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Pre-Restoration Habitat Use by Chinook Salmon in the Nisqually Estuary Using Otolith Analysis

By Angela Lind-Null, Kimberly Larsen, and Reginald Reisenbichler

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Introduction

The Nisqually Fall Chinook population is one of 27 stocks in the Puget Sound evolutionarily significant unit listed as threatened under the federal Endangered Species Act. The preservation of the Nisqually delta ecosystem coupled with extensive restoration of approximately 1,000 acres of diked estuarine habitat is identified as the highest priority action for the recovery of naturally spawning Nisqually River Fall Chinook salmon (*Oncorhynchus tshawytscha*) in the Nisqually Chinook Recovery Plan.

In order to evaluate the response of Chinook salmon to restoration, a pre-restoration baseline of life history diversity and estuary utilization must be established. Otolith analysis has been proposed as a means to measure Chinook salmon life history diversity, growth, and residence in the Nisqually estuary. Over time, the information from the otolith analyses will be used to: (1) determine if estuary restoration actions cause changes to the population structure (i.e. frequency of the different life history trajectories) for Nisqually River Chinook, (2) compare pre and post restoration residence times and growth rates, and (3) suggest whether estuary restoration yields substantial benefits for Chinook salmon.

Otoliths are calcium carbonate structures in the inner ear that grow in proportion to the overall growth of the fish. Daily growth increments can be measured so date and fish size at various habitat transitions can be back-calculated. Careful analysis of otolith microstructure can be used to determine the number of days that a fish resided in the estuary as a juvenile (increment counts), size at entrance to the estuary, size at egress, and the amount that the fish grew while in the estuary. Juvenile Chinook salmon can exhibit a variety of life history trajectories – some enter the sea (or Puget Sound) as fry, some rear in the estuary before entering the sea, and some rear in the river and then move rapidly through the estuary into the sea as smolts.

The purpose of this study is to evaluate and use analysis of otolith microstructure as a tool for characterizing the importance of the estuary to Chinook salmon in the Nisqually River before and after restoration efforts at the Nisqually National Wildlife Refuge (NNWR). This tool is used to quantify changes in habitat use and help assess restoration benefits to the federally threatened Nisqually River Chinook salmon population.

Analysis of otolith microstructure typically is superior to the alternative of traditional mark-recapture methods. The latter are extremely expensive or inadequate in estuary habitats, typically are biased and substantially underestimate use, and do not directly reveal the importance or contribution to adult recruitment (i.e., they do not account for differential survival afterward in Puget Sound or the ocean). Analysis of otolith microstructure for these purposes, while new, is proving highly successful in a similar study that USGS and partners are conducting in the Skagit River estuary system located in northern Puget Sound. This work has been based on research by Neilson et al. (1985). We expect to use the Skagit River data as a reference for the before/after restoration comparison in the Nisqually River.

Objectives

Objective #1: Develop a Nisqually-specific signature of otolith microstructure growth patterns and checks that allow us to distinguish growth and residence of juvenile salmon in the estuary from growth in the river (upstream) and in Puget Sound (seaward). Evaluate between-year variation in these characters by comparing otoliths collected in 2004 with those collected in 2005.

Objective #2: Determine whether distinct growth patterns on the otoliths of hatchery and wild salmon in the Nisqually River allow us to recognize unmarked hatchery fish and separate them from wild fish.

Objective #3: Analyze the otoliths of returning adults in order to catalog the juvenile life-history trajectories of these "successful" fish and provide a preliminary estimate for the proportions and numbers of wild and hatchery adults that reared in the delta and estuary as juveniles.

Objective #4: Describe the relationship between juvenile salmon size or date of entry to the estuary with the fish's growth rate or residence time in the estuary.

Methods

Unmarked and marked juvenile Chinook salmon were collected by the Nisqually tribe and U.S. Fish and Wildlife Service – NNWR in March through October of 2004 and February through October of 2005 from various sites within the Nisqually River mainstem, tidal delta, nearshore, and associated habitats (Table 1). No Chinook were caught in the nearshore or EEM (Animal fyke trap) catch during February of 2005. The fish were collected by beach seining in the following distinct habitat zones (Cowardin et al. 1979; Figure 1):

1. *Freshwater (FW)* – forested slow water habitat on the mainstem Nisqually River without tidal influence.
2. *Forested Riverine Tidal (FRT)* – riparian forest, mud/silt substrate, and tidal influence.
3. *Emergent Forested Transition (EFT)* – scrub/shrub and marsh vegetation, mud/silt substrate, and tidal influence.
4. *Estuarine Emergent Marsh (EEM)* – low and high salt marsh vegetation, mud substrate, and full tidal influence.
5. *Delta Flats (DF)* – sparse to no vegetation, mud and/or gravel/cobble substrate, and large tidal fluctuations.
6. *Nearshore (NS)* – areas outside of Nisqually tidal delta, vegetation and substrate variable.
7. *Pocket Estuary (PE)* – sand spit enclosed estuary with salt marsh vegetation, sand and mud substrate, and forested bluffs.

A few sites within the EEM habitat were sampled with fyke nets. Each fish was euthanized and measured for length and weight. The fish were preserved in alcohol and sent to USGS where the sagittal otoliths of unmarked fish were extracted, sectioned, and polished according to the Western Fisheries Research Center's (WFRC) standard protocols.

Table 1: Number of otoliths from unmarked juvenile Chinook collected in 2004 – 2006 and used for otolith analysis. Additional fish were collected in 2005 and 2006 but not listed here.

		March	April	May	June	July	August	September	October	TOTAL
2004	**FRESHWATER**	10	20	10	11	0	0	6	1	58
	FRT	17	18	3	7	0	1	0	0	46
	EFT	2	1	9	9	0	0	0	0	21
	EEM	7	0	40	44	17	0	0	0	108
	NEARSHORE	0	0	0	2	0	0	0	0	2
	DELTA FLATS	0	13	3	17	3	0	0	0	36
	POCKET ESTUARY	0	3	0	0	0	0	0	0	3
2005	**EEM (Animal Fyke Trap)**	1	10	12	11	10	4	0	0	48
	NEARSHORE	0	0	2	5	1	0	0	0	8
2006	**NEARSHORE**	0	1	13	1	2	2	0	0	19
	TOTAL	37	66	92	107	33	7	6	1	349

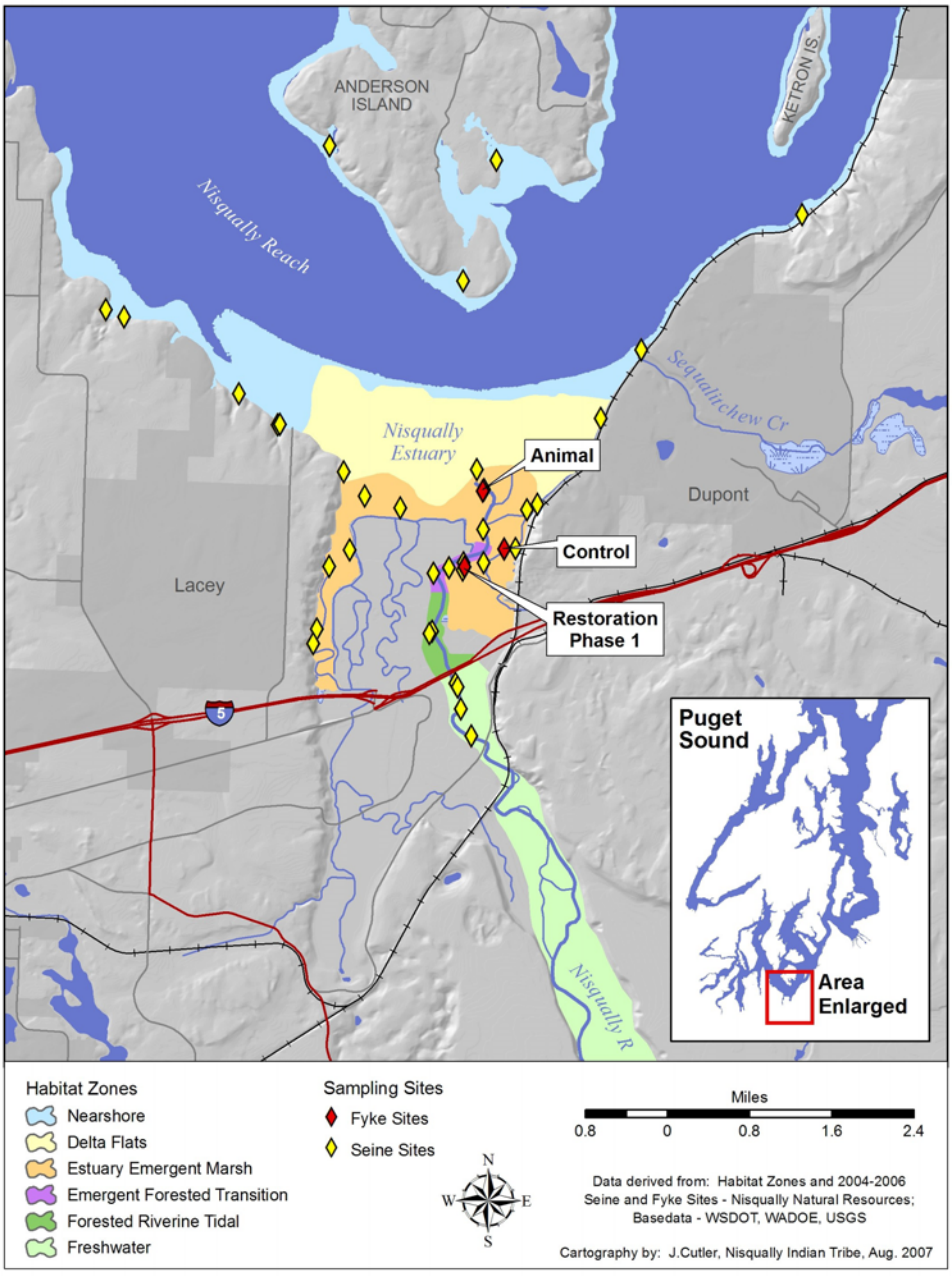


Figure 1: Nisqually field sampling sites.

A total of 167 juvenile Chinook salmon were collected in 2004 directly from the various South Sound hatcheries less than two weeks prior to hatchery releases for determination of unique patterns specific to individual hatcheries. An average of 15 fish per hatchery were sacrificed and the otoliths of at least 8 fish from select hatcheries were processed. These particular hatcheries corresponded to "high incidence" hatchery populations composing at least 5% of the CWT catch for this study in 2004 (shown in italics): *Clear Creek 44.6%, Kalama 28.0%, White River 6.5%, Garrison/Chambers Creek 5.8%, Voights Creek 5.8%*, Lost CWT 5.0%, Soos Creek 1.4%, Tumwater 2.2%, and Clark's Creek 0.7%.

In 2004, a total of 274 pairs of otoliths were collected from unmarked fish. All fish otoliths (one from each pair) were processed and sorted as "high incidence" hatchery or wild fish (Tables 2 and 3). Samples identified as "high incidence" were not analyzed. If the sample was not obviously hatchery or wild, the fish was categorized as "unknown origin" and was not analyzed further. Fish from the pocket estuary also were not analyzed due to small sample size (n=3). A total of 97 fish were identified as hatchery, 119 as wild, and 58 as unknown origin. A total of 97 samples were analyzed out of the 119 available wild fish. Some samples were not suitable for analysis because of: (i) presence of vaterite (a morph of the calcium carbonate structure), (ii) poor initial quality, (iii) uneven microstructural growth along the radial axis or (iv) processing error.

Table 2: Number of otoliths analyzed / processed. With the exception of the 2005 Animal fyke trap, the number analyzed does not include unmarked hatchery fish separated from the catch.

		March	April	May	June	July	August	September	October	TOTAL
2004	**FRESHWATER**	4 / 10	12 / 19	4 / 10	2 / 10	0 / 0	0 / 0	0 / 6	0 / 1	22 / 56
	FRT	8 / 17	10 / 17	1 / 3	2 / 7	0 / 0	1 / 1	0 / 0	0 / 0	22 / 45
	EFT	1 / 1	1 / 1	2 / 9	1 / 9	0 / 0	0 / 0	0 / 0	0 / 0	5 / 20
	EEM	5 / 7	0 / 0	11 / 36	10 / 44	9 / 17	0 / 0	0 / 0	0 / 0	35 / 104
	NEARSHORE	0 / 0	0 / 0	0 / 0	1 / 2	0 / 0	0 / 0	0 / 0	0 / 0	1 / 2
	DELTA FLATS	0 / 0	5 / 13	1 / 3	6 / 17	0 / 3	0 / 0	0 / 0	0 / 0	12 / 36
	POCKET ESTUARY	0 / 0	0 / 3	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 3
2005	**EEM (Animal Fyke Trap)**	1 / 1	7 / 10	11 / 11	7 / 9	7 / 10	4 / 4	0 / 0	0 / 0	37 / 45
	NEARSHORE	0 / 0	0 / 0	0 / 2	1 / 5	1 / 1	0 / 0	0 / 0	0 / 0	2 / 8
2006	**NEARSHORE**	0 / 0	0 / 1	4 / 13	1 / 1	0 / 2	0 / 2	0 / 0	0 / 0	5 / 19
	TOTAL	19 / 36	30 / 64	33 / 87	25 / 104	17 / 33	5 / 7	0 / 6	0 / 1	141 / 338

Table 3: Number of unmarked hatchery samples separated from the catch.

		March	April	May	June	July	August	September	October	TOTAL
2004	**FRESHWATER**	0	0	6	7	0	0	5	1	19
	FRT	1	1	1	5	0	0	0	0	8
	EFT	0	0	5	5	0	0	0	0	10
	EEM	1	0	14	24	5	0	0	0	44
	DELTA FLATS	0	5	1	8	2	0	0	0	16
	NEARSHORE	0	0	0	0	0	0	0	0	0
2005	**NEARSHORE**	0	0	2	4	0	0	0	0	6
	EEM (Animal Fyke Trap)	0	0	3	2	3	0	0	0	8
2006	**NEARSHORE**	0	1	7	0	0	2	0	0	10
	TOTAL	2	2	38	47	8	2	5	1	121

In 2005, a total of 333 pairs of otoliths were collected from unmarked fish. The majority of samples from the 2005 collection were not analyzed due to limited funding in the current funding contract. At the request of our cooperators and project officer we processed otoliths from one particular site in the EEM habitat (Animal fyke trap) (Table 2). A total of 48 pairs of otoliths were collected from unmarked fish at the Animal fyke site. One otolith from each pair was processed and sorted as to hatchery or wild. A total of 8 fish were identified as hatchery, 32 as wild, and 8 as unknown origin. Both hatchery and wild fish were analyzed for a total of 37 out of 45 suitable otoliths, however only wild fish were included in the analyses.

The nearshore collection was supplemented with samples collected in 2005 (n=8) and 2006 (n=19) due to small sample size in 2004 (n=2) (Table 1). A total of 16 fish were identified as hatchery, 10 as wild, and 3 as unknown origin. A total of 8 samples were analyzed out of the 10 available wild fish.

Adult samples were collected from the fishery and spawning grounds by the Nisqually tribe in 2005 and 2006. In 2005, a total of 176 samples were collected from the fishery and 125 were collected from the spawning grounds. In 2006, a total of 189 samples were collected from the fishery and 34 were collected from the spawning grounds. No adult samples were processed or analyzed due to limited funding under the current funding contract. Collections are archived for future funding opportunities.

Fish collected from freshwater showed a pattern which was used as a reference pattern on the otoliths. This reference pattern did not have any “checks” beyond the recognizable emergence and first feed checks. Checks are generally referred to as a consistently prominent mark or pattern on the otolith which interrupts the normal sequence of otolith deposition (Campana 1983). Each increment was interpreted as daily growth for the fish. Otoliths from fish collected in all other habitat types were visually analyzed for additional patterns, checks, or increased growth beyond the identifiers observed on the freshwater residence portion of the otoliths.

Daily growth increments and checks in the otolith microstructure were measured with the aid of a digital imaging system, Image-Pro. We selected a standardized radial axis for measurements at 85 ± 5 degrees ventral of the longitudinal axis passing through an identifiable and preferred nucleus. Distances along the radial axis and individual increment widths between checks or increase in growth representing change in habitat, were recorded for each fish.

Growth rates (mm/day) in the tidal delta were calculated from lengths based on the Fraser-Lee method (Murphy and Willis 1996):

$$L_i = \frac{L_c - a}{S_c} S_i + a$$

where

L_i is the back-calculated length of the fish at the beginning of the tidal delta check,

L_c is the length of the fish at capture, S_c is the radius of the otolith at capture,

S_i is the radius of the otolith at the beginning of the tidal delta check, and

a is the intercept from the overall regression of capture fork length verses otolith radius (Figure 2).

Average growth rate and mean increment widths (MIW) were determined for all habitat types. Residence time and fork lengths upon entry to the tidal delta and delta flats/nearshore habitat zones were also calculated.

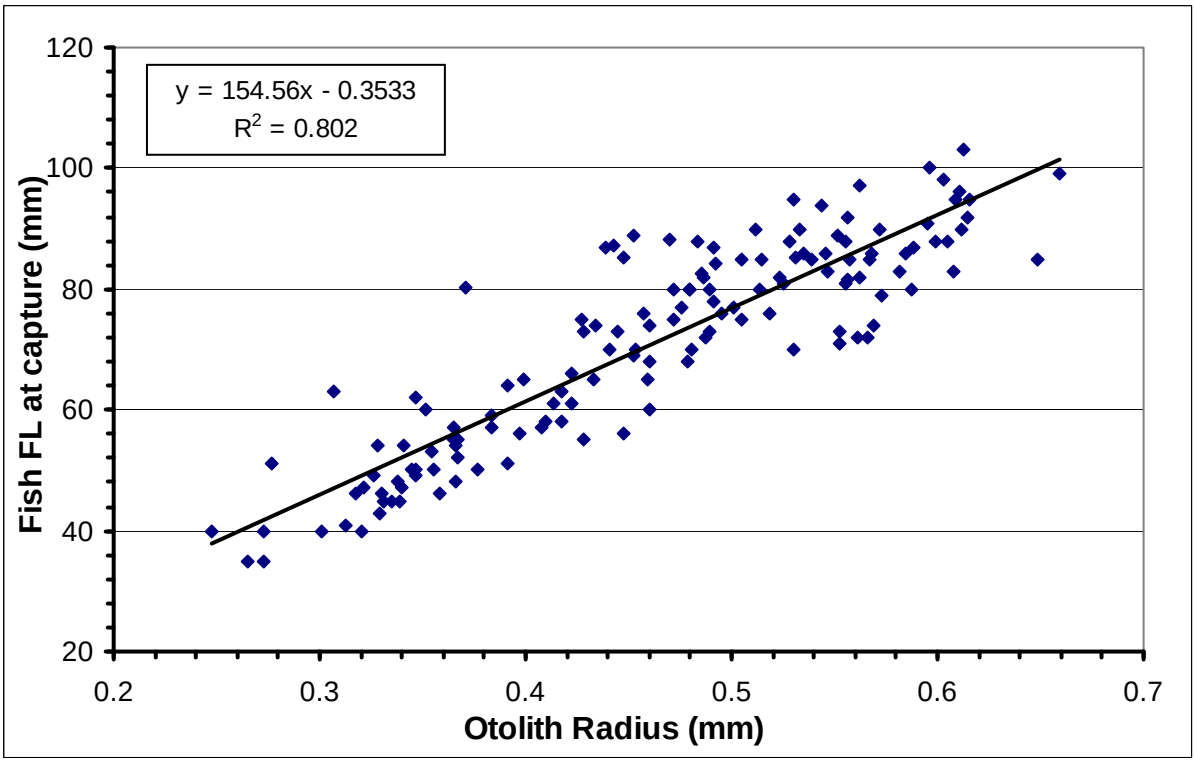


Figure 2: Relationship between fish fork length (mm) and otolith radial distance (microns).

Results

Otolith microstructure pattern varied little over the years in 2004 – 2006; however the timing (i.e. month) of check formation did vary. After first feed, the increments on all otoliths became more legible and consistent across the radial axis (Figure 3). An interruption in the microstructure pattern, designated as a tidal delta check (TDCK), was detected on samples collected within tidal delta habitats EFT and EEM, indicating the fish's transition from freshwater to the estuarine habitats (Figure 4). Increments were consistently narrow across the radial axis until the tidal delta check appeared where consistently wider increments indicated increased growth. No tidal delta check or increased growth was seen on otoliths from fish collected in the freshwater or the upper most tidal delta habitat (FRT).

In 2004, a tidal delta check was not observed on samples collected in March from EFT and EEM habitats. Insufficient sample sizes in April precluded analysis of the tidal delta check. In mid to late May, the tidal delta check appeared on samples from EFT and EEM habitats. In 2005, the tidal delta check first appeared on samples collected in the EFT and EEM in early June, but was barely detectable on some samples in late May (2 out of 8).

In addition to the tidal delta check, an additional interruption was seen on otoliths collected in the nearshore habitat beginning in June and in the delta flats habitat in April. We called this check a delta-flats check (DFCK). It indicated the fish's transition from estuarine habitat to the nearshore habitat (Figure 5). This check looked identical to the nearshore check located on Chinook in the Skagit River system (Beamer et al. 2000). Due to classification of sites, we called this check a delta-flats check instead of a nearshore check. The check was abbreviated in some samples (3 out of 11), possibly due to the fish being caught immediately upon entrance into the habitat. Insufficient samples were available to determine whether a delta-flats check was visible on samples collected in the nearshore in March or April. The number of samples containing a delta-flats check that were collected in the nearshore habitat were considerably low (1 out of 8).

With samples analyzed from multiple years, a one-way ANOVA was run to test for differences between years among tidal delta and freshwater MIW and growth rates. A significant difference occurred for MIW in the freshwater portion of the otolith for Animal fyke samples ($P<.05$). Therefore, the 2005 Animal fyke trap samples were excluded from the freshwater portion of the analysis.

No difference was visually observed in the microstructure pattern between EFT and EEM. To further validate this observation, a one-way ANOVA was run to test for significant differences between EFT and EEM. No significant differences occurred in growth rate or MIW ($P>0.05$) and therefore the data were combined and classified as "tidal delta." FRT was not included as part of the tidal delta habitat for analysis because visually the microstructure pattern did not differ from freshwater samples nor was an additional check or increased growth ever observed.

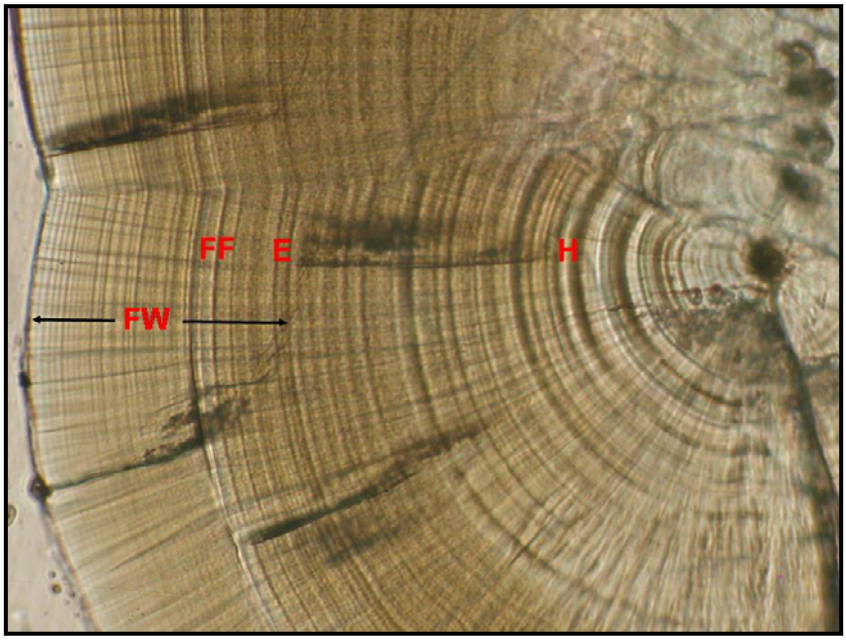
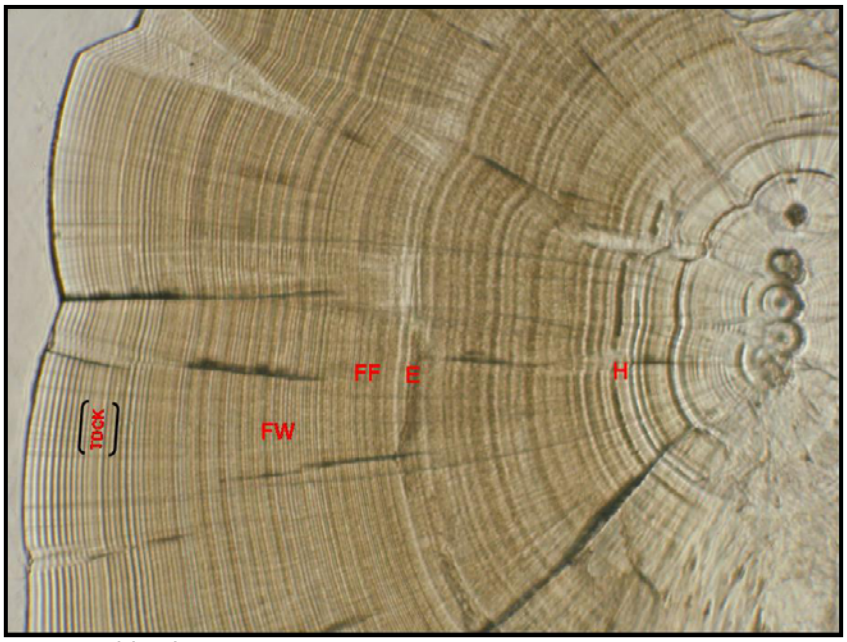
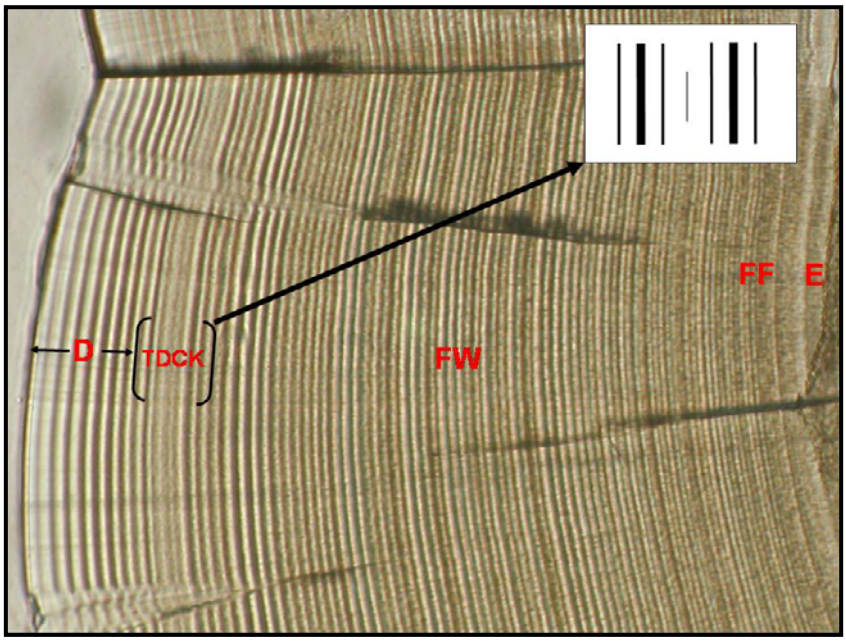


Figure 3: Representative sample of freshwater growth. The letters below represent: H = hatch, E = emergence, FF = first feed, FW = freshwater residence.



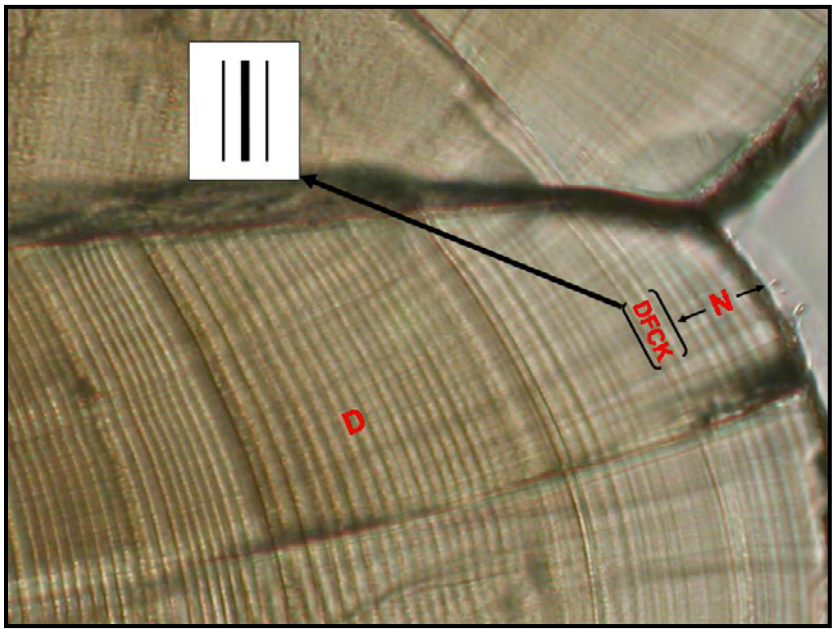
20x objective



40x objective

Figure 4: The tidal delta check (TDCK) was seen on samples collected in the tidal delta in mid to late May (2004) and mid-June (2005). The check was bold and prominent consisting of two thin dark bands encompassing two wide bright bands containing a thick dark band between them. This sequence was then repeated following approximately 1 increment. Beyond the tidal delta check, increments were consistently wider indicating increased growth. The letters below represent: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, TDCK = tidal delta check, and D = tidal delta residence.

10x objective



40x objective

Figure 5: The delta-flats check (DFCK) was seen on samples collected in the nearshore beginning in mid-June and the delta flats in April. The check was bold and prominent consisting of two thin dark bands encompassing two wide bright bands containing a thick dark band between them. Beyond the delta-flats check, increments were consistently wider indicating increased growth. The letters below represent: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, TDCK = tidal delta check, D = tidal delta residence, DFCK = delta-flats check, and N = delta flats/nearshore residence.

We tested for differences in MIW in freshwater and tidal delta portions of the otoliths (Figure 6). One-way ANOVA showed a significant difference ($P<0.05$) across habitats. On average, delta flats habitat had the lowest freshwater and tidal delta MIW. Overall, the MIW of the freshwater portion of all otolith samples was lowest followed by the tidal delta and nearshore habitats, respectively.

The equivalent results for growth rate were that the freshwater growth rates (mean= 0.42 mm/day) were lower compared to the tidal delta growth rates for fish residing in the tidal delta (mean = 0.57 mm/day), nearshore (mean = 0.57 mm/day), and delta flats (mean = 0.66 mm/day) habitats, with a 36% increase in growth from freshwater habitat to tidal delta habitat. The delta flats/nearshore growth rate for fish caught in the delta flats was the same as the tidal delta growth rate. No significant difference was found between tidal delta and delta flat/nearshore growth rates (one-way ANOVA, $P>0.05$).

The average fork length upon entry to the tidal delta was 72.8 mm. Fish caught in the tidal delta spent an average of 16 days with a minimum residence time of 10 days and a maximum of 35. These fish samples provided a minimum estimate of residence because the fish were sacrificed prior to entering the Sound. Evaluation of those fish caught in the delta flats and nearshore habitats exhibited an average residence time of 21 days in the tidal delta (n = 10). This value represents a truer estimate of residence time in the tidal delta, however the sample size was quite small. Fish caught in the delta flats were on average 60.2 mm when they entered the tidal delta and 69.5 mm upon exit, whereas fish caught in the nearshore were 73.1 mm upon entrance to the tidal delta. A positive relationship existed between the growth rate and the date the fish entered the tidal delta or nearshore (Figure 7). This could not be explained by a difference in size at entrance into the tidal delta and nearshore habitats.

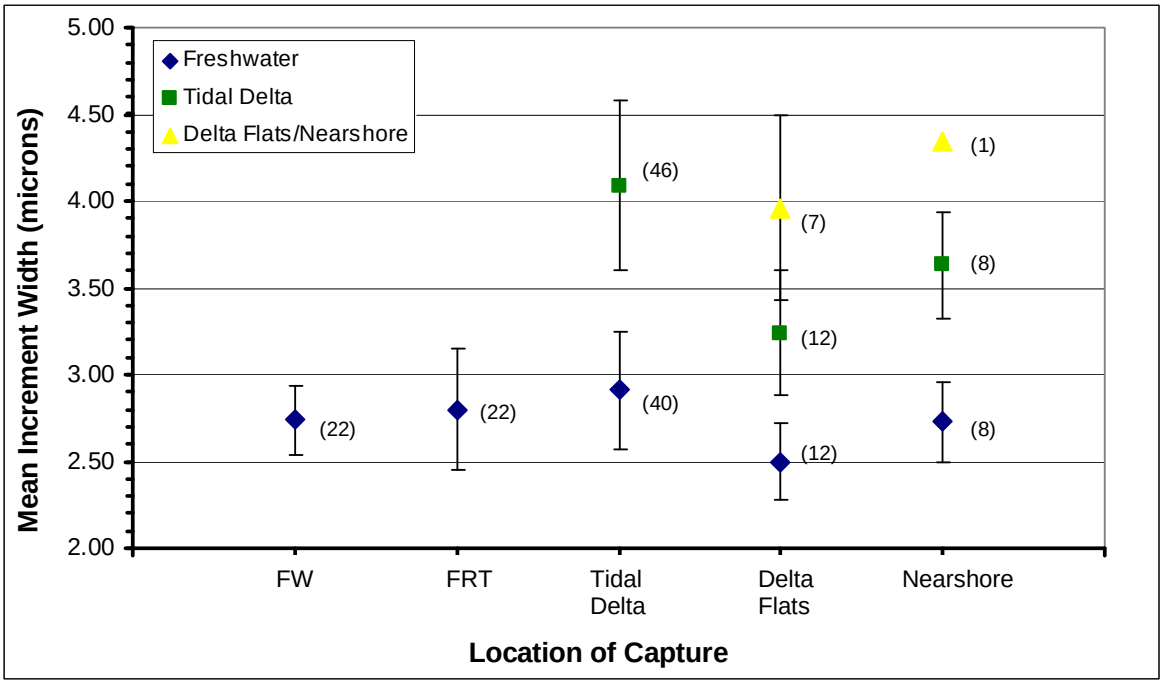


Figure 6: Mean Increment width (microns) for freshwater, tidal delta, and delta flats/nearshore residence within each habitat. Two samples collected in the delta flats were excluded from the delta flats/nearshore portion of the MIW analysis because residence time was only one day. The number of samples are represented in parentheses. Error bars represent ±1 standard deviation.

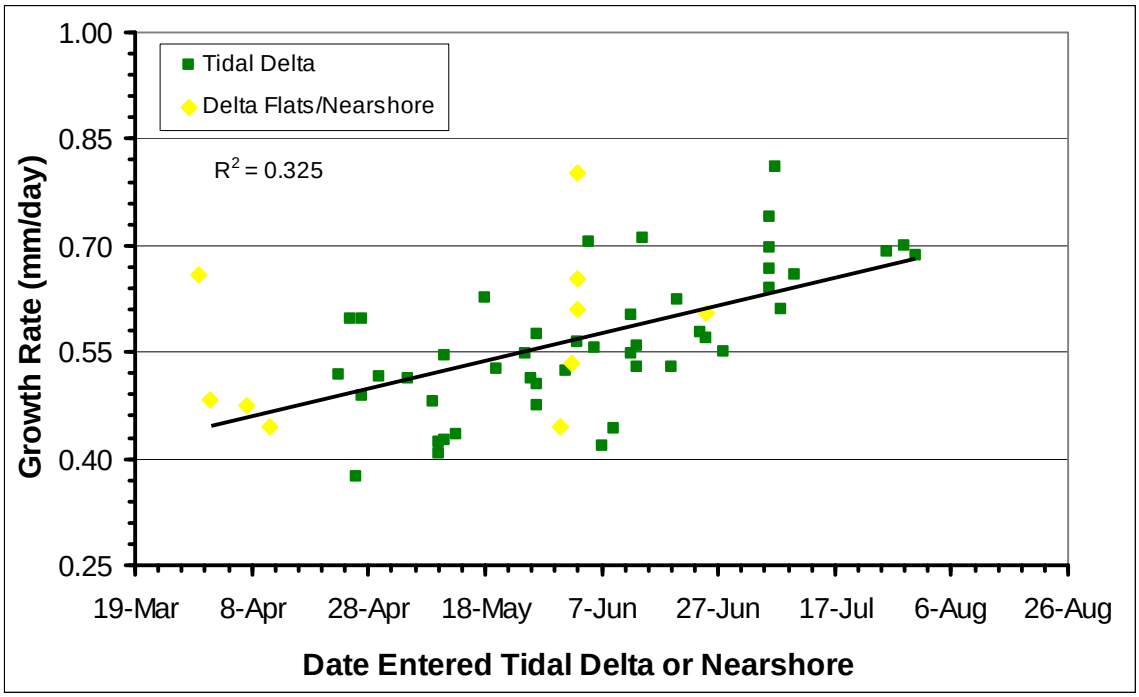


Figure 7: Relationship between the growth rate (mm/day) and the date the fish entered the tidal delta or delta flats/nearshore habitat.

Discussion

Hatchery Chinook salmon vastly outnumber wild salmon in the Nisqually River; however distinct microstructure patterns unique to each hatchery allowed us to recognize and separate unmarked hatchery fish from wild. The majority of unmarked hatchery fish were seen in natural habitats during May and June subsequent to hatchery release. Few hatchery strays were seen in March and April prior to release.

We characterized a Nisqually-specific signature of otolith microstructure growth patterns and checks for wild fish that allowed us to distinguish entry into the tidal delta and nearshore habitats. However, we were not able to distinguish between all habitat types prior to mid-May (2004) or early-June (2005) when the tidal delta check first appeared. The tidal delta check was not visible on freshwater or FRT samples regardless of when they were caught nor on samples collected in March in the EEM and EFT. We do not know whether a tidal delta check occurs in April because we had an insufficient sample size (n=1). Samples collected in March displayed few (x = 7) increments following the freshwater pattern which indicated that the fish were collected at or very early after entrance to the habitat and may not have had sufficient time to develop a visible check. This could be clarified by substantially increasing early season (March) sample size or by the addition of otolith microchemical analysis of Sr:Ca ratios (Fowler et al. 1995) in the hypothesized freshwater/tidal delta transition zone.

The delta-flats check first appeared in early June in nearshore samples and in April in delta flats samples. It is unclear whether a delta-flats check appeared in nearshore samples in April due to limited sample size (n=1).

We saw no visual difference in the microstructure pattern between otoliths collected in 2004, 2005, and 2006. However, analysis revealed differences in MIW for freshwater residence and when the tidal delta check first appeared between the 2004 and 2005 Animal fyke samples. The majority of samples from the 2005 collection have not been analyzed to date due to limited funding. We focused our efforts for this reporting period on one sampling year (2004) rather than divide the effort across two years, lowering the sample size further and possibly missing potential characterization of some life history types.

Mean increment widths generally increased as the fish moved from freshwater to the nearshore habitats. The magnitude of the difference in MIW between the tidal delta and nearshore habitats probably is underestimated and may be an artifact of low sample size compounded by the brief time spent in the nearshore habitat for a large proportion of the fish.

Overall, the growth rate increased as the fish migrated from one habitat to another. Fish were growing faster (36%) in the tidal delta compared to freshwater, but this increase is significantly less than that seen in the Skagit River (U.S. Geological Survey, unpublished data). Our analysis revealed that fish grew at the same rate in the nearshore as in the tidal delta. This may be due to small sample size or that the majority of fish were caught soon after arrival (mean number of days residing before capture = 8; 8 out of 10 residing less than 10 days) in the nearshore habitat.

Funds and allocated time were insufficient to accomplish the analysis of adults during the current funding period. As previously mentioned, it is important to establish baseline information of life history trajectories from the juveniles, and then proceed to examining that portion of the adult otolith corresponding to the juvenile stage. Our resources were exhausted in working with the juveniles so we were not able to proceed to the adult samples. Otoliths collected from carcasses in Fall of 2006 were the first adult collections to correspond to the 2004 collection of juvenile outmigrants (i.e. 2003 brood year) in the estuary. Sampling will be attempted through 2010 to exhaust possible adult returns from 2003 – 2006 brood years.

Our results suggest that otolith microstructure analysis can be a valuable tool to establishing a baseline for use of the Nisqually River estuary habitats by juvenile Chinook salmon under existing conditions. However, this study provides limited information due to small samples sizes in some months, and only looks at contributions of wild-origin fish. The sample sizes contributed to uncertainty about whether Nisqually salmon deposit various habitat transition checks on the otoliths in all months. This uncertainty occurs for both tidal delta checks and delta-flats checks. Collection and analysis of additional fish especially in tidal delta and nearshore habitat zones should be addressed. Furthermore, these collections should occur over several years to allow adequate evaluation of inter-annual variation in microstructure growth patterns and checks, and may reveal additional life history types. Analysis of otolith microchemistry in conjunction with microstructure would provide an additional avenue for identifying early entry (March and April) into the tidal delta and perhaps the nearshore. Of course, further work should include analysis of adults because they show the proportions and numbers of adults that reared in the estuary as juveniles.

Acknowledgments

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