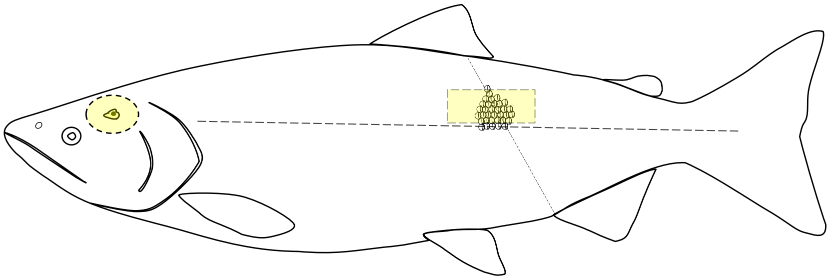
Final Report

Investigating Juvenile Life History and Maternal Run Timing of Chehalis River Spring and Fall Chinook Salmon using Otolith Chemistry



By

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Executive Summary

Understanding life history diversity of Chinook salmon in the Chehalis River, Washington and the factors maintaining this diversity were identified as data gaps by the Aquatic Species Enhancement Plan Technical Committee of the Chehalis Basin Strategy (Aquatic Species Enhancement Plan Technical Committee, 2014). Improved understanding of Chinook salmon diversity is needed to inform ongoing discussions among stakeholder groups regarding strategies for flood control and aquatic species restoration in this basin. The current study used otolith chemistry of adult Chinook salmon (*Oncorhynchus tshawytscha)* collected from the Chehalis River to describe successful juvenile life history strategies (as measured in surviving adults) and to assign spring/fall run timing to adult Chinook salmon based on maternal origin.

We explored the effects of summer stream temperature and distance between the spawning stream and estuary on the size at which juvenile Chinook salmon entered the saline portions of the Grays Harbor estuary. We found evidence of both fry (< 60 mm fork length, FL) and subyearling parr (> 60 mm FL) life histories in adults returning to Chehalis sub-basins. The proportion of the fry life history was very low (0-5%) in returning adults in the two sub-basins furthest from saltwater that included the upper Chehalis River (Rkm 148) and the Newaukum River (Rkm 121), but made up a substantial proportion (8-24%) of the returning adults in the sub-basins closer to saltwater than included the Wynoochee, Satsop, Black and Skookumchuck rivers. Mean size at estuary/ocean entrance was correlated with sub-basin distance from the estuary and year, but not with summer stream temperature. The results show clear evidence that a portion of the fish that leave their natal rivers and enter the saline Grays Harbor as small fry can and do survive and return to spawn. We hypothesize that the correlation between successful

juvenile life histories and distance from a specific habitat (in this case estuary) describes a life history cline, where proximal habitats play a larger role in early juvenile rearing then distant habitats. Though this research was conducted on many individual adult Chinook (n=305) our inference to differences between years (n=2) or between sub-basins (n=7) is limited. To strengthen this research and to further test these finding, complete brood years should be analyzed in order to test outmigration year effects while controlling for age/life history bias.

We used the chemistry (strontium:calcium) of the otolith core to infer maternal run timing and compared the field and otolith chemistry assignments of adult Chinook salmon (n=303). Based on the chemistry of the otolith core, maternal run timing was assigned as either a stream maturing-spring or ocean maturing-fall Chinook. We found evidence of the fall Chinook salmon run type in all sub-basins and evidence of the spring Chinook salmon run type in samples from the Skookumchuck, Newaukum, and upper Chehalis sub-basins only. Field identification of spring Chinook salmon corresponded weakly with otolith results in 2015 (33% agreement) and moderately in 2016 (~50%). However, field identification of fall Chinook salmon run type agreed with otolith determinations 93% and 99% of the time in 2015 and 2016 respectively. Caution is advised when interpreting the results of spring and fall classification via otolith chemistry. We found freshwater strontium:calcium levels in the otolith to be elevated in the Skookumchuck, Newaukum, and Upper Chehalis (~ 1.2 mmol mol^{-1}). These elevated levels may interfere with the ability to use otolith chemistry to assign spring or fall run type. To increase the accuracy of otolith chemistry assignments of Chinook salmon run types in the Chehalis River basin, we would recommend expanding/including genetic and otolith isotope analysis (^{86}Sr /^{87}Sr) and collecting known spring and summer (if present) Chinook salmon as reference. For example, reference samples collected in spring fisheries would be immensely informative. With consideration of the above mentioned issues, this research finds low agreement between field and otolith methods for assigning the spring Chinook run type. If the otolith results are assumed to be accurate, spring Chinook are misidentified more frequently than fall Chinook salmon in the field.

Introduction

Diversity within a species is generally thought to improve the resilience of a population to environmental stressors such as droughts, floods, and poor ocean conditions (Greene et al. 2009, Schindler et al. 2010). For Chinook salmon, diversity is expressed in the duration of time that individuals spend in freshwater, estuary, and ocean habitats (Healey 1991, Quinn 2011). In rivers of the Pacific northwest, juvenile Chinook salmon spend varying amounts of time in freshwater and estuary habitat before migrating to the ocean and studies have identified that multiple juvenile life histories contribute to seaward migration (Campbell 2010, Volk et al. 2010) and returning adult populations (Miller et al. 2010, Campbell and Claiborne 2017). In addition to juvenile diversity, adult life histories of Chinook salmon are distinguished by the timing and maturation state at which adults return to freshwater from the ocean (Healy 1991, Quinn et al. 2015). Populations of Chinook salmon are named according to this return timing (spring-run, summer-run, fall-run, winter-run) and often coexist in the same river systems.

Populations of both spring and fall Chinook salmon are recognized in the Chehalis River, a low gradient, coastal river in southwest Washington. Information on the current status and ecology of Chinook salmon in the Chehalis River is needed to inform ongoing discussions among stakeholder groups regarding strategies for flood control and aquatic species restoration in this basin. An understanding of Chinook salmon diversity and the factors that maintain diversity were recently identified as data gaps by the Aquatic Species Enhancement Plan Technical Committee of the Chehalis Basin Strategy (Aquatic Species Enhancement Plan Technical Committee, 2014).

Evaluating the status of spring and fall Chinook salmon in the Chehalis River is complicated by spatial and temporal overlap in their spawning activities. Furthermore, little is known about the juvenile life histories that contribute to the returning spawning populations. In general, juvenile Chinook salmon in western Washington rivers emigrate to saltwater in their first year of life. ‘Fry’ migrants emerge from the gravel and immediately emigrate to saltwater, ‘subyearling parr’ migrants rear in freshwater for three to six months prior to emigrating to saltwater, and ‘yearling smolts’ are a minor component of the outmigration (Topping and Zimmerman 2012, Lamperth et al. 2014, Zimmerman et al. 2015, Hillson et al. 2017).

Among the many factors that may influence the expression of juvenile life histories are length of migration to the ocean and the availability of suitable rearing temperatures. In the Chehalis River, distances that juvenile Chinook salmon travel between emergence from the gravel and saltwater entry may vary by more than one hundred and fifty kilometers. We hypothesize that juveniles travelling further to reach saltwater have a longer exposure to freshwater habitats resulting in a positive correlation between distance traveled and size at entry to the estuary. Stream temperatures may also determine how long juveniles remain in their natal tributaries prior to emigration. Several studies have shown a positive relationship between increases in daily stream temperature and movements or smoltification of subyearling Chinook salmon (Sykes and Shrimpton 2010, Winkowski and Zimmerman 2017). Although subyearling Chinook salmon are often observed to emigrate through the month of August (Lamperth et al. 2014, Winkowski and Zimmerman 2017), summer temperatures in many areas of the Chehalis River basin exceed 18˚C for prolonged periods of time during the summer months (Liedtke et al. 2016). We hypothesize that the duration of freshwater rearing may be shorter in sub-basins where warm stream temperatures trigger earlier emigration behavior and smoltification.

Otolith chemistry is a tool used to reconstruct natal and maternal origin and life history in Pacific salmon (*Oncorhynchus* spp) that can be used to corroborate field assignments of spring versus fall run types and to test hypotheses about juvenile life histories of Chinook salmon in the Chehalis River. The migratory behavior and maternal origin can be described in diadromous fishes because the element Strontium (Sr) is generally found in lower quantities in freshwater habitats then estuary/ocean environments (Kraus and Secor 2004; Miller et al. 2010) and is incorporated into otoliths in approximate proportion to its abundance in water (Campana 1999; Bath et al. 2000; Kraus and Secor 2004; Brown and Severin, 2009, Miller et al. 2011). Coupled with a relationship between fish size and otolith size, Sr may be used to reconstruct juvenile size at migration to brackish/marine waters (Campbell 2010, Miller et al. 2010, Tomaro et al. 2012, Claiborne and Campbell 2016). Similarly, Sr incorporated into the core of the otolith during egg development varies in part, in relation to its abundance in the environment, and the duration of residence in freshwater prior to spawning (Volk et al. 2000, Donohoe et al. 2008). For example the ratio of Strontium to Calcium (Sr:Ca) in the otolith core has been used to distinguish between the progeny of spring and fall Chinook salmon (*O. tshawytscha)* (Miller and Kent 2009), and the progeny of resident and anadromous O. *nerka* (Rieman et al. 1994) and O. *mykiss* (Zimmerman and Reeves 2000, Berejikian et al. 2013).

The objectives of this study where: 1) to evaluate the successful juvenile life histories (e.g. fry, parr subyearling, yearling) of adult spring and fall Chinook salmon returning to the Chehalis River basin and 2) provide an independent estimate of maternal run timing (spring or fall) for individuals that were classified as spring or fall in the field. We used a combination of carcass recovery surveys, otolith microchemistry and back-calculations models to accomplish these objectives.

Methods

Study Area

The Chehalis River is a 6,889 km^2 coastal watershed in southwestern Washington State (Figure 1). The main stem river flows 193 km from the headwaters in the Willapa Hills through the cities of Chehalis and Centralia and enters the Grays Harbor estuary near the town of Aberdeen. The watershed is comprised of multiple sub-basins that drain from the Willapa Hills in the south (Upper Chehalis and South Fork Chehalis sub-basins), foothills of the Cascade Mountains in the east (Newaukum and Skookumchuck sub-basins), and foothills of the Olympic mountains in the north (Satsop and Wynoochee sub-basins). An additional sub-basin, the Black River, does not gain elevation but flows out of Black Lake into the Chehalis River. The hydrology of all sub-basins is rain dominant characterized by extended periods of low flow in the summer months and peak winter flows that can be three orders of magnitude larger than base summer flows.

Between 1996 and 2015, spawning escapements averaged 15,991 for fall Chinook salmon and 2,417 for spring Chinook salmon. Spawning of fall Chinook salmon occurs throughout the basin including the main stem Chehalis River (Rkm 45 to 108 and Rkm 142 to 174), Humptulips, Johns, Hoquaim, Wishkah, Wynoochee, Satsop, Black, Newaukum and Skookumchuck rivers as well as Cloquallum and Porter creeks. Spawning of spring Chinook salmon is more spatially limited than the fall run; the majority of spawning occurs in the Skookumchuck, Newaukum, South Fork Chehalis and the mainstem Chehalis rivers (Rkm 53 to

Rkm108 and Rkm 130 to 182) with some spawning observed in the Black River and in Elk and Stillman creeks.

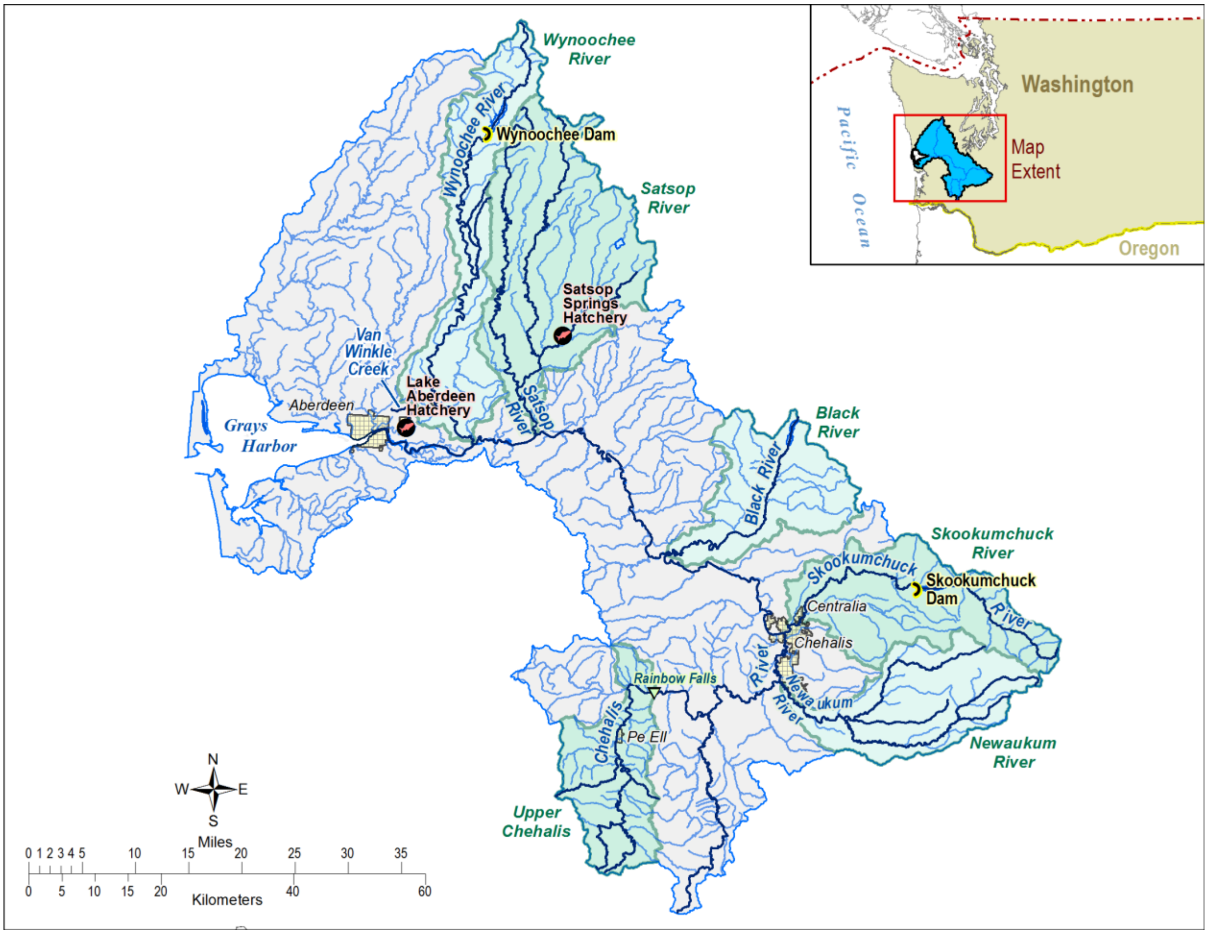

Figure 1. Map of the Chehalis River Basin showing the sub-basins sampled for spring and fall Chinook salmon carcasses in 2015 and 2016. The Black, Skookumchuck, and Upper Chehalis sub-basins were sampled in 2015 and the Wynoochee, Van Winkle Creek, Satsop, Skookumchuck, Newaukum, and Upper Chehalis sub-basins in 2016 (map courtesy of Dale Gombert, WDFW).

Sub-basin Temperature Classification

For the purpose of this study, we classified each sub-basin as having ‘cool’ or ‘warm’ summer stream temperatures in order to test the hypothesis that stream temperatures were related to the size at which juvenile Chinook salmon emigrated to saltwater. The threshold used to make this distinction was an average July and August temperature of 16˚C, which is the temperature criteria used to define ‘core summer salmonid habitat’ by the Washington State Department of Ecology (Water Quality Program 2002).

Stream temperature data is sparse in the Chehalis River; however, the Washington Department of Fish and Wildlife installed an array of fixed loggers between 2014 and 2016 to monitor steam temperatures associated with spring Chinook salmon distribution (Liedtke et al. 2016) and juvenile salmon and steelhead rearing (Winkowski et al. 2017). Based on mean daily temperatures in July and August, the Skookumchuck and Newaukum sub-basins were classified as ‘cool’ (14˚C to 16˚C) and the Satsop (excluding the East Fork), Black, South Fork Chehalis, and upper Chehalis sub-basins were classified as ‘warm’ (19˚C to 20˚C). The Wynoochee River was also classified as ‘warm’ based on available information from this watershed (Cleland et al. 1999). Van Winkle Creek, a small tributary to the Chehalis River downstream of the Wynoochee River, which was also categorized ‘warm’ based on summer stream temperatures available at Lake Aberdeen hatchery (K. Burns, WDFW, personal communication).

Fish Collection

Chinook salmon were sampled during spawning ground surveys or at adult traps in the fall of 2015 and 2016. Spawner surveys were conducted weekly between early September and late November. Carcasses were sampled for length, sex, age (scales), genetics (fin clip), and mark status (presence or absence of adipose fin and Coded Wire Tag CWT). Sagittal otoliths from unmarked fish (adipose fin intact, no CWT) were surgically removed from the skull and retained for laboratory analysis. In 2015, otolith samples were obtained from the Black and Skookumchuck rivers as well as the upper Chehalis mainstem (above Rkm 147) in 2015 (Table 1). In 2016, the study area was expanded to include VanWinkle Creek, Wynoochee, Satsop, Skookumchuck, Newaukum and South Fork Chehalis rivers and the upper Chehalis River mainstem (above Rkm 147). Several collections were from unmarked (wild) Chinook that recruited to adult traps at hatcheries or dams. Collections from VanWinkle Creek were from the Lake Aberdeen hatchery trap, collections from the Wynoochee River were from the trap at the Wynoochee Dam, collections from the Satsop included Chinook from Satsop Spring Ponds, and collections from the Skookumchuck River were from the trap at the Skookumchuck Dam.

Field identification of spring and fall Chinook

For Washington North Coastal Chinook stocks, the field identification for a spring-run versus fall-run Chinook is determined based on the threshold date of October 15th: live fish or redds observed on or before October 15th are called spring Chinook and live fish or redds observed after October 15th are called fall Chinook. In the Chehalis River basin, the threshold date is one consideration taken in field identification of spring and fall Chinook salmon. The spawn timing of spring Chinook salmon takes place from early September to mid-October, with fall Chinook salmon spawning from early to mid-October to mid-December. Unlike the North Coast, the Chehalis spring and fall Chinook can overlap in spawn distribution and timing. Field determination of the run type of Chinook carcasses is particularly difficult in the month of October (statistical week 40 to 44) when the last of the spring Chinook salmon and first of the fall Chinook salmon overlap in the same spawning reaches. Field calls forChinook salmon in the month of October are based on the threshold date, live fish conditional observation, stream flow conditions, prior week's activity, and condition of the carcass. Spring Chinook carcasses are identified as having at least two of three following characteristics: dull/dusky appearance, not bright/shiny colors; fungus present on carcass and edges of snout, fins showing wear; and soft caudal peduncle. Fall Chinook carcasses are identified as having at least two of three following characteristics: bright/shiny vivid colors; no or minimal amounts of fungus/wear; and firm caudal peduncle. Adjustments can be made to field calls based on the threshold date and the biologist's knowledge of the basin.

Otolith Analysis

Otolith chemistry was analyzed to reconstruct the juvenile life histories and maternal run timing of adult Chinook salmon sampled on the spawning grounds during return year 2015 and 2016 (Figure 1). Otoliths were prepared for chemical analysis by thin sectioning in the sagittal plane, where otolith material was removed from both the distal and proximal surfaces until primordia were clearly visible (Volk et al. 2010, Campbell et al. 2015, Claiborne and Campbell 2016). All otolith chemistry was conducted at the Keck Collaboratory for Plasma Mass Spectrometry at Oregon State University. We used Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS) to collect otolith chemical data. Specifically, instrumentation consisted of a Thermo X series II ICPMS coupled with a Photon Machines G2 193-nm excimer laser. Ablated material was transported from the laser to the mass spectrometer using Helium as the carrier gas. The LA-ICPMS operating conditions were as follows: 13 L/min cooling gas, 0.95 L/min auxiliary gas, 0.75 L/min Helium. The laser beam diameter was set at 30 microns, scanned

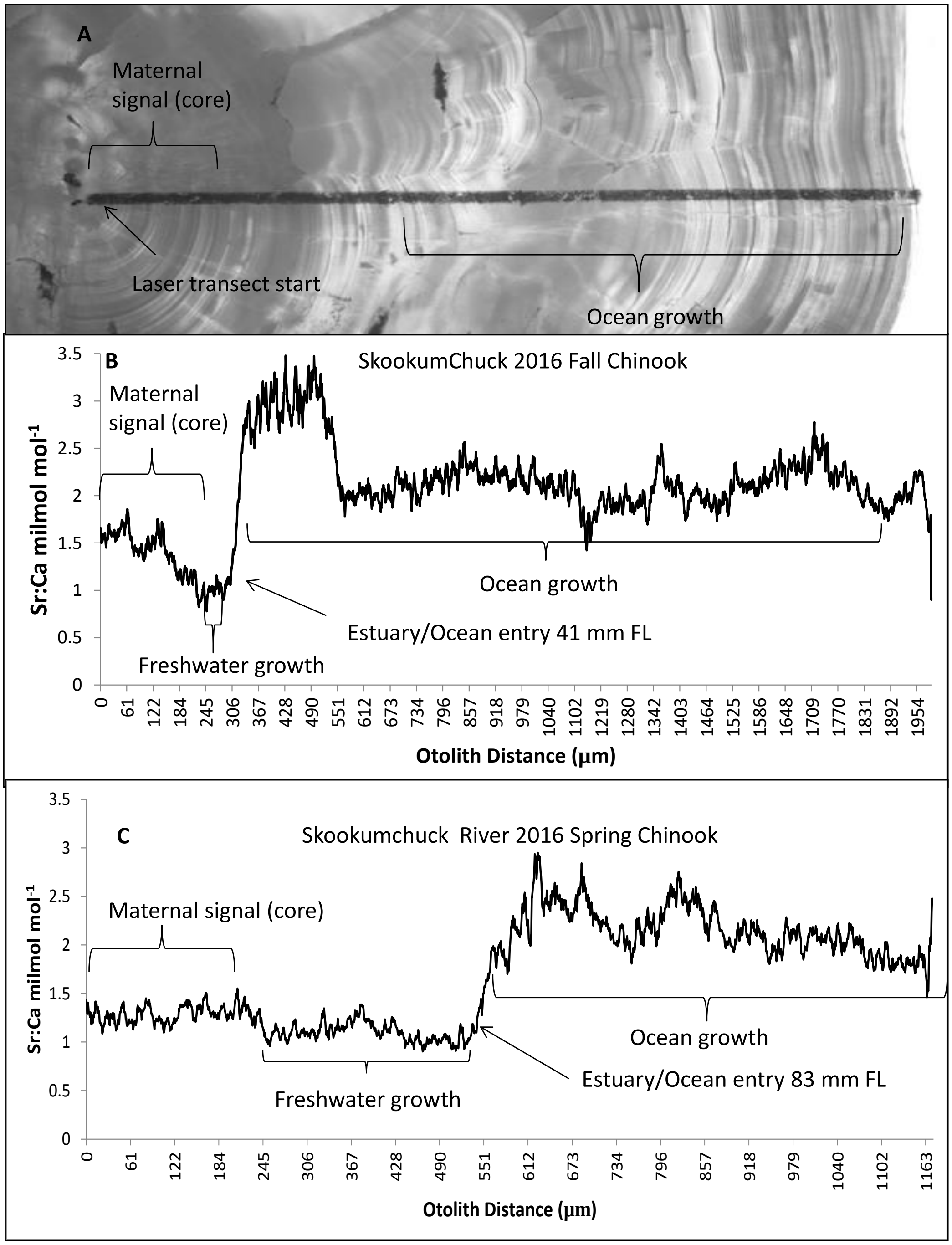

Figure 2. A) Sectioned adult Chinook salmon otolith with laser scar and approximate location of the maternal signal, freshwater and ocean phases; The ratio of Strontium to Calcium (Sr:Ca) and approximate location of maternal signal, freshwater and ocean phases for fall (B), and spring (C) run Chinook salmon.

at 5 microns/second at a pulse rate of 8 Hz. Laser transects were analyzed from the otolith core to the otolith edge in the dorsal/posterior quadrant, ~25º off the midline (Figure 2A). Normalized ion ratios were converted to elemental concentration using a glass standard from the National Institute of Standards and Technology (NIST 610) and finally converted to molar ratios for analysis. NIST scans were run every ten samples to quantify instrument drift. The point of estuary/ocean entrance for each otolith was determined as the point of inflection in ratio of Strontium to Calcium (Sr:Ca), defined here as the point of rapid Sr:Ca increase from a baseline freshwater signal (Figure 2B & C). We used a fish size/otolith size relationship from the Green River in Puget Sound WA ($y = 0.1468x - 0.5545$, $n = 211$ Ruggerone and Volk 2004) and otolith radius at Sr:Ca inflection to back-calculate fish size at estuary/ocean entrance. Maternal run timing was determined for each otolith by comparing freshwater Sr:Ca to Sr:Ca in the otolith primordia (Figure 2B & C). For each fish, we quantified mean Sr:Ca from the otolith primordia out 50 µm, and from exogenous feeding to the point of Sr:Ca inflection (Figure 2B & C). A fish was designated as the progeny of a fall spawning adult if Sr:Ca in the otolith primordia was two standard deviations greater than the average freshwater value and designated the progeny of a spring run fish, if primordia Sr:Ca was less than two standard deviations higher than the freshwater average (Berejikian et al. 2013). On average otolith core Sr:Ca that was 0.77 milmol mol^{-1} higher than freshwater values for fish determined to be fall and 0.07 milmol mol^{-1} higher than freshwater values for fish determined to be spring.

Statistical Analysis

To evaluate the successful juvenile life history's (e.g. fry, parr subyearling, yearling) of adult spring and fall Chinook salmon returning to the Chehalis River basin we used estimates of size at estuary/ocean entry to infer broad life history characteristics defined as: fry (< 60 mm

FL), parr subyearling (> 60 mm FL) and yearlings (presence of a scale or otolith annulus). Due to non-normality and unequal variance of the samples we compared median back-calculated size at estuary/ocean between populations using a Kruskal-Wallis then Dunn’s Test. We also used multiple linear regression to explore how sub-basin distance from the estuary, return year, and a categorical factor of stream temperature (warm or cool) in each sub-basin may be related to juvenile size at estuary/ocean entrance. Mean size at estuary/ocean entry for each sub-basin was the dependent variable, and distance from the sub-basin to estuary, sub-basin water temperature, and return year were independent variables in full and reduced models. Finally, we used simple linear regression to examine the relationship between sub-basin distance from the estuary and the proportion of fry, and mean size at estuary/ocean entry for each sub-basin (2016 only due to only three sub-basins sampled in 2015).

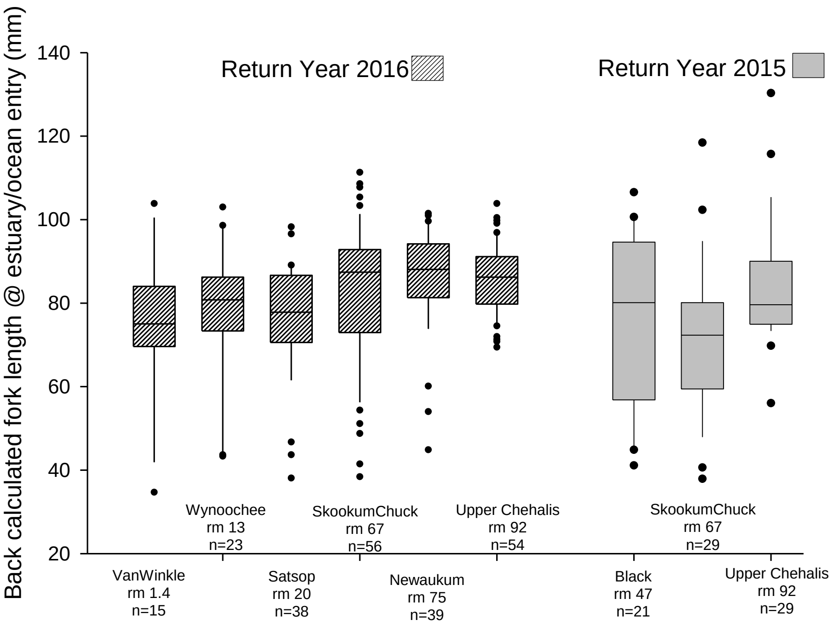

Figure 3. Box plot of back-calculated size at estuary/ocean entry for Chinook salmon recovered in sub-basins throughout the Chehalis River basin in 2015 and 2016.

Table 1. Summary statistics otolith chemistry results for Chinook salmon collected in sub-basins of the Chehalis River watershed in 2015 and 2016. Sample size (n), median and standard deviation of size at estuary/ocean entry, proportion of fry, proportion of parr, presence or absence of Spring Chinook (Yes or No), summer temperature profile (warm or cool), and the distance in river kilometers from spawning locations in each sub-basin to the saline portion of the estuary are shown.

Tributary	**n**	**Median size at ocean/estuary (Fl-mm)**	**Size at estuary/ocean (StDev)**	**Fry (<60mm)**	**Parr (>60mm)**	**Spring Chinook (Y/N)**	**Temp profile**	**Distance to estuary (Rkm)**
Vanwinkle (LAH) 2016	15	75.04	17.83	0.13	0.87	N	warm	2.25
Wynoochee 2016	23	80.80	17.15	0.17	0.83	N	warm	20.92
Satsop 2016	38	77.83	13.39	0.08	0.92	N	cool	32.51
Skookumchuck2016	56	87.41	17.05	0.13	0.88	Y	cool	107.83
Newaukum 2016	39	88.09	12.08	0.05	0.95	Y	cool	121.34
SF Chehalis 2016	1	79.78			1.00		warm	
Upper Chehalis River 2016	54	86.23	8.27	0.00	1.00	N	warm	147.58
Black2015	21	80.12	19.90	0.24	0.76	N	warm	75.64
SkookumChuck2015	29	72.32	18.60	0.24	0.76	Y	cool	107.83
Upper Chehalis River 2015	29	79.61	14.51	0.03	0.97	Y	warm	147.58

value and designated the progeny of a spring run fish, if primordia Sr:Ca was less than two standard deviations higher than the freshwater average (Berejikian et al. 2013). On average otolith core Sr:Ca that was 0.77 milmol mol^{-1} higher than freshwater values for fish determined to be fall and 0.07 milmol mol^{-1} higher than freshwater values for fish determined to be spring.

Statistical Analysis

To evaluate the successful juvenile life history's (e.g. fry, parr subyearling, yearling) of adult spring and fall Chinook salmon returning to the Chehalis River basin we used estimates of size at estuary/ocean entry to infer broad life history characteristics defined as: fry (< 60 mm FL), parr subyearling (> 60 mm FL) and yearlings (presence of a scale or otolith annulus). Due to non-normality and unequal variance of the samples we compared median back-calculated size at estuary/ocean between populations using a Kruskal-Wallis then Dunn's Test. We also used multiple linear regression to explore how sub-basin distance from the estuary, return year, and a categorical factor of stream temperature (warm or cool) in each sub-basin may be related to juvenile size at estuary/ocean entrance. Mean size at estuary/ocean entry for each sub-basin was the dependent variable, and distance from the sub-basin to estuary, sub-basin water temperature, and return year were independent variables in full and reduced models. Finally, we used simple linear regression to examine the relationship between sub-basin distance from the estuary and the proportion of fry, and mean size at estuary/ocean entry for each sub-basin (2016 only due to only three sub-basins sampled in 2015).

Results

Juvenile life histories of returning spawners in sub-basins of the Chehalis River

We evaluated otoliths from 305 returning unmarked adult Chinook salmon classified as either spring or fall stocks in the Chehalis River basin and found evidence that juvenile size at estuary/ocean entrance varied between year and sub-basins. For return year 2015, we evaluated samples collected from three sub-basins – Upper Chehalis (warm summer temperature, furthest from estuary), Skookumchuck (cool summer temperature, intermediate distance to estuary) and Black River (warm summer temperature, closest to estuary). Among all three sub-basins, 76-97% of fish emigrated to the estuary/ocean as parr subyearlings. In the Black and Skookumchuck rivers, 24% of returning fish had emigrated as fry whereas only 3% (n = 1) of fish returning to the Upper Chehalis River in 2015 emigrated as fry (Table1). No yearling migrants were observed in the returning spawners. For return year 2015 we found a statistical difference (p = 0.01, Dunn's Test) between the median size at estuary/ocean entrance of Chinook salmon sampled in

	Comparison	**p**
RY 2015	Upper Chehalis River vs Skookumchuck	**0.01**
	Upper Chehalis River vs Black	0.41
	SkookumChuck vs Black	0.24
RY 2016	Newaukum vs Satsop	**<0.01**
	Upper Chehalis River vs Satsop	**0.02**
	Newaukum vs Vanwinkle (LAH)	**0.04**
	Skookumchuck vs Satsop	0.07
	Newaukum vs Wynoochee	0.08
	Upper Chehalis River vs Vanwinkle (LAH)	0.09
	Upper Chehalis River vs Wynoochee	0.20
	Skookumchuck vs Vanwinkle (LAH)	0.21
	Skookumchuck vs Wynoochee	0.51
	Wynoochee vs Vanwinkle (LAH)	1.00
	Upper Chehalis River vs Newaukum	1.00
	Newaukum vs Skookumchuck	1.00
	Satsop vs Vanwinkle (LAH)	1.00
	Upper Chehalis River vs Skookumchuck	1.00
	Satsop vs Wynoochee	1.00

Table 2. Results (Bonferroni corrected p-values) of Dunn's Test for multiple comparisons of median size at estuary/ocean entry from run years 2015 and 2016 for various sub-basins in the Chehalis River watershed.

the Skookumchuck (72 mm FL) vs the Upper Chehalis (80 mm FL) (Table 1 & 2). Though a relatively small sample size with no brood year/age class overlap (only one return year), it appeared that surviving juveniles from the Upper Chehalis (warm summer temperatures, further from estuary) entered brackish/marine waters at a larger size than surviving juveniles from the Skookumchuck River (cool summer temperature, closer to estuary) (Figure 3).

In return year 2016 we expanded our sample size to include three additional lower river sub-basins (Wynoochee River, VanWinkle Creek, and Satsop River) and one additional upriver sub-basin (Newaukum River) (Figure 1). For return year 2016, 83-100% of fish emigrated to the estuary/ocean as parr subyearlings while 3-17% of fish emigrated as fry, and 0% as yearlings (Table 1). Similar to 2015, we observed that fry migrants were more commonly observed in Chinook salmon returning to the more downstream sub-basins. An average of 13% (SD = 3.89) of fish returning to the Wynoochee River, VanWinkle Creek, Satsop River, and Skookumchuck River emigrated as fry compared to only 0-5% from the Newaukum and Upper Chehalis River (Table 1). For the two sub-basins with samples collected in return year 2015 and 2016, we found a statistical difference between estimated fork length at estuary ocean entrance for the Skookumchuck ($p < 0.01$, Wilcoxon Rank Sum Test) but not for the Upper Chehalis ($p = 0.07$, Wilcoxon Rank Sum Test).

Relationship between life history expression, return year, distance from the estuary, and temperature

There was evidence that size at estuary/ocean entrance significantly differed among Chinook salmon returning to the lower and upper sub-basins in the 2016 such that carcasses sampled in the Satsop River and Vanwinkle Creek were smaller at estuary/ocean entrance then carcasses sampled in the Newaukum and Upper Chehalis sub-basins (Figure 3, Table 2). We observed that size at estuary/ocean entry was associated with sub-basin distance from the estuary ($p < 0.01$, extra sum of squares F-test), even after accounting for differences between return years ($p = 0.02$, extra sum of squares F-test). We observed no effect of our categorical measure of summer stream temperature on the size at estuary/ocean entrance ($p = 0.23$, extra sum of squares F-test) after accounting for sub-basin distance and return year. Specifically in return year 2016 we found a positive relationship between fish size at estuary/ocean entry and distance from the estuary, where sub-basins furthest from the estuary were larger at marine entry (Figure 4, simple linear regression, $p < 0.01$, $R = 0.985$). A similar relationship with distance was found between the where sub-basins furthest from the estuary were larger at marine entry (Figure 4, simple linear regression, $p < 0.01$, $R = 0.985$). A similar relationship with distance was found between the proportion of fry migrants in each sub-basin sample and distance to the estuary, although this result was not significant (Figure 4, simple linear regression, $p = 0.09$, $R = 0.75$).

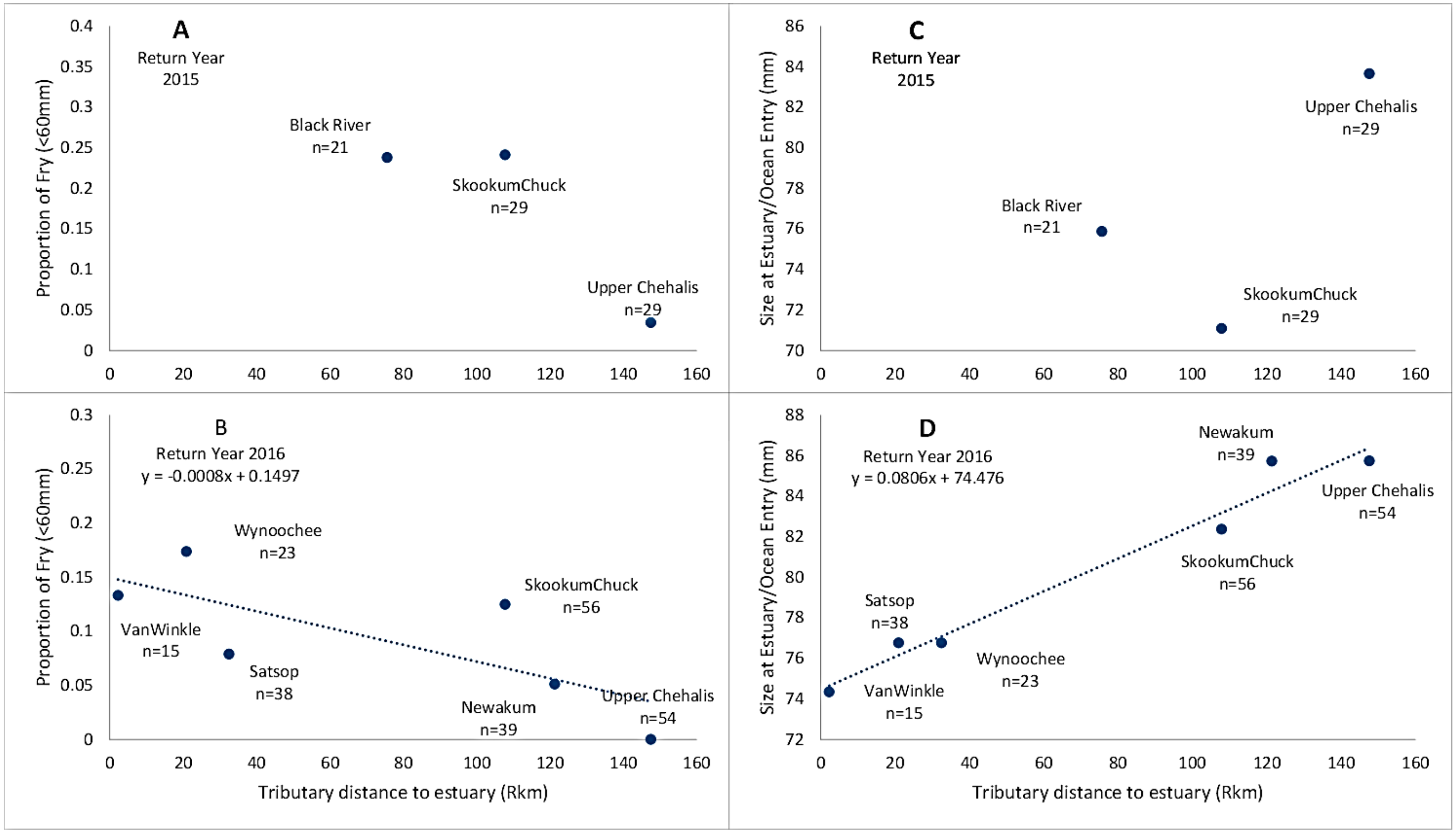

Figure 4. Relationship between the proportion of fry (FL<60mm) estimated using otolith chemistry of returning adults in each sub-basin and distance from the estuary in A) 2015 and B) 2016. Relationship between mean size at estuary/ocean entry (FL<60mm) estimated using otolith chemistry of returning adults in each sub-basin and distance from the estuary in C) 2015 and D) 2016.

Table 3. Field determinations of run timing based on carcass condition and date in comparison to otolith based determination of maternal run timing using the ratio of Strontium to Calcium. Green cells indicate the number of fish whose run timing was similar based on otolith chemistry and field calls.

		Field Determination Run Timing	
Run Year 2015		**Fall**	**Spring**
Otolith Maternal Run Timing	**Fall**	54	14
	Spring	4	7
Run Year 2016			
Otolith Maternal Run Timing	**Fall**	213	5
	Spring	1	5

Quantifying otolith Sr:Ca to estimate maternal spawning life history (spring vs fall)

We examined a total of 305 otoliths for maternal run timing from Chehalis River sub-basins in 2015 and 2016 of which two individuals had too little freshwater residence between exogenous feeding, and estuary/ocean entry to determine freshwater otolith Sr:Ca values. Of the remaining 303, 31 samples were identified as spring Chinook salmon and 272 were identified as fall Chinook

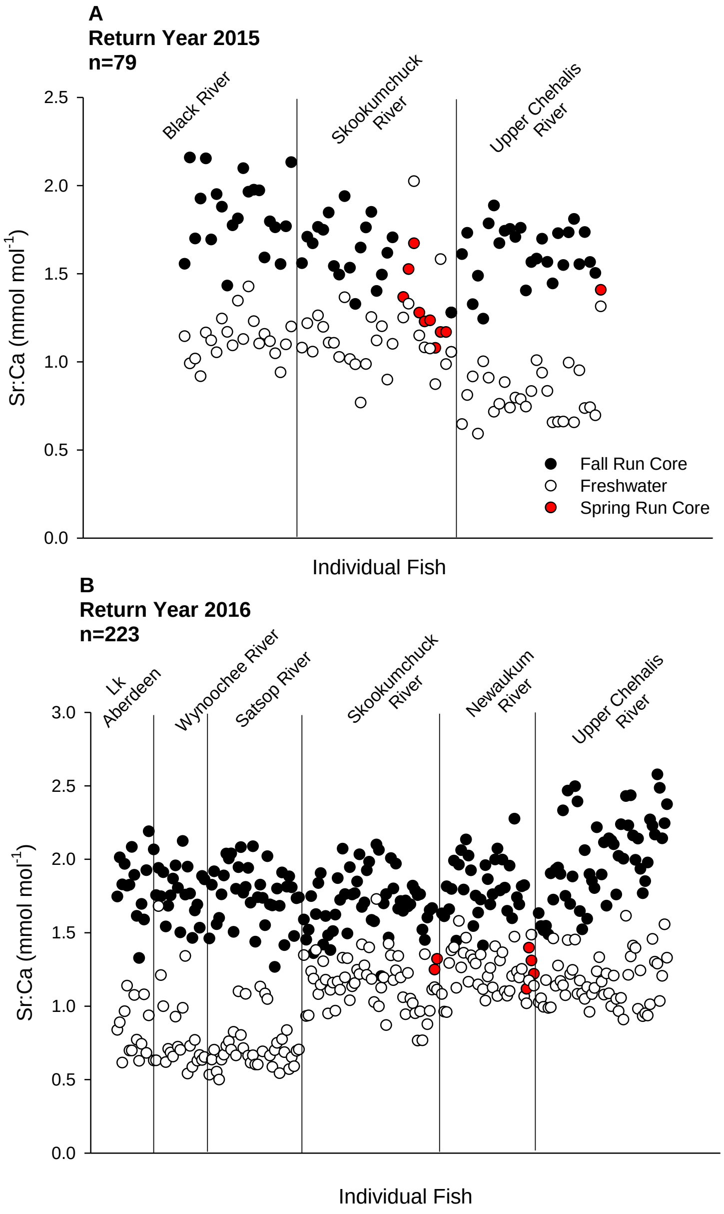

Figure 5. The ratio of Strontium to Calcium (Sr:Ca) during freshwater (open circle), and the core portions of the otolith (filled circles) for Chinook salmon classified as spring or fall run from sub-basins in the Chehalis River basin in A) 2015 and B)2016.

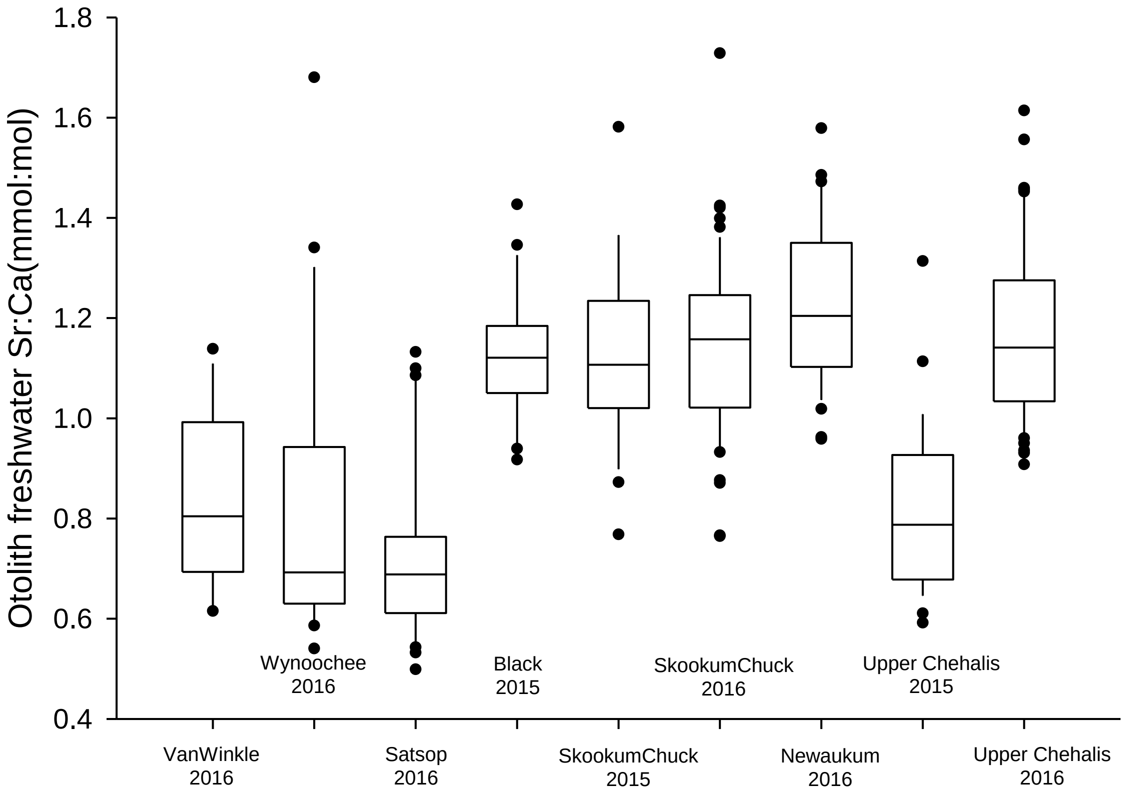

Figure 6 Box plots for otolith material formed during juvenile freshwater rearing (proxy for water chemistry). Results showing differences in lower and upper Chehalis subbasins.

salmon in the field using run timing and carcass condition criteria. The correspondence between field and otolith assignments of fall Chinook salmon was high (93 - 99% agreement) in both years (Figure 5, Table 3). In the 2015 samples, we found weak correspondence (33% agreement) between field identification and the otolith assignment of run type; 67% of the carcasses identified as a spring Chinook salmon in the field were assigned as a fall Chinook salmon based on otolith chemistry that included a high maternal Sr:Ca signal consistent with an ocean-maturing fish. In 2016, field identification and otolith chemistry assignment of spring Chinook salmon agreed 50 % of the time suggesting that field assignment of run type also overrepresented the proportion of spring Chinook salmon in this year. We found that otolith Sr:Ca levels associated with freshwater were relatively high for the Skookumchuck, Newaukum, and Upper Chehalis rivers (~ 1.2 mmol mol^{-1}) (Figure 6). These elevated levels may interfere with our ability to classify stream and ocean maturing samples using elemental Sr:Ca values, without examining isotopes of Sr (Figure 5 & 6).

Summary

- We found evidence that Chinook salmon entering the Grays Harbor estuary as small fry sized fish (< 60 mm FL) survived early migration and returned to spawn as adults in the Chehalis basin. The survival through, and the use of estuary/marine habitat during this early life stage is consistent with results from Puget Sound, the Columbia River, Coastal Oregon and the Sacramento River where juvenile Chinook have been observed entering into and residing for extended periods or documented returning in the adult populations (Volk et al. 2010, Miller 2010, Campbell et al. 2010, Campbell and Claiborne 2017).

- Our results are consistent with the hypothesis that early fry migrants survive leaving their natal streams and may make up a substantial portion of the adult population while emphasizing the importance of protecting and restoring habitat critical to small fish.

- The proportion of fry migrants returning in the adult spawning collections decreased in sub-basins further upriver from the estuary. In addition, we found a positive relationship between the median size at estuary/ocean entrance and distance from the estuary. We hypothesis that the correlation between successful juvenile life histories and distance from a specific habitat (in this case estuary) describes a life history cline, where proximal habitats play a larger role in early juvenile rearing then distant habitats.

- Our results suggest that the numbers of spring Chinook salmon may be overestimated and the numbers of fall Chinook salmon may be underestimated based on current methods used to identify run types. The correspondence between field identified spring Chinook salmon (using spawn timing and carcass condition) and spring Chinook salmon identified with otolith chemistry was weak in 2015 (33% agreement, n = 7/21) but increased to 50% agreement in 2016 (50% agreement, n = 5/10). In comparison, the correspondence between field and otolith assignments of fall Chinook salmon was high (93 - 99% agreement) in both years.

- Over return year 2015 and 2016, we found freshwater otolith Sr:Ca values to approach marine values (~1.0-1.5 Sr:Ca mmol:mol) for the Black, Skookumchuck, Newaukum, and upper Chehalis (2016 only) (Figure 5 &6). These relatively high freshwater Sr:Ca levels likely decrease our accuracy in assigning progeny from stream maturing (Spring stock) fish. Known origin spring Chinook, such as those captured in a fishery in May/June would greatly inform the range of Sr values we would expect to see from potential spring Chinook on the spawning grounds. In addition supplementing this work with genetic analysis and otolith isotope analysis (Sr86/Sr87) would help refine our predictive ability.

- We found little evidence that temperature, categorized by warm and cool summer temperatures, explained differences in size at estuary/ocean entry or the proportion of fry found in the returning adults (Table 1). Sub-basin temperature did seem to be related to the presence of spring Chinook salmon identified by otolith chemistry; the majority (n = 16) of otolith-assigned spring Chinook salmon were found in the cool (Skookumchuck and Newaukum) sub-basins but not in the warm sub-basins. One exception to this conclusion is the one spring Chinook identified in the upper Chehalis in 2015. However this individual sample had a freshwater chemical signal significantly different from other upper Chehalis basin fish and thus may have been a stray. The Satsop sub-basin in the lower Chehalis (rKM 32) is unique in that the East Fork Satsop is a spring-fed system with cooler water than the neighboring West Fork and Middle Fork Satsop rivers (Winkowski et al. 2017), but spring Chinook salmon are not known from this sub-basin, nor were any found in our otolith analysis.

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