish from the Fox River estuary and 35 from the Anchor River estuary for laboratory analysis.

Three age classes of coho salmon were captured in both estuaries (0, 1, 2), though the relative dominance of age classes within the measured group differed significantly between estuaries ($\chi^2 = 338.4$, $P < 0.001$, 2 DF, Table 1, Figure 5). Fish captured in the Fox River estuary were primarily composed of younger age classes (age-0 and age-1 fish), with less than five percent of the catch composed of age-2 fish. The Anchor River estuary measured fish group was composed of over 20 percent age-2 fish and had a smaller proportion of age-1 fish than that of the Fox River (Table 1). We were restricted in retaining age-2 fish for individual analysis from the Fox River estuary due to low catch rates of this age class in the system.

Table 1. Age composition summary. Data for measured and laboratory fish groups for the Fox River and Anchor River estuaries, Alaska. The number of fish showing estuary use is denoted for the laboratory fish group.

| Measured Group Composition | | | | |
|---|---|---|---|---|
| | Age-0 | Age-1 | Age-2 | Total |
| Fox | 785 | 760 | 76 | 1621 |
| Anchor | 291 | 133 | 108 | 532 |
| Total | 1076 | 893 | 184 | 2153 |
| Laboratory Group Composition | | | | |
| | Age-0 | Age-1 | Age-2 | Total |
| Fox (with estuary signature) | 24 (6) | 45 (17) | 4 (1) | 73 (24) |
| Anchor (with estuary signature) | 9 (3) | 14 (11) | 12 (10) | 35 (24) |
| Total (percent with estuary signature) | 33 (41) | 59 (48) | 16 (69) | 108 (44) |

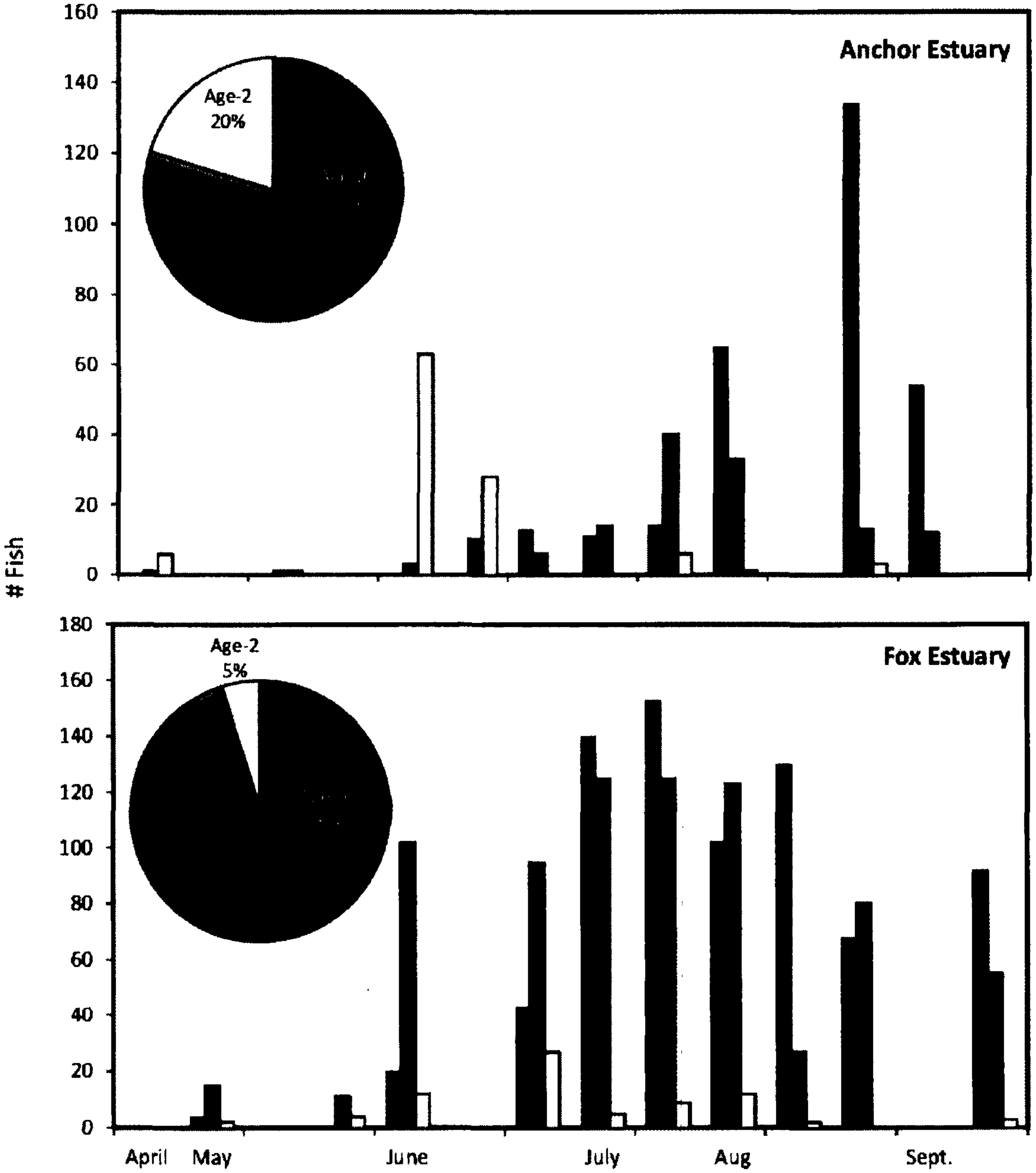

Figure 5. Total catch and age composition plots. Comparison of total catch of coho salmon separated by age class in the Fox and Anchor River estuaries, Alaska. Inset pie charts illustrate age composition of catch; colors delineate ages: age-0 (black), age-1 (grey), age-2 (white).

A substantial proportion of laboratory group fish displayed elevated Sr:Ca signatures, indicating growth within the estuary (44%, n = 48 of 108 collected fish). Of these, ten individuals overwintered in saline environments (either estuarine or near-shore environments) according to Sr:Ca ratios; 13 exhibited summer season use patterns of residence in saline environments followed by use of fresher water environments (e.g. Figure 2A). Of the 35 Anchor River fish and 73 Fox River fish analyzed, 24 from each exhibited estuary use signatures; the Fox River fish showed a significantly lower proportion of fish with estuary signatures ($\chi^2 = 22.7$, $P < 0.001$, 2 DF; Table 1). Only two fish from the Fox River estuary showed overwintering signatures (one individual from age classes 1 and 2).

Between estuaries, use differed among age classes of juvenile coho salmon. Most age-2 individuals were captured in April through June; age-1 individuals dominated the June and early July catches, and age-0 individuals were not captured until later in June. Disparity in patterns of capture, residence times, and entry dates were apparent in comparisons of fish captured in the two estuaries. The highest total capture of coho salmon occurred in late August and late July for the Anchor River and Fox River estuaries, respectively (Figure 5). Fish using the Anchor River estuary entered earlier in the summer season and had shorter and less variable residence times than those captured in the Fox River estuary; however, these differences were not statistically significant for pooled data (pooled, catch composition weighted data for laboratory group with estuary signatures, entry dates: $F = 1.71$, $P = 0.20$, DF= 46; residence: $F= 2.06$, $P = 0.16$, DF= 463.69, Table 2). Some variables were significant when analyzed by age class (age-0, Entry date: $t = -2.50$, DF = 30, $P = 0.02$; Condition: $t = -1.92$, DF = 30, $P = 0.06$), but this was not common.

Generally, fish captured and measured within the two estuaries differed in size, weight, and body condition (Table 3). Anchor River mean size (FL) at age was significantly larger and less variable for all age classes of fish separately in the measured group (Age-0:$t = -151.15$, $P < 0.01$, 306 DF; Age-1:$t = -6.22$, $P < 0.01$, 889 DF; Age-2: $t= -3.35$ $P < 0.01$, 108 DF, Table 4, Figure 6) when compared to fish captured in the Fox River estuary. Fish in the laboratory group from the Anchor River estuary were generally larger and had higher weights and body condition; however, these differences were not statistically significant between estuaries with pooled, weighted data but could be noted when comparisons were made by separate age classes (Table 4).

Table 2. Estuary use data summary. Mean and median residence times and entry dates for the laboratory group of coho salmon captured in the Fox and Anchor rivers, Alaska. Note that the entry date for fish lacking an estuary signature was calculated using the capture date.

| | Average Summer Season Residence | | |
|---|---|---|---|
| | Age-0 | Age-1 | Age-2 |
| Fox | 49.3 | 39.2 | 6.0 |
| Anchor | 36.3 | 29.7 | 14.8 |
| | Mean Entry dates (laboratory fish) | | |
| Fox | 7/10/2011 | 7/8/2011 | 6/12/2011 |
| Anchor | 8/6/2011 | 6/24/2011 | 5/25/2011 |
| | Mean Entry dates (estuary signature) | | |
| Fox | 7/3/2011 | 6/26/2011 | 5/22/2011 |
| Anchor | 7/2/2011 | 6/17/2011 | 5/21/2011 |
| | Mean entry date (no estuary signature) | | |
| Fox | 7/13/2011 | 7/18/2011 | 6/17/2011 |
| Anchor | 8/23/2011 | 7/13/2011 | 7/4/2011 |

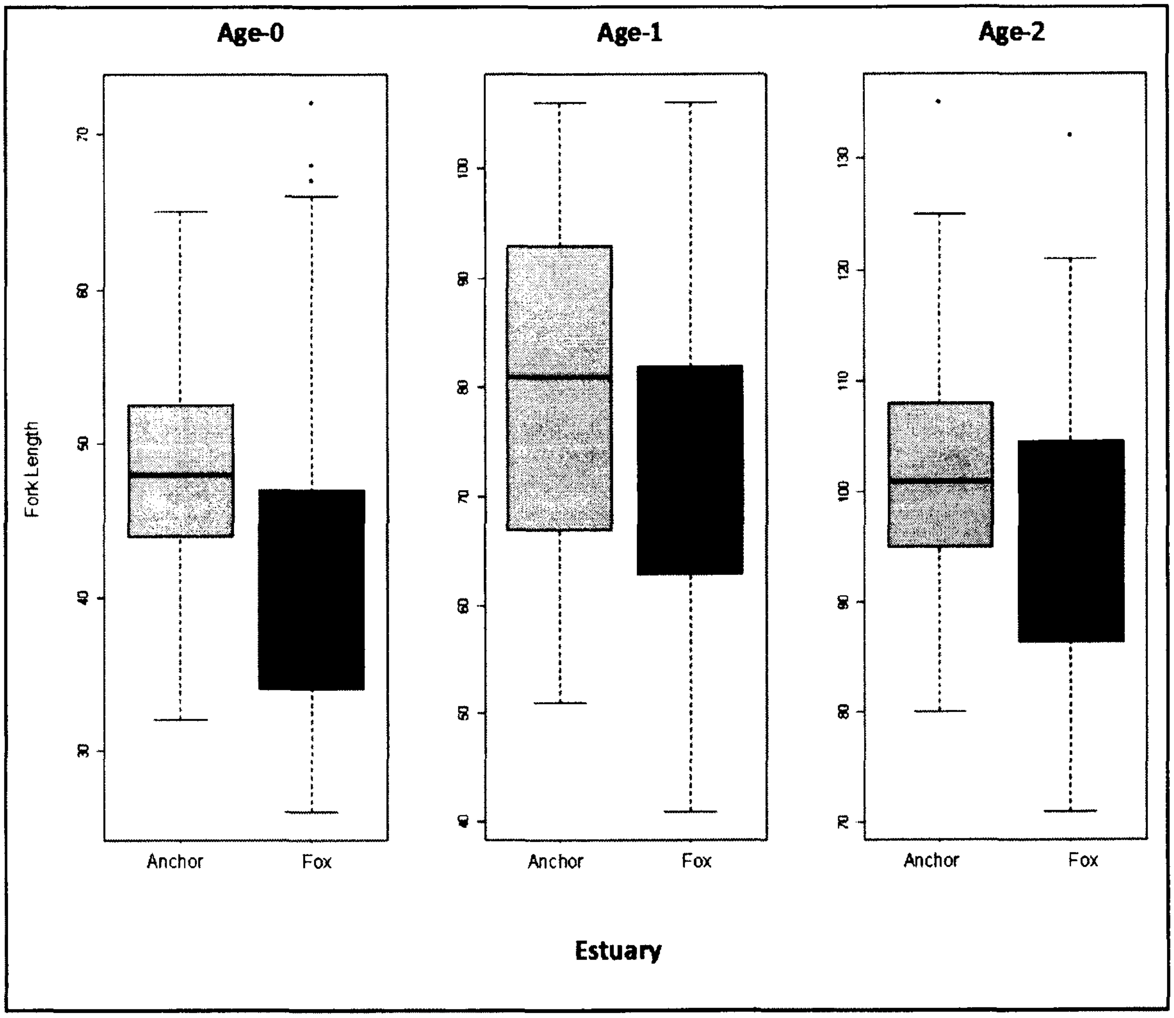

Figure 6. Box plots of trait differences of coho salmon (estuary groups). Statistically significant differences in trait patterns of comparisons of fish between estuaries by age class in the Fox river (dark bars) and Anchor river (light bars) estuaries, Alaska.

Table 3. Coho trait data summary. Mean size, weights, and condition for measured (n=2153) and laboratory (n=108) groups of coho salmon captured from the Anchor and Fox Rivers, as indicated, Alaska.

| | Age-0 | Age-1 | Age-2 |
|---|---|---|---|
| Mean size (measured group) | | | |
| Fox | 40.68 | 72.86 | 85.34 |
| Anchor | 48.07 | 80.75 | 101.40 |
| Mean size (laboratory group) | | | |
| Fox | 41.91 | 76.11 | 90.75 |
| Estuary signature | 51.50 | 79.10 | 80.10 |
| No signature | 38.50 | 74.10 | 93.00 |
| Anchor | 48.22 | 77.90 | 99.75 |
| Estuary signature | 53.30 | 79.10 | 98.10 |
| No signature | 45.67 | 71.50 | 108.00 |
| Mean dry weight (laboratory group) | | | |
| Fox | 0.15 | 1.13 | 1.47 |
| Estuary signature | 0.30 | 1.22 | 0.96 |
| No signature | 0.09 | 1.08 | 1.60 |
| Anchor | 0.25 | 1.04 | 1.99 |
| Estuary signature | 0.32 | 1.11 | 1.91 |
| No signature | 0.18 | 0.70 | 2.40 |
| Mean condition (Fulton's, laboratory group) | | | |
| Fox | 0.91 | 1.08 | 1.05 |
| Estuary signature | 1.12 | 1.12 | 1.14 |
| No signature | 0.84 | 1.06 | 1.03 |
| Anchor | 1.05 | 1.12 | 1.00 |
| Estuary signature | 1.12 | 1.15 | 1.00 |
| No signature | 1.02 | 0.99 | 1.02 |
| Mean water weight (laboratory group) | | | |
| Fox | 0.59 | 4.02 | 5.72 |
| Estuary signature | 1.11 | 4.26 | 4.02 |
| No signature | 0.41 | 2.87 | 6.15 |
| Anchor | 0.89 | 3.96 | 7.30 |
| Estuary signature | 1.24 | 4.15 | 6.80 |
| No signature | 0.71 | 2.89 | 9.79 |

Table 4. Statistical results for fish trait comparisons between estuaries. Results for coho salmon for each age class. Fish were captured in the Fox River and Anchor River estuaries in south-central Alaska. Only tests with probability values <0.10 are reported.

| Metric | Statistical significance | Pattern |
|---|---|---|
| | Age-0 | |
| Size | $t = 61.27$, $P < 0.01$, 751.30 DF | Measured fish, Anchor fish larger |
| Entry Date | $t = -2.50$, $P = 0.02$, 30 DF | Laboratory fish, Anchor fish earlier entry date |
| Condition (Fulton's) | $t = -1.92$, $P = 0.06$, 30 DF | Laboratory fish, Anchor fish higher condition |
| | Age-1 | |
| Size (FL) | $t = -5.95$, $P < 0.01$, 889DF | Measured fish, Anchor fish larger |
| | Age-2 | |
| Size (FL) | $t = -3.36$, $P < 0.01$, 182 DF | Measured fish, Anchor fish larger |

The age and presence or absence of an estuary signature (over estuary type, e.g. glacial or snowmelt-fed) significantly contributed to variability between traits (size, condition, dates of entry, and weights). When fish with estuary use signatures were compared to those lacking signatures, fish lacking estuary use had significantly earlier and more variable dates of entry ($F = 13.44$, $P < 0.01$, 103 DF; Table 4, Figure 7). Fish using the estuary were significantly greater in size, water weights, and had higher condition when samples from both estuaries were pooled (size: $F = 5.75$, $P < 0.01$, 103 DF; water weight: $F = 3.79$, $P = 0.05$, 103 DF; condition: $F = 13.12$, $P < 0.01$, 103 DF, Table 5, Figure 7).

In summary, the greatest variability in fish traits could be accounted for by the age class of the fish and the presence or absence of an estuary use signature. Patterns of trait differences between estuaries were apparent, though not statistically significant given our limited sample sizes of estuary residents. Fish using the Anchor River estuary showed a higher proportion of overwintering use, and summer season composition of residents was higher in older individuals with greater body conditions, larger sizes, earlier entry dates, and shorter residence periods when compared to fish in the Fox River estuary. All fish exhibiting estuary use were significantly larger, had greater weights, and higher body condition than those lacking estuary use signatures.

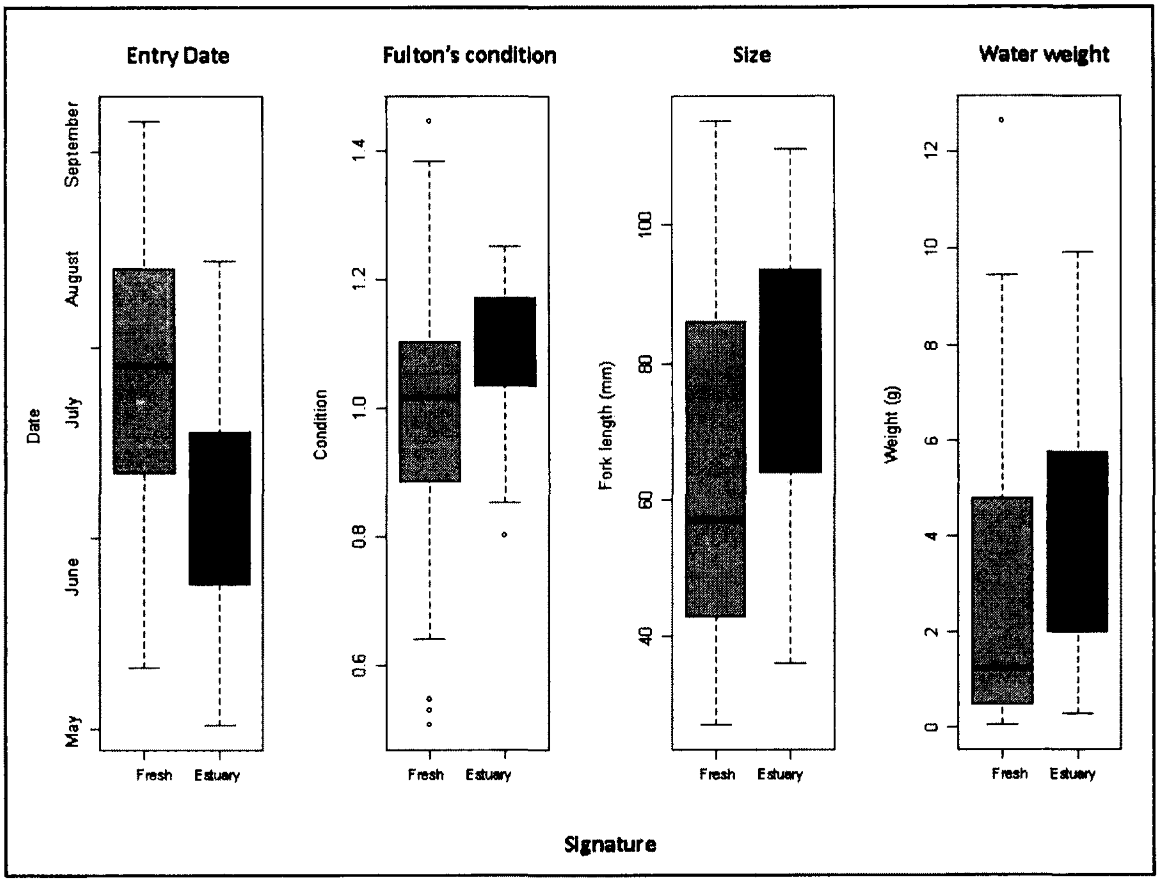

Figure 7. Box plots of trait differences between coho salmon (signature groups). Statistically significant differences in trait patterns of comparisons between fish with (dark bars) and without (light bars) estuary signatures captured in the Fox and Anchor River estuaries, Alaska.

Table 5. Statistical results for fish trait comparisons between signatures. Results are shown for pooled, catch-weighted by age analyses for coho salmon evidencing estuary use and those lacking use patterns in the Fox River and Anchor River estuaries in south-central Alaska.

| Metric | Statistical significance | Pattern in estuary-signature fish |
|---|---|---|
| | Both estuaries, pooled and catch-weighted | |
| Condition (Fulton's) | $F = 13.12$, $P < 0.01$, 103 DF | Higher, less variable condition |
| Dry weight | $F = 3.34$, $P = 0.07$, 103 DF | Higher, less variable dry weight |
| Water weight | $F = 3.79$, $P = 0.05$, 103 DF | Higher, less variable water weight |
| Entry date | $F = 13.44$, $P < 0.01$, 103 DF | Later entry dates |
| Size (FL) | $F= 5.75$, $P = 0.02$, 103 DF | Larger, less variable size |

### *Discussion*

The estuaries in our study play far more important and prolonged roles than previously discussed for juvenile coho salmon (McMahon and Holtby 1992; Thorpe 1994; Magnusson and Hillborn 2003). Juvenile coho salmon in all cohorts used estuaries for extended periods of time, including overwintering in estuaries or near-shore areas, and these patterns of use differed between estuaries. The smaller, snow-melt spring-fed estuary had larger, older fish that overwintered in the estuary or near shore environments, and these fish used the estuary for shorter and earlier summer season periods prior to outmigration than juvenile coho in the glacial estuary. Fish in the Fox River estuary were composed of younger age classes with longer residence times and few estuarine overwintering fish. Direct measurements of residence of older cohorts (ages 1-2) described in other bodies of work were substantially shorter than those in our findings (up to 16 days [Chittenden et al. 2008] and up to 18 days [Miller and Sadro 2003]). Our findings also suggest variable use of estuaries by young of year and older cohorts of coho salmon. Miller and Sadro (2003) and Koski (2009) discuss the potentially important role of the 'nomad' or young-of-year coho salmon that spend up to eight months in the upper estuary ecotone and then return to freshwater to overwinter. Although a large proportion of young of year migrants exhibited summer season patterns of movement between freshwater and estuaries, we observed no evidence of movement to freshwater environments to overwinter. The discrepancy here could be due to differences in the relative size and the definition of the estuary ecotones between our study and others and also by differences in methods; our work uses otolith microanalyses to directly measure use of higher salinity environments as opposed to inference from mark-recapture, which may be biased to smaller time periods and short-distance movements (Gowen et al. 1994)

The overwintering strategy found in our study is of particular interest in both its theoretical application regarding life history variability and in its apparent uniqueness to central Alaskan coastal populations, although few studies exist to address it. This estuarine/marine overwintering life history pattern may be in response to a saturated or poor-quality lower-river rearing habitat (Murphy et al. 1997) or, conversely, high estuarine habitat quality; alternatively, it could represent exploitation of higher coastal productivity, forage, and near shore habitat quality. We note that incorporation of materials into the otolith matrix and our sampling regime do not allow us to distinguish between overwintering in the estuary channels themselves or the near shore environments of Kachemak Bay and Cook Inlet. The possibility exists that one life history tactic of coho salmon is to enter the near-shore marine environment and rear by moving between a number of fjords and estuary habitats such as those that exist along the shoreline of Kachemak Bay and Cook Inlet. Further research is necessary for an understanding of the drivers and full range of overwintering areas used by these estuarine-resident juveniles.

Although we did not examine the mechanisms driving differential patterns of estuarine habitat use, we speculate that differences in timing of use among estuaries may be due to spatial variability in water turbidity, temperature regimes, and channel depths. Channel depths are an important documented factor relating to the use of estuary side channels by juvenile salmon (Miller and Simenstad 1997; Webster et al. 2001; Hering et al. 2010). Water depth in the Fox River estuary channels increased gradually from mid-June to late August, when glacial run-off peaks, leading to cooler and less variable water temperatures. Anchor River estuary channels were deepest in early spring during peak snow-melt and become most shallow and warm in mid-July and early August until the fall rains began in late August. We captured most fish in late August and late July in the Anchor and Fox River estuaries, respectively, suggesting a suitable combination of water temperature and channel depth to accommodate most estuary use.

Our study raised several interesting questions regarding the importance of the freshwater environment and watershed characteristics and their influence on exploitation of the estuarine environment– a point for future investigation. The influence of the watershed type and availability of suitable upstream rearing habitat may play a role in estuary use. Murphy et al. (1997) discuss the importance of lower-river freshwater areas in large glacial river systems for juvenile salmon rearing. The Fox River watershed is located in a smaller, more constrained valley and lacks the lower-river freshwater back-channel areas discussed in Murphy's work, whereas the Anchor River has numerous side-channel lower-river areas that may contribute to the differences in fish size and residence times we observed. The Anchor River estuary had a large proportion of older, larger resident fish with early entrance dates and shorter summer residence times, whereas the Fox River estuary had a smaller proportion of younger residents entering later and staying longer. This suggests that more suitable and extensive freshwater rearing habitat upstream may exist in the Anchor watershed (to allow for greater growth prior to estuary entry) and that temperature differences (cold glacier melt water versus warmer snow-melt and spring water) may contribute to patterns in growth and emergence timing. All but two of the Anchor river age-2 fish exhibited estuary overwintering, implying that the estuary environments may play an important role in this system, despite its small extent. We did not determine the overall proportion of fish using the estuary during the juvenile phase in each population. It is possible that fish using the estuary for any amount of time may only contribute small numbers to the overall population within each river and probable that this varies from year to year. Regardless of the proportion of the whole-river reproductive population that these strategies compose, they contribute a unique suite of tactics that increase trait diversity of each river's coho population, diversity that would contribute to the resilience of the population to environmental change (Schindler et al. 2010).

Prolonged use of estuary habitats (months during the summer and throughout the winter) may represent a distinct life history tactic that contributes to the overall population life history portfolio (Schindler et al. 2010). It follows, then, that pristine, functioning estuary habitats can contribute to resilience of salmon populations to environmental changes in two ways: first, by providing a place for individuals to increase size and condition prior to ocean entry to improve survival; and second, by providing for an alternative life history strategy. Maximizing both the availability of supplemental habitats and life history diversity is particularly important given increasing human populations that stress land and water resource development and fishery resource use. Managers require a thorough understanding of the suite of environmental factors that influence the structure and survival of exploited fish populations to make decisions that provide the greatest benefit to all stakeholders (Bottom et al. 2009). Gaps in our understanding of environmental influences on life history expression arise from the fact that many of the highly studied salmon ecosystems in the Northeast Pacific are disturbed or substantially altered in some manner that has caused centralization or loss of variability in life history traits within stocks (Bottom et al. 2005b; Healey 2009). These stocks have lost variability of life history trait expression associated with the loss of habitat complexity (Miller and Simenstad 1997; Cornwell et al. 2001; Magnusson and Hillborn 2003; Bottom et al. 2005a; Bottom et al. 2005b), highlighting the importance of understanding functioning watersheds to inform management of endangered or threatened stocks. Some interesting directions for future work include investigating the mechanisms for the differences in size, condition, residence times, and age composition found between fish using contrasting estuaries. A broader understanding of the importance of estuaries to different runs of salmon could be ascertained by determining the proportion and variability of estuary residents in adult returns. Additionally, an understanding of the connections between the watershed, estuary, and near shore environments during early marine rearing in coho salmon will facilitate strategic and knowledge-based management of these fragile and dynamic areas, thereby providing for resilient fisheries.

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# Chapter 3. Genetic Differentiation in Coho Salmon Occupying Two Contrasting Estuaries: Is Phenotypic Variability in Estuarine Occupancy Reflected in Genetic Differences[5]?

## *Abstract*

Expression of life history diversity in salmon populations is largely driven by environmental dynamics and spatial variability in habitat conditions. Strong, consistent environmental pressures lead to canalization of traits and, over time, can lead to population divergence; alternatively, highly variable stochastic environments lead to generalization and phenotypic plasticity. We examined the linkages between environmental variability, trait expression, and genetic divergence through investigation of two coho salmon populations that rear in contrasting estuarine environments. Using microsatellite markers, we determined that genetic divergence exists between these two populations sufficient to show reproductive isolation. Our work implies that differing physical processes between adjacent juvenile rearing environments (freshwater and estuarine) could drive genetic structuring and population differentiation in coho salmon.

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### *Introduction*

Salmon populations demonstrate genetic and phenotypic variation in response to environmental variability over their geographic range (Adkison et al. 1996; Smith et al. 2001; Quinn 2005; Wood et al. 2008). Selection pressures act on phenotypic traits with trait expression in offspring resulting from a balance between gene flow and phenotypic plasticity related to the frequency, magnitude, duration and predictability of events that lead to mortality and selection (Sultan and Spencer 2002; Waples et al. 2009). For example, sockeye salmon *Oncorhynchus nerka* in Bristol Bay with variable spawning behaviors presented patterns of single stock dominance over time periods that corresponded to regional climate conditions (Hilborn et al. 2003). Each individual stock possessed a suite of traits that made it best suited to survive through a particular climate regime, leading to an alternating pattern of dominance that buffered the population against major climatic changes over the past century (Hilborn et al. 2003).

Patterns of trait dominance and genetic diversity and structuring can be expected in response to specific life history strategies and environmental dynamics through space and time (Wood et al. 2008). Salmon populations that experience frequent local extirpations (e.g., sea type form of sockeye salmon) will exhibit weak spatial divergence over short distances, high genetic diversity, and greater phenotypic plasticity. Conversely, stocks that experience less frequent localized extirpations (e.g. lake type form of sockeye salmon) will exhibit increased spatial divergence and structure over small distances reflective of adaptive radiation, as well as reduced genetic diversity and local adaptation (Sultan and Spencer 2002; Wood et al. 2008; McPhee et al. 2009). Environmental conditions, such as watershed size, prevalence of watershed features (lakes, waterfalls), and climate conditions have been shown to influence levels of gene flow among adult salmon populations (e.g. Dionne et al. 2008). These environmental gradients, such as temperature and salinity, are of particular interest to consider, not only with population structuring, but also with regard to the influence of climate change on anadromous populations (Olsen et al. 2011).

Coho salmon populations, in particular, have exhibited phenotypic and genetic variation that make them an ideal candidate to test hypotheses regarding the influence of environmental dynamics through space and time on trait expression and genetic divergence and within-population diversity (Small et al. 1998; Smith et al. 2001; Wood et al. 2009). Most work investigating trait expression and genetic diversity in anadromous salmon has focused on adult fish and spawning habitats (Dionne et al. 2008, Wood et al. 2008, McPhee et al. 2009). We conducted this study on two populations of juvenile coho rearing within a glacial-fed and a snow-melt, spring-fed estuary that show significant differences in rearing habitats used during the juvenile stage (e.g. duration of estuary use, body size and conditions, and age; see previous chapters of this dissertation). Our objective was to understand if the coho salmon using these watersheds were composed of one stock (with great phenotypic plasticity in estuarine use and juvenile life history) or two genetically distinct stocks. This work represents the final stage of a study investigating if differences in rearing environments of two adjacent, but contrasting, watersheds translate into differences in estuary habitat use and life history tactics employed by juvenile coho salmon. We determined if the differences observed in juvenile ecology were reflected in genetic distinctness between the two stocks or if they were better reflected by a single stock that expresses considerable phenotypic plasticity in juvenile life history and habitat use.

### *Study Area*

Juvenile coho salmon were captured in the Anchor and the Fox River estuaries in Kachemak Bay, located at the southern end of the Kenai Peninsula in south-central Alaska (Figure 1). Adult fish were angled from the mouth of Clearwater Slough, a small snowmelt, spring-fed tributary of the Fox River (Figure 1). Fish were captured primarily within the middle-transitional zone of each estuary, downstream of highest tide line to channels upstream of the lowest tide line, bordered by mud flats and vegetation. The wide tidal range of Kachemak Bay and Cook Inlet (over 5.5 m) (NOAA 2012) creates large ecotonal regions with diverse habitat conditions, particularly in glacial rivers with heavy silt deposition zones. The Anchor River delta is a bar-built estuary that abruptly transitions into the marine environment, with an approximate estuary length of 0.8 km (measured from mean high water tide line to its confluence with the Cook Inlet). The Fox River delta, in contrast, is an open glacial-fed estuary located at the head of Kachemak Bay, approximately 27 km east of Homer and 29 km south of the mouth of the Anchor River estuary. The Fox River transitions through a large, approximately 6 km delta into Kachemak Bay, which results in a more gradual and extended estuarine ecotone between the marine environments of Cook Inlet and freshwater environments of the Fox River (Figure 3.1). Adult returning coho salmon were counted by sonar on the Anchor River as they returned to spawn, with a six-year range of 2,692-18,977 fish (years 2004-2009; Kirkvliet and Booz 2012). Juvenile outmigration has been estimated since 2010 using a rotary screw trap located approximately 1 km above the high tide line (Anderson and Stillwater Sciences 2011). Fox River salmon escapement data exist only as annual single-pass aerial counts in a small tributary of the upper river; full escapement data have not been collected to date. Some information is available regarding spawning migration and timing (Faurot and Palmer 1992), but limited data exist for juvenile coho salmon abundance and outmigration in the Fox River (Walker et al. 2009).

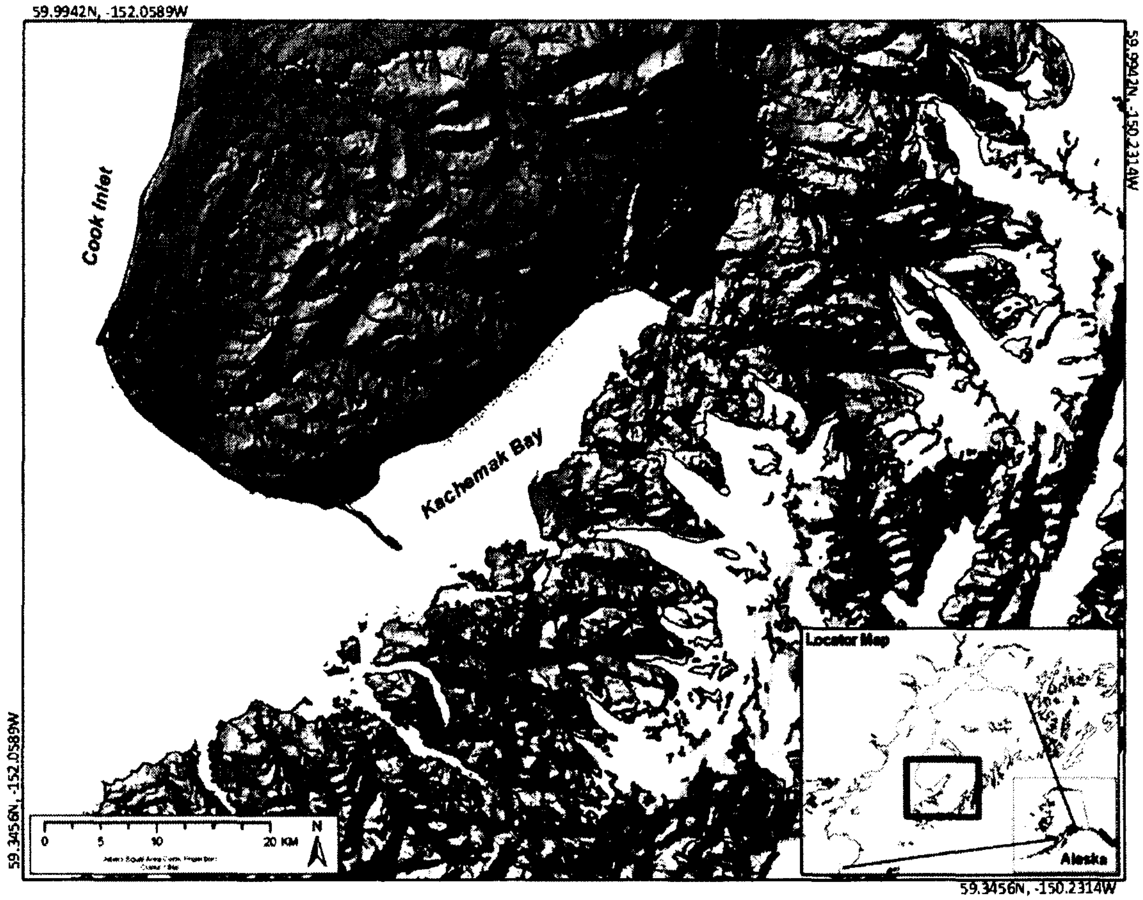

Figure 1. Study area map. Locations of coho salmon sample collection in the lower Kenai Peninsula, Alaska are shown with symbols indicating areas: Anchor River (triangle) estuary, Fox River (square) estuary, and Clearwater slough (circle).

### *Methods*

#### *Tissue collection*[6].—

Samples were collected from 101 fish (43 from the Anchor River and 58 from the Fox River), with seven fish from adult returns to the Clearwater Slough in the Fox River (Figure 1). Juvenile and adult coho salmon were collected in estuary and river habitats from August through November from the Fox and Anchor rivers in 2010. Tissue samples were taken from adult fish by lifting the dermal tissue on the operculum and cutting a small piece (1cmx1cm) with a scalpel. Juvenile samples consisted of pieces of caudal fin (1cmx1cm) clipped with a scalpel and stored in 99% ethanol or from rayed fins taken from freeze dried fish and stored in plastic vials.

#### *Laboratory analysis.*—

Individuals were genotyped at 19 microsatellite markers at the U.S. Fish and Wildlife Service Conservation Genetics Laboratory in Anchorage, Alaska. Individual fin clips were biopsied under laboratory conditions using a 3-mm diameter Harris Micro-Punch™ (Sigma Aldrich, St. Louis, MO). Fin tissue punches were placed in dedicated wells on sterile 96-well reaction plates. Total genomic DNA was extracted in parallel using the QIAxtractor DNA tissue procedure with DX Reagents following manufacturer's protocol (Qiagen Inc., Valencia, CA). DNA extracts were stained with

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[6] UAF Institutional Animal Care and Use Permit number 149489-4

the intercalating fluorochrome PICOGreen® (Molecular Probes, Eugene, OR) and visualized with a VICTOR™X3 2030 Multilabel Plate Reader married to a JANUS Automated Workstation (PerkinElmer, Waltham, MA). If necessary, a buffer solution was added to dilute samples with relatively high yields of DNA to a standard concentration of 30 ng/µl. This quality control measure ensured each sample was at the same concentration and contained extremely clean DNA prior to downstream analyses.

Genetic variation was assayed at nineteen microsatellite loci (Table 1). Using MJResearch PTC-200 or Bio Rad Tetrad 2 Peltier Thermal Cyclers, polymerase chain reaction (PCR) amplifications of these markers were carried out in 10µl reaction volumes containing approximately 30-50ng template DNA, 1X buffer, 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.1-0.6 µM labeled forward primer, 0.1-0.6 µM unlabeled reverse primer, and 0.05 U/µl NovaTaq™ DNA polymerase (EMD4Biosciences, Billerica, MA). Cycling conditions began with 1 cycle of 2 min at 92 °C, followed by 30 cycles of 15 sec at 92 °C, 15 sec at each marker's respective Ta, 30 sec at 72 °C; and a final extension for 10 min at 72 °C. Markers were multiplexed or pseudoplexed whenever possible. PCR products were size fractioned using an Applied Biosystems 3730 Genetic Analyzer. Applied Biosystems GeneScan™600 LIZ® size standard, with 36 fragments ranging in size from 20 to 600 bases, was loaded in all capillaries containing samples for use with internal lane standards. Markers were developed for salmonids from multiple sources (Scribner et al. 1996; Beacham et al. 1998;

Condrey and Bentzen 1998; Olsen et al. 1998; Small et al. 1998; Smith et al. 1998; Buchholz et al. 1999; Greig and Banks 1999; Nelson and Beacham 1999; Cairney et al. 2000; Williamson et al. 2002, Greig et al. 2003; Spies et al. 2005; Table 1).

Table 1. Summary of microsatellite loci information. Name, sequence, primer, and reference information for microsatellite markers used to compare the Fox and Anchor River coho salmon genetic samples.

| Name | Repeat sequence | Primers | Ta (°C) | Accession number | Publication |
|---|---|---|---|---|---|
| Ocl8 | (GT)$_{46}$ | F: TAGTGTTCCGTGTTCGCCTG<br>R: CCCTGTCCCTTCCATCTCT | 56 | AF028697 | (Condrey and Bentzen 1998) |
| Ogo2 | (GA)$_{24}$ | F: ACATCGCACACCATAAGCAT<br>R: GTTTCTTCGACTGTTTCCTCTGTGTTGAG | 56 | AF009794 | (Olsen et al. 1998) |
| Oke2 | (GT)$_{44}$ | F: AGGGCCAGAGAAAAGTCTCACTAT<br>R: GTCAGTCCTGCCCTCTGTGTCCTA | 56 | AF330219 | (Buchholz et al. 1999) |
| Oke3 | (TCCCTCTCGTCTC)$_{8}$ | F: ACCCTGAGAGCAATCAAC<br>R: TCAGGGATATGCAGTAAATAGTA | 54 | AF330330 | (Buchholz et al. 1999) |
| Oke4 | (CA)$_{4}$(A)$_{1}$(CA)$_{9}$ | F: AGGCCCAAAGTCTGTAGTGAAGG<br>R: GATGAATCGAGAGAATAGGGACTGAAT | 56 | AF330221 | (Buchholz et al. 1999) |
| Oki1 | (CTGT)$_{18}$ | F: AGGATGGCAGAGCACCACT<br>R: CACCCATAATCACATATTCAGA | 56 | AF055427 | (Smith et al. 1998) |
| Oki3 | (CAC)$_{9}$ | F: CACCCATAATCACATATTCAGA<br>R: CACCCATAATCACATATTCAGA | 54 | AF055432 | (Smith et al. 1998) |
| Oki11 | (GT)$_{16}$ | F: TCTGAGACAGGCAAATGCAC<br>R: GTTTTAAACCTCACCATTGAGT | 54 | AF055436 | (Smith et al. 1998) |
| Omy1011 | (CAGA)$_{11}$ | F: AACTTGCTATGTGAATGTGC<br>R: GACAAAAGTGACTGGTTGGT | 54 | AY518334 | (Spies et al. 2005) |
| Oneμ3 | (GA)$_{18}$ | F: TCTCCTTGGTCTCTCTGTCCCTT<br>R: CTATCAGCCAATCGCATCAGGAC | 54 | U56702 | (Scribner et al. 1996) |
| Oneμ13 | (GA)$_{20}$ | F: TCATACCCCATGCCTCTTCTGTT<br>R: GATGAGTGAAAGAGAGGGAGCGA | 58 | U56713 | (Scribner et al. 1996) |
| Ots101 | unknown | F: ACGTCTGACTTCAATGATGTTT<br>R: TATTAATTATCCTCCAACCCAG | 48 | unknown | (Small et al. 1998) |
| Ots103 | (GACA)$_{\text{unknown}}$ | F: AGGCTCTGGGTCCGTG<br>R: TGATATGGTGTGATAGCTGG | 58 | unknown | (Beacham et al. 1998) |
| Ots105 | (GTCT) or (ATCT) | F: GAGGATCTATCAACATTATC<br>R: GCAGCACCAGCTTCCC | 54 | AF069677 | (Nelson and Beacham 1999) |
| Ots213 | (CATA)$_{28}$ | F: CCCTACTCATGTCTCTATTTGGTG<br>R: AGCCAAGGCATTTCTAAGTGAC | 56 | AJ534363 | (Greig et al. 2003) |
| Ots2M | unknown | F: GCCTTTTAAACACCTCACACTTAG<br>R: TTATCTGCCCTCCGTCAAG | 56 | AF107030 | (Greig and Banks 1999) |
| OtsG422 | (GATA)$_{24}$ | F: GCTTGCTCGCTCAATCTTCTTATT<br>R: GAGGCAATGAGGGAGGATGGTGAG | 58 | AF393197 | (Williamson et al. 2002) |
| P53 | unknown | F: TGACACATATCCTCGCTTTCTCC<br>R: CAACTCTCTTGGTGAGGC | 58 | unknown | unknown |
| Ssa407 | (GACA)$_{37}$ | F: TGTGTAGGCAGGTGTGGAC<br>R: CACTGCTGTTACTTTGGTGATTC | 54 | AJ402724 | (Cairney et al. 2000) |

*Statistical analyses.—*

Genotypes were checked for conformation to Hardy-Weinberg proportions and gametic disequilibrium using GenePop v. 4.0.10 (Raymond and Rousset 1995). We calculated $\Theta_{ST}$ (Weir and Cockerham 1984) between the two rivers (in GenePop) and used factorial correspondence analysis (in Genetix v. 4.05, Belkhir et la. 2004) to examine differences in combined allele frequencies between populations.

## *Results and Discussion*

One marker, *Oke2*, did not amplify for most fish and thus was removed from analyses (Buchholz et al. 1999). All populations were homozygous for one marker (*Oke2*, Buchholz et al. 1999), and four of the six cohorts (Fox: age-0, age-1, age-2, adult; Anchor: age-1, age-2) were homozygous for *Oki1* and *Oke3*. We observed some conflicts in Hardy-Weinberg (HW) expected values within the Fox River coho salmon; however, these were most likely related to small sample sizes for each cohort. When all cohorts were pooled for the Fox River, loci frequencies deviated significantly from HW expectations at two loci (*Oneu13* and *Ots103*). We therefore ran sample data by each individual cohort for each estuary to determine if deviations may be related to cohort composition. Separate analyses by cohorts showed the Fox River age-1 coho salmon significantly deviated from HW expectations for four of the 18 loci respectively (*Ocl8, Oki1, Oki11,* and *Oneu13*); however the sample size was prohibitively limited for each individual cohort from which to draw conclusions (Table 2). The remaining cohorts conformed to HW expectations and the deviations we noted are most likely related to the small sample size for each cohort and do not impact the interpretation of reproductive isolation between populations; however, they do raise some interesting questions for future work.

Examination of $F_{ST}$ values by cohort illustrates that fish from each estuary were more closely linked to cohorts within each respective estuary than to those from the comparative estuary (Table 3). Factorial correspondence analysis clearly showed spatial differentiation in allele frequencies between the Fox and Anchor River fish for all cohorts when pooled by estuary with 76.4 % of the variation accounted for within the first three axes (Figure 2). The adult fish sample from the Fox River accounted for 17.4 % of the variation in the sample (axis 2, Figure 2), raising questions for future work.

Table 2. Summary of Hardy Weinberg tests. Sample sizes and test results for Hardy Weinberg expected frequencies by cohort for the Fox and Anchor River estuaries.

| Cohort | N | Chi-square | HW probability |
|---|---|---|---|
| Anchor Age 1 | 30 | 69.58 | <0.05 |
| Anchor Age 2 | 13 | 42.48 | 0.10 |
| Fox Age 0 | 11 | 44.68 | 0.07 |
| Fox Age 1 | 31 | Inf. | <0.05 |
| Fox Age 2 | 8 | 32.28 | 0.45 |
| Fox Adult | 7 | 30 | 0.63 |
| Anchor pooled | 43 | 36 | 0.55 |
| Fox pooled | 57 | Inf. | <0.05 |

Table 3. Pair-wise genetic distances ($F_{ST}$ values) for each coho salmon cohort. Data are from fish analyzed in the Fox and Anchor river genetics sampling. Sample sizes for each cohort are shown in parentheses.

| Cohort (n) | Anchor age-1 (30) | Anchor age-2 (14) | Fox age-0 (11) | Fox age-1 (31) | Fox age-2 (8) | All Fox (57) |
|---|---|---|---|---|---|---|
| Anchor age-2 (14) | -0.003 | | | | | |
| Fox age-0 (11) | 0.048 | 0.056 | | | | |
| Fox age-1 (31) | 0.046 | 0.053 | 0.005 | | | |
| Fox age-2 (8) | 0.033 | 0.047 | 0.011 | 0.013 | | |
| Fox adult (7) | 0.053 | 0.072 | 0.010 | 0.017 | 0.006 | |
| All Anchor (44) | | | | | | 0.056 |

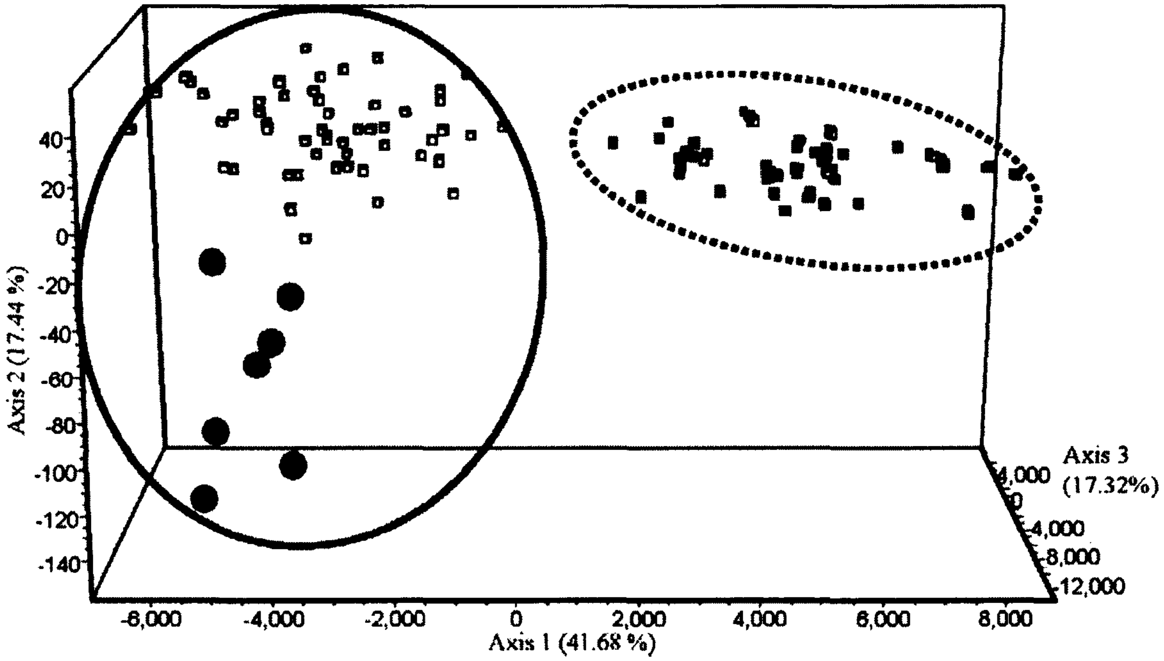

Figure 2. Factorial correspondence analysis results plot. Graphical illustration of three primary axes of factorial correspondence analysis of allele frequencies for coho salmon captured in the Fox (solid ellipse) and Anchor (dashed ellipse) river estuaries. Fox River adult fish shown with solid circles (●)

Some interesting findings in our work are that the adult fish sample from the Fox accounted for 17.44 % of the variation in the sample (axis 2, Figure 2). These fish were angled from one of two small snow-melt, spring fed tributaries to the glacial Fox River that raise the question of whether genetic differences within this population correspond to environmental heterogeneity within the watershed (glacial vs. spring-fed) –interesting questions for future work. Our work showed phenotypic and behavioral differences between these two populations (see Chapter 2 of this dissertation) and genetic divergence observed suggests reproductively isolated. The differences observed in phenotypic traits suggest that these two populations have specific run characteristics that may respond differently under alternative climate scenarios. This highlights the importance of freshwater and estuarine habitat conditions and their affect on life history diversity and further emphasizes the need to consider and investigate how freshwater, marine, and estuarine environments influence survival for exploited diadromous fishes.

Our previous work in these two estuaries revealed significant differences in life history characteristics of juvenile coho salmon, including size at estuarine entry, estuarine residence time, age class composition, and timing of estuary entry (see Chapter 2 of this dissertation). Environmental conditions were also significantly different between these two estuaries (see Chapter 2 of this dissertation), particularly seasonal patterns of water temperature and water depth, which may influence the suitability of rearing habitat for juvenile salmon (Webster et al. 2001;

Miller 2007; Hering et al. 2010). In this case, the geographic separation is quite small, but the environmental contrast is large -primarily related to how the watershed is supplied. Fish using these two geographically adjacent watersheds to spawn and rear exhibit evidence of reproductive separation (pair-wise $F_{ST}$ [between rivers] = 0.056m, Table 3) and pair-wise $F_{ST}$ values between these two populations within the Cook Inlet region are similar to those found in previous studies of other populations using many of the same loci (Olsen et al. 2004).

Individual stock analysis has been employed by fishery managers to focus or center harvest and management strategies (Waples 1998). It follows then, that management strategies of exploited populations such as types of gear employed, and the allocation and magnitude of harvest are different for each stock depending on its life history characteristics (i.e. run timing, fish body size, timing and duration of occupancy of various habitats). Over time, environmental conditions may shift to favor one stock over another; it therefore becomes important to manage salmon populations to maintain the diversity of individual stock function (or response) rather than simply the abundance of the overall population itself. In essence, maintaining adaptive potential of the population is as crucial as maintaining total harvest for sustainable salmon fisheries. Collectively, this work implies that estuaries contribute to this adaptive potential and genetic diversity, driving stock separation as well as life history diversity.

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## Dissertation Conclusions and Implications

Wild fisheries compose some of the last remaining animal populations in the world vulnerable to mass harvest pressures established through regulations based on human consumption and economies (Waples 1998). We require cautious and informed decisions regarding harvest and conservation thresholds of wild salmon because wild populations are influenced not only by human harvest, but by regional conditions and dynamics outside of human control. This contrasts sharply with the confined and domesticated conditions for agricultural production under which much of our food base lies. When we consider fisheries management and harvest, the manager's decision can be distilled down to one rather broad but pertinent question: on average, how many individuals may be harvested each year from a fish stock to ensure long term sustainable harvest under the given environmental and ecological pressures (definition: maximum sustainable yield, derived from 2012 NOAA fisheries: http://www.nmfs.noaa.gov/om2/glossary.html)? Several important pieces of information are crucial to the manger's ability to make this decision: what is the 'stock' and what are the environmental and ecological conditions that affect the stock over time? To discuss the implications of my work, I borrowed from Waples' (1998) as his discussion addresses the issue of stock delineation and some considerations to make each time we report our findings and make decisions regarding fisheries resources.

The first key piece of information for informed harvest decisions is the definition or delineation of the 'stock'. This issue has plagued fisheries managers and biologists for decades because it is instrumental to how we manage the populations (Waples 1998). We have clearly defined 'stock' in fisheries management legislation as:

> "...a species, subspecies, geographical grouping, or other category of fish capable of management as a unit."
>
> Gutiérrez (2007)

I do not attempt to answer the question of 'what is a stock', but rather discuss the consequences of the delineation of this term related to my work. Essentially, when fish are all considered one stock, we manage them and develop our level of harvest based on the population size of that stock (Waples 1998). In salmon, there can be multiple populations of fish (often delineated by small geographic boundaries such as spawning locations, creeks, rivers, or watersheds) that support larger stocks, often delineated by a larger geographic boundaries such as a bay fed by multiple watersheds (Hilborn et al. 2003). These stocks can play different roles in the level of response to environmental and ecological conditions in any year (Hilborn et al. 2003; Schindler et al. 2010). At any point, a single run may contribute proportionally little to the overall population numbers; however, under differing environmental conditions, that same run could contribute a large proportion of the overall population abundance. Since, over time, environmental conditions may shift to differentially favor one stock over another, it becomes important to manage salmon populations to maintain the diversity of individual stock function (or response) rather than simply the abundance of the population itself.

In this dissertation, I considered the role that estuaries played in the response of a salmon stock or stocks to specific environmental conditions: those encountered during early marine entry. I did not address mechanisms for the responses, but wanted to consider several preliminary questions that need to be addressed prior to addressing mechanistic questions. The first was to determine if estuaries are being used by juvenile salmon for growing and therefore can impose environmental pressures that may influence growth and development. This issue is critical because it requires a direct measure of growth during residence in the estuary to show that the individual was vulnerable to environmental conditions within the estuary habitats. The second was to determine if fish using estuaries did or did not show some sort of a trait or behavior from estuary use that would affect the early marine entry phase (i.e. changes in size or condition). From there, we determined whether there was a response to the environmental conditions within estuaries that may change regionally or seasonally. Finally we determined whether those responses were a product of plasticity (or flexibility of responses within a group that appears genetically similar) or if the groups were reproductively isolated (therefore having potential trait differentiation with a genetic basis). It is impossible to remove the watershed influence from the response of the fish at the scale of our study (in terms of timing and size at marine entry) because the function of the watershed drives the habitat conditions within the estuary. However, we did demonstrate that fish were using estuaries as places in which to rear or grow, that the duration of this use was substantial -over a year of the life of some individuals-, that environmental conditions corresponded to patterns of fish use, and that these two systems had groups of fish exhibiting genetic distances from one another indicative of reproductive isolation. We would therefore consider these two groups different stocks in that they also provide for differential functions (sizes, ages, and times of marine entry) in the Cook Inlet population.

In short, this dissertation led to the following conclusions:

1) Juvenile coho salmon are using habitats within estuaries for rearing for extended periods of time and estuary use benefits fish with residents showing increased body condition and weight over non-residents
2) Patterns of use by juvenile coho salmon corresponded to variability in environmental conditions within estuaries and differed between estuaries with contrasting environmental conditions.
    a. Habitats showing variation in water temperature and depth were occupied by fewer fish and for shorter periods of time than those that were more stable;

   b. Habitats with high water temperatures (>20°C) and shallow water depths (<0.4 m) were used less frequently during periods when these conditions were present;
   c. Patterns of use corresponded to patterns of stream flow in contrasting estuarine environments that provided for adequate water temperatures and channel depths for rearing:
      i. In the snowmelt and spring-fed estuary, cooler and deeper water conditions in the early spring corresponded to high estuarine abundances;
      ii. In the glacial-fed estuary, cooler and deeper water conditions in mid-summer corresponded to higher fish abundances;
      iii. Summer seasonal abundance was greater for longer periods of time in the glacial fed estuary channels, with cooler and less variable water temperatures and depths.

3) Salmon occupying adjacent, environmentally contrasting estuaries exhibited differential traits and behavior patterns and were genetically distinct populations.

### *Future research*

One of many questions that were raised from our work was to what degree does life history diversity exist within each of these two stocks? We found evidence to indicate that diversity in estuarine residency may be a strategy employed by coho salmon populations. We assert that, regardless of the strategy, estuaries provide an opportunity for coho salmon to diversify the age, body size, body condition, and timing of marine entry.

In Alaska, we are fortunate that much of our freshwater habitats are relatively pristine and unaltered by development. However, human populations are continuing to increase and changing climate conditions are altering habitats for animals, including changes in the magnitude and frequency of regional precipitation events, drying and wind patterns (Hinzman et al. 2005). These combined pressures hold the potential to reduce available habitats and thus the ability for salmon to respond to interannual variability of environmental conditions. Preserving the greatest range in diversity- such as a wide variety of watersheds with a range of conditions- will continue to enhance the ability for these fishes to use and colonize stochastic environments. We need to proceed with development cautiously and give consideration to whole watershed effects of alterations in stream flow that will affect characteristics of estuarine habitats –such as temperature, depth, and salinity– that provide rearing habitats for juvenile salmon. Finally, in addition to managing salmon stocks for productivity and overall sustainability, we must also consider factors driving their adaptive potential and the evolutionary processes that lead to long term persistence of salmon in the dynamic and changing environments of the Pacific Northwest.

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