Final Report presented to the Nisqually Indian Tribe

Characterization of Estuary Use by Nisqually Hatchery Chinook Based on Otolith Analysis

By Angie Lind-Null, Kim Larsen, and Reg 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 (ESA). Preservation and extensive restoration of the Nisqually delta ecosystem are planned to assist in recovery of the stock. A pre-restoration baseline including life history types, estuary residence time, growth rates, and habitat use are needed to evaluate the potential response of hatchery and wild Chinook salmon to restoration.

Otolith analysis has been selected as a means to examine Chinook salmon life history, 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, 3) suggest whether estuary restoration yields substantial benefits for Chinook salmon through (1) and (2), and 4) compare differences in habitat use between hatchery and wild Chinook to further protect ESA listed stock.

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 hatchery Chinook salmon are generally released as smolts that move quickly through the delta with much shorter residence times than for many wild fish and are not dependent on the delta as nursery habitat (Myers and Horton 1982; Mace 1983; Levings et al. 1986).

The purpose of this study is to use and evaluate otolith microstructure analysis as a tool for assessing the role of the estuary in the life history of hatchery Chinook salmon in the Nisqually River before and after restoration efforts at the Nisqually National Wildlife Refuge (Nisqually NWR). This tool is used to quantify changes in rate of growth, length of residence and habitat use to help predict restoration benefits to the federally threatened Nisqually River hatchery and wild Chinook salmon populations.

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 any differential survival afterward in Puget Sound or the ocean). Analysis of otolith microstructure for these purposes is proving successful for the Nisqually wild Chinook stock as well as a similar study that USGS and partners are conducting in the Skagit River estuary system located in northern Puget Sound. This work is based on research by Neilson et al. (1985). We expect to use the Skagit River as a reference for the before/after restoration comparison in the Nisqually River.

Objective

Characterize the importance of the Nisqually estuary to hatchery Chinook salmon by 1) estimating growth rates, 2) residence times, and 3) size at entry to the tidal delta and nearshore habitats.

Methods

Unmarked and marked juvenile Chinook salmon were collected by the Nisqually Tribe and the U.S. Fish and Wildlife Service – Nisqually NWR in March through October of 2004 from various sites in the lower Nisqually River, the tidally influenced region of the estuary near the river's mouth (hereafter referred to as tidal delta), and the shallow sub-tidal and intertidal areas (accessible by beach seine; hereafter referred to as nearshore) outside of Nisqually delta complex (Table 1). Most 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 (uppermost portion of the tidal delta).
3. *Emergent Forested Transition* (EFT) – scrub/shrub and marsh vegetation, mud/silt substrate, and tidal influence (tidal delta).
4. *Estuarine Emergent Marsh* (EEM) – low and high salt marsh vegetation, mud substrate, and full tidal influence (lowermost portion of the tidal delta).
5. *Delta Flats* (DF) – sparse to no vegetation, mud or gravel/cobble substrate, and large tidal fluctuations.
6. *Nearshore* (NS) – saltwater, shallow sub-tidal and intertidal areas, 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.

Table 1: Number of marked juvenile Chinook providing otoliths. All fish were collected in 2004. Only marked fish were used in this study.

	March	April	May	June	July	August	September	October	TOTAL
FW	0	0	19	7	0	0	0	0	26
FRT	0	0	5	14	1	0	0	0	20
EFT	0	0	31	5	0	0	0	0	36
EEM	0	0	107	61	24	0	0	0	192
DF	0	4	15	25	9	0	1	0	54
NS	0	0	7	7	0	0	0	0	14
PE	0	0	1	0	0	0	0	0	1
TOTAL	0	4	185	119	34	0	1	0	343

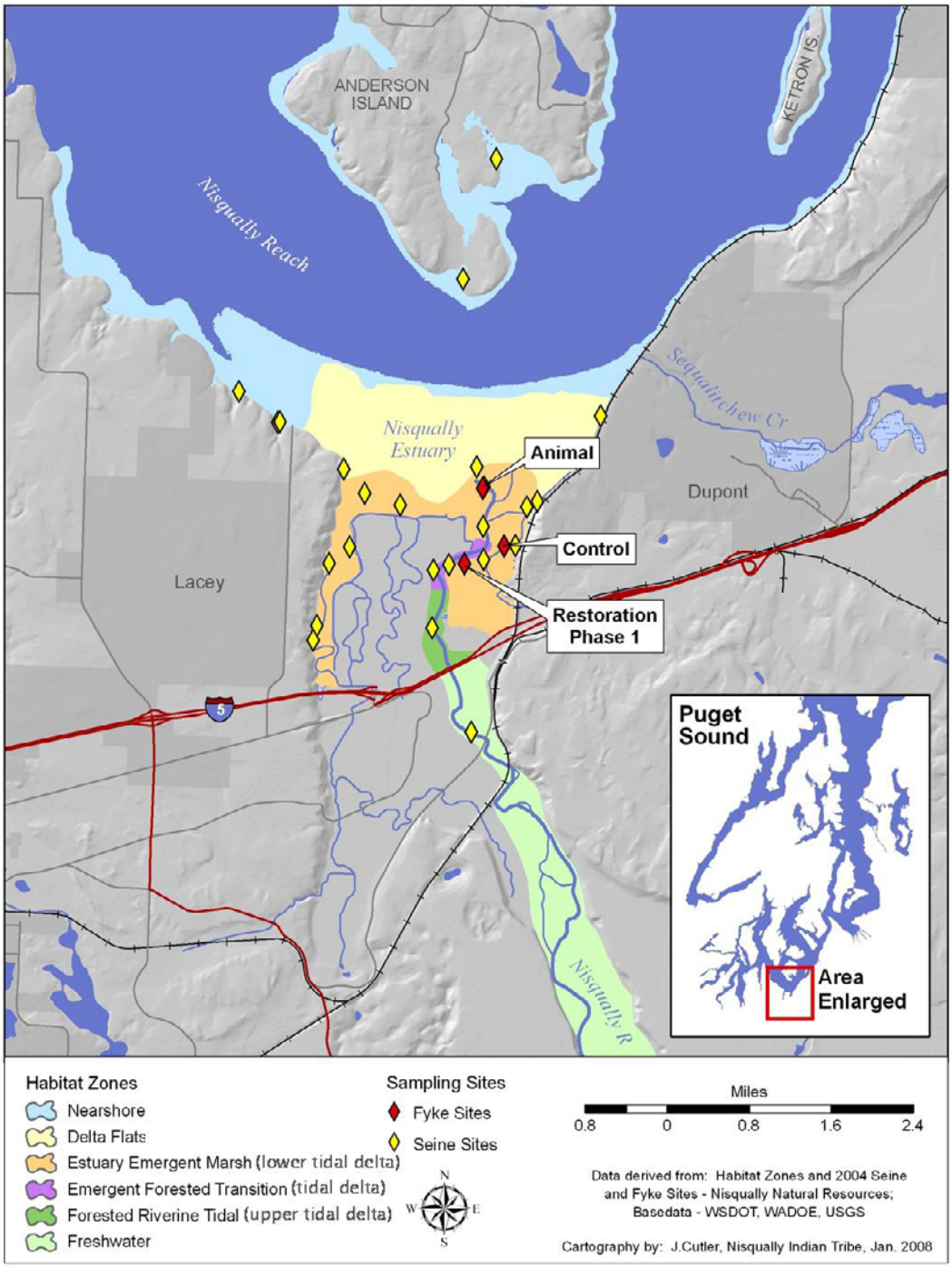

Figure 1: Nisqually field sampling sites.

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 marked fish were extracted.

A total of 343 pairs of otoliths were collected from marked fish. All fish otoliths (one from each pair), excluding those with a coded wire tag from other than the Nisqually River (n=31) and the single fish collected from PE habitat (n=1) were processed according to the Western Fisheries Research Center's (WFRC) standard protocols (Table 2). An additional 59 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. In total, 252 samples were analyzed out of the 296 processed marked fish.

Fish collected from FW showed a consistently recognizable pattern which was used as a reference pattern for all fish otoliths collected below FW habitat. This reference pattern had no checks beyond the recognizable button-up and first feed checks. A check is a consistently prominent mark or pattern on the otolith which interrupts the normal sequence of otolith deposition (Campana 1983). Each increment was interpreted as one day's growth for the fish (Stevenson and Campana 1992). 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 FW 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 an increase in growth representing change in habitat, were recorded for each fish.

Growth rates (mm/day) in the tidal delta and DF/NS habitats were calculated from lengths based on the Fraser-Lee method (DeVries and Frie 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 a habitat transition, 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 a habitat transition, 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 zones. Residence time and fork lengths upon entry to the tidal delta and DF/NS habitat zones were also calculated.

Table 2: Number of otoliths (one per fish) analyzed / processed.

	March	April	May	June	July	August	September	October	TOTAL
FW	0 / 0	0 / 0	17 / 18	7 / 7	0 / 0	0 / 0	0 / 0	0 / 0	24 / 25
FRT	0 / 0	0 / 0	5 / 5	12 / 14	1 / 1	0 / 0	0 / 0	0 / 0	18 / 20
EFT	0 / 0	0 / 0	21 / 28	4 / 5	0 / 0	0 / 0	0 / 0	0 / 0	25 / 33
EEM	0 / 0	0 / 0	85 / 101	40 / 47	12 / 15	0 / 0	0 / 0	0 / 0	137 / 163
DF	0 / 0	0 / 2	12 / 13	19 / 22	5 / 5	0 / 0	0 / 1	0 / 0	36 / 43
NS	0 / 0	0 / 0	7 / 7	5 / 5	0 / 0	0 / 0	0 / 0	0 / 0	12 / 12
PE	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
TOTAL	0 / 0	0 / 2	147 / 172	87 / 100	18 / 21	0 / 0	0 / 1	0 / 0	252 / 296

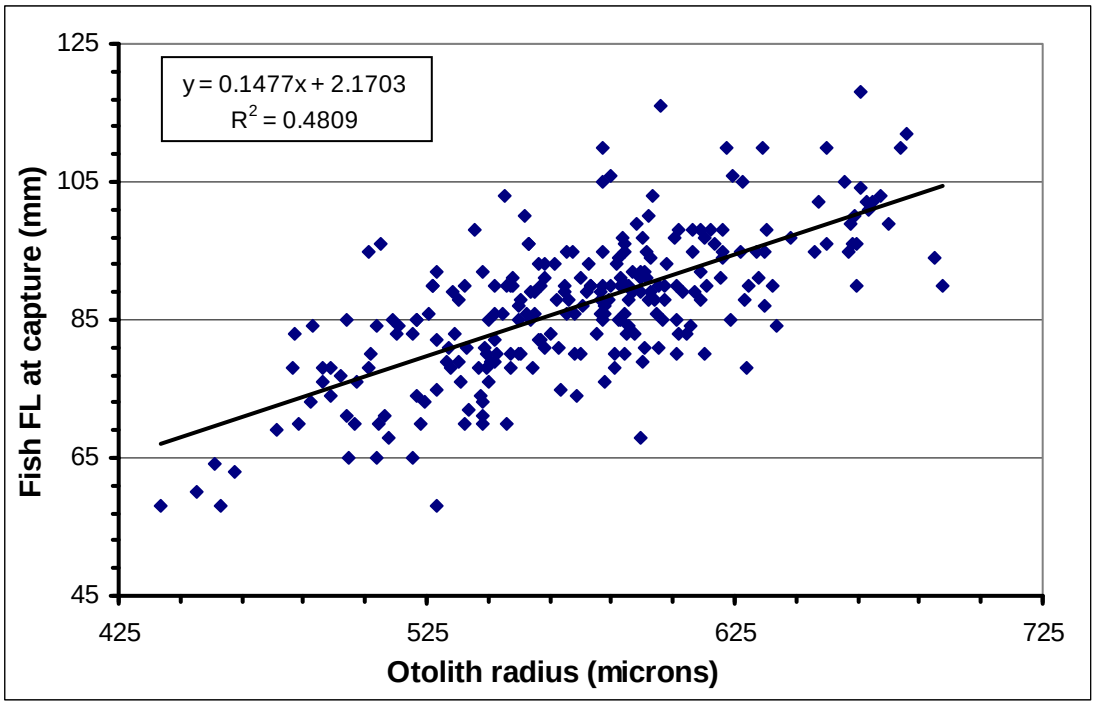

Figure 2: Relationship between fish fork length (mm) and otolith radial distance (microns).

Results

A check coincident with release from the Clear Creek (6 May – 4 June 2004) and Kalama (14 May – 1 June 2004) hatcheries was difficult to determine because the fish were volitionally released or perhaps a release check does not exist. Lack of a release check might reflect direct and rapid movement to the tidal delta once fish leave the hatchery; however we have no direct evidence for this possibility. Since a release check was not identified, FW growth included for analysis represents time spent in the hatchery prior to release and time spent in the river after hatchery release.

The increments on all otoliths became more legible and consistent across the radial axis beyond the first-feed check (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 beginning in mid to late May indicating transition to tidal delta habitat following volitional release from the hatcheries (Table 3, Figure 4). The check was abbreviated in some samples (n=16), possibly because the fish were caught immediately upon entrance to the habitat. Increments were consistently thin with narrow spacing across the radial axis until the TDCK appeared. At this point, the increments became consistently thicker with wider spacing, indicating an increase in growth with habitat transition from FW habitat to tidal delta habitat. No TDCK or increase in growth was seen on otoliths from fish collected in FW or FRT. Hereafter, the tidal delta is in reference to EFT and EEM habitats only.

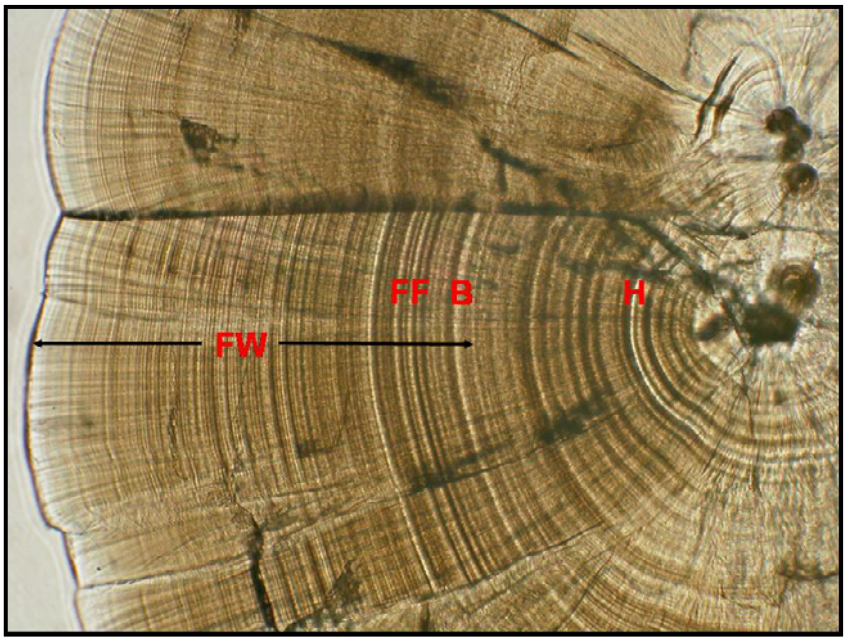

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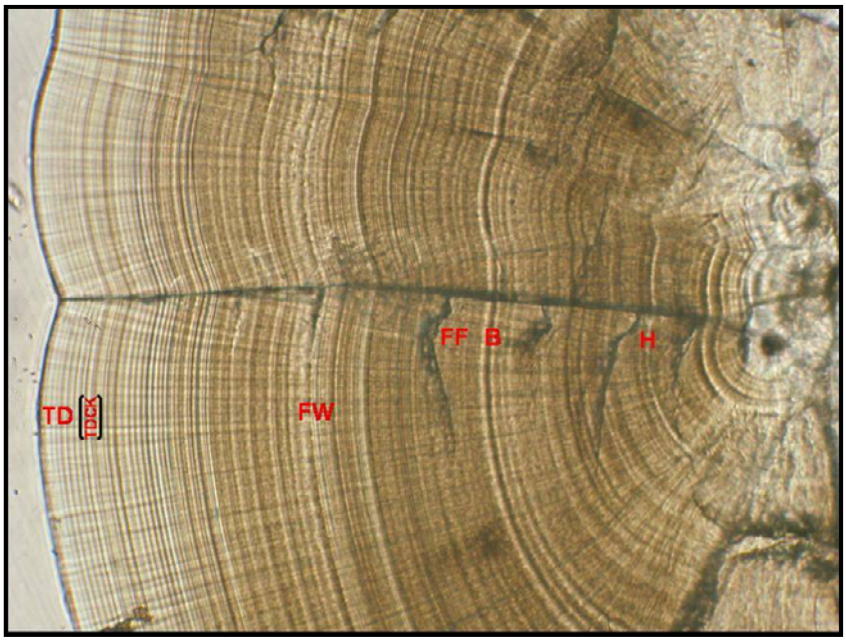
Figure 3: Representative sample of FW growth. The letters below represent: H = hatch, B = button-up, FF = first feed, FW = FW residence.

Table 3: Number of otoliths (one per fish) with a tidal delta check (TDCK) or delta-flats check (DFCK). Dashes indicate where a check should not be expected

	May		June		July	
	TDCK	DFCK	TDCK	DFCK	TDCK	DFCK
FRT	0	-	0	-	0	-
EFT	3	-	4	-	0	-
EEM	10	-	36	-	12	-
DF	0	0	14	0	4	3
NS	6	0	4	0	0	0
TOTAL	19	0	58	0	16	3



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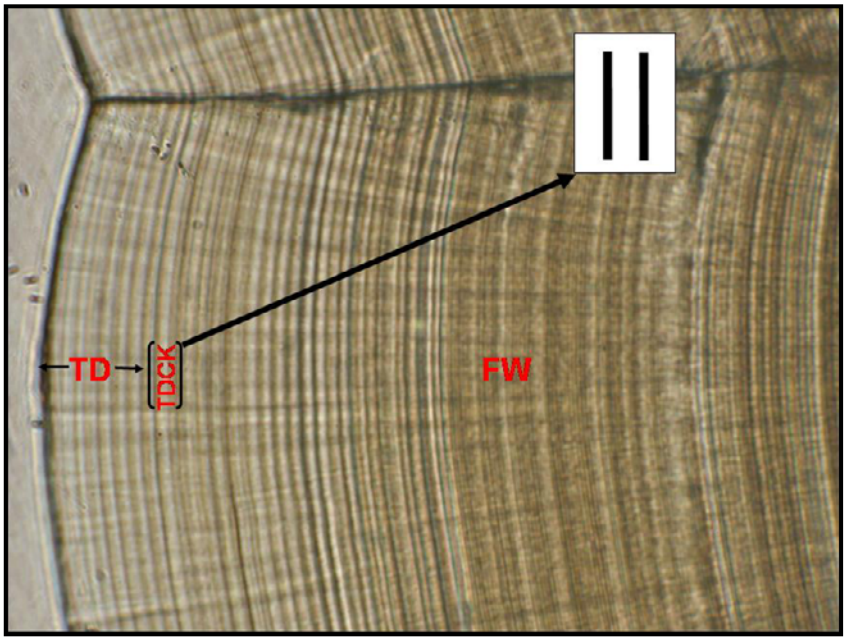

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Figure 4: The tidal delta check (TDCK) was seen on samples collected in the EFT and EEM in mid to late May. The check was bold and prominent consisting of two thick dark bands with a wider white space between. Beyond the TDCK, increments were consistently wider indicating an increase in growth. The letters below represent: H = hatch, B = button-up, FF = first feed, FW = FW residence, TDCK = tidal delta check, and TD = tidal delta residence.

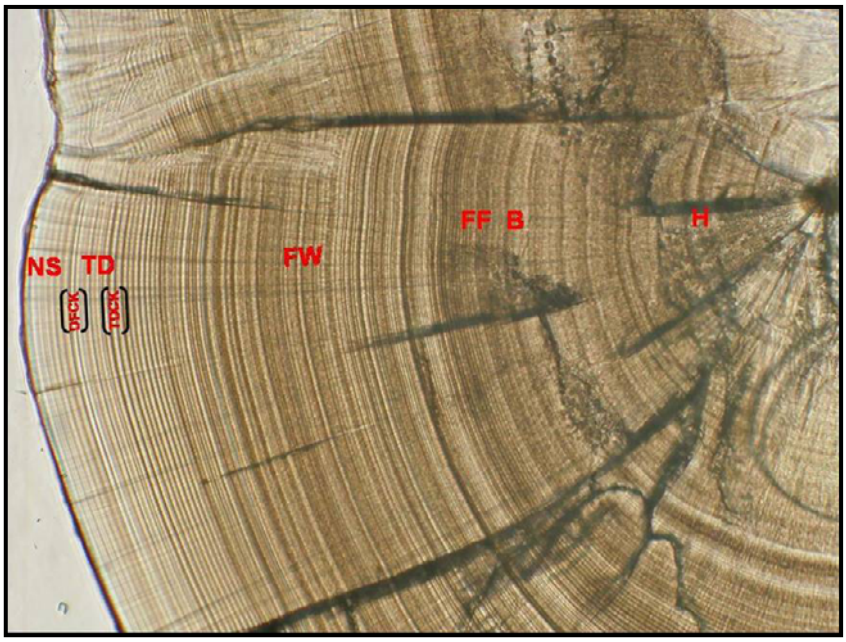
In addition to the TDCK, an additional check was seen on some otoliths collected in DF habitat beginning in early July. We called this check a delta-flats check (DFCK). It indicated the fish's transition from tidal delta habitat to the DF/NS habitat (Figure 5). This check looked identical to the NS check located on Chinook in the Skagit River system (Beamer et al. 2000). Due to classification of sites, we called this check a DFCK instead of a NS check. The number of samples containing a DFCK that were collected in the DF habitat was considerably low (3 out of 36). A DFCK was not seen on samples collected in NS habitat possibly due to the lack of samples from later in the season.

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 difference occurred in the MIW ($P>.05$) and therefore the MIW data were combined and classified as "tidal delta." There was a significant difference ($P<.05$) in the growth rate and therefore the growth rate data were not combined. This apparent discrepancy seems to have resulted because the otoliths from EFT fish were small for the size of the fish (5 out of 7). Any given increment width of these fish corresponded to a greater growth on body size compared to the EEM fish. FRT was not included as part of the tidal delta habitat for analysis because visually the microstructure pattern did not differ from FW samples nor was an additional check or an increase in growth ever observed on FRT samples as stated previously.

We tested for differences in MIW in the FW and tidal delta portions of the otoliths (Figure 6). One-way ANOVA showed a significant difference ($P<.05$) across habitats for the FW portion and no significant difference ($P>.05$) for the tidal delta portion. On average, fish from FRT and FW habitats had the smallest FW MIW. In general, the MIW of the FW portion of all otolith samples was smallest followed by the tidal delta and DF/NS habitats, respectively.

The equivalent results for growth rate were that the FW growth rates (mean= .40 mm/day) were lower compared to the tidal delta growth rates for fish residing in the EFT (mean = .67 mm/day), EEM (mean = .59 mm/day), DF (mean = .61 mm/day), and NS (mean = .55 mm/day) habitats, with a 50% increase in growth from FW habitat to tidal delta habitat. No significant difference was found between tidal delta and DF/NS growth rates (one-way ANOVA, $P>.05$). The increase in growth from the tidal delta habitat to the DF habitat was 2%, however sample size was small (n=3).

The average fork length upon entry to the tidal delta was 86.3 mm. Fish caught in the tidal delta spent an average of 9 days with a minimum residence time of 4 days and a maximum of 20. These samples provided a minimum estimate of residence because the fish were sacrificed prior to entering the Sound. Evaluation of those fish caught in the DF habitats exhibited an average residence time of 8 days in the tidal delta (n = 3). Normally, this value would represent a truer estimate of residence time in the tidal delta, however sample size was small (n=3). Fish caught in the DF were on average 88 mm when they entered the tidal delta whereas fish caught in the NS were 83.1 mm upon entrance to the tidal delta. We were able to measure size at entrance (78.8 mm) and exit (82.4 mm) for the three fish caught in the DF with a DFCK.



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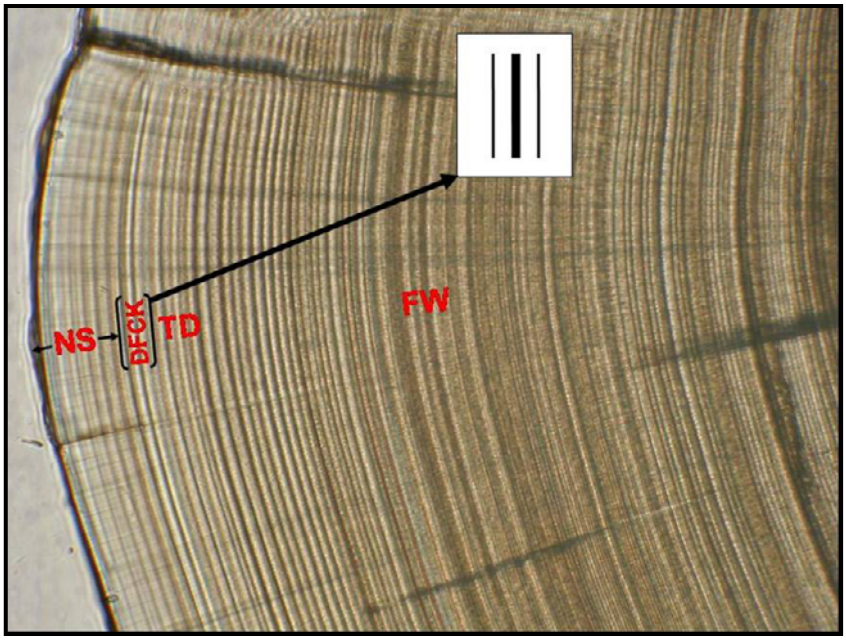

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Figure 5: The delta-flats check (DFCK) was seen on samples collected in DF beginning in early July. The check was bold and prominent consisting of two thin dark bands encompassing two wide bright bands containing a thick dark band between them. Beyond the DFCK, increments were consistently wider indicating an increase in growth. The letters below represent: H = hatch, B = button-up, FF = first feed, FW = FW residence, TDCK = tidal delta check, TD = tidal delta residence, DFCK = delta-flats check, and NS = DF/NS residence.

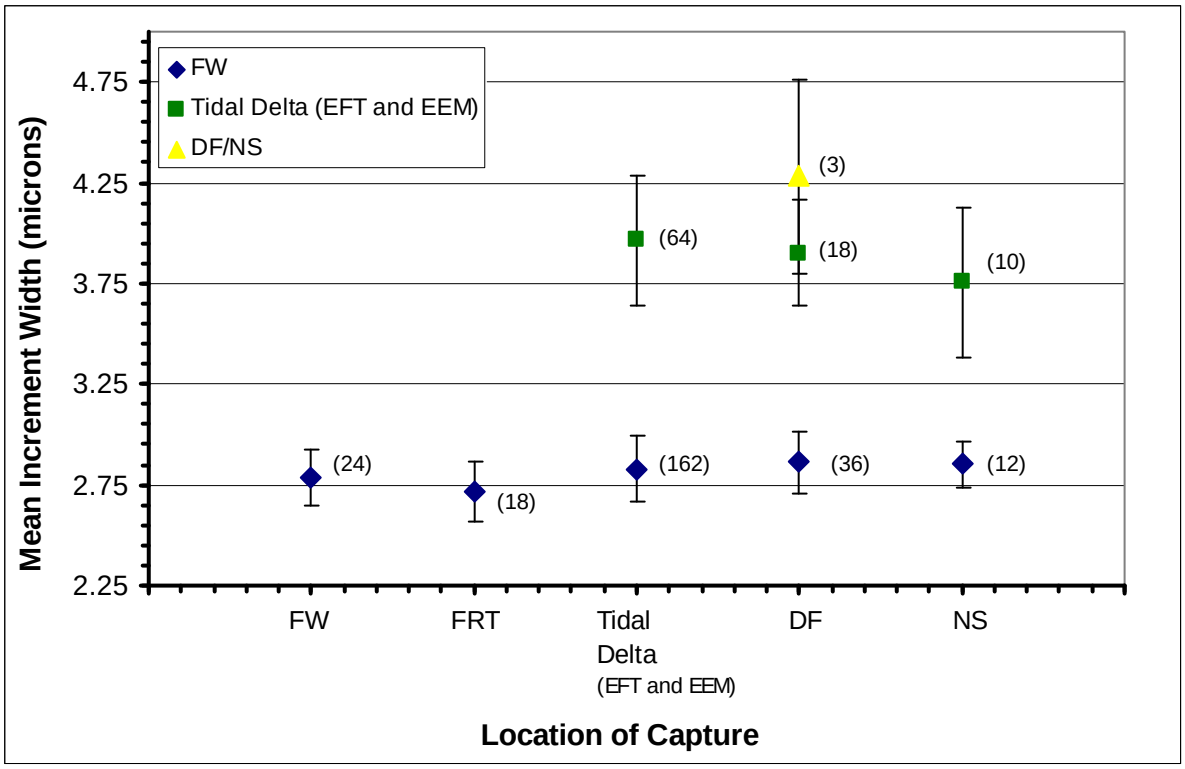

Figure 6: Mean Increment width (microns) for FW, tidal delta, and DF/NS residence within each habitat. One sample collected in the tidal delta was excluded from the tidal delta portion of the MIW analysis because residence time was only one day. The number of samples are represented in parentheses. Error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

Discussion

A check associated with release from the Clear Creek and Kalama hatcheries could not be identified on samples possibly due to volitional releases. Collection of samples directly from the hatchery prior to ponding events may help us to identify the release check. Presence of a release check will allow us to determine how quickly hatchery fish move to the delta and estimate FW growth in the river following release.

We characterized a Nisqually-specific signature of otolith microstructure growth patterns and checks for hatchery Chinook that allowed us to distinguish entry into the tidal delta and DF/NS habitats following volitional release from the hatcheries. The TDCK first appeared in mid to late May on samples from EFT and EEM habitats whereas the DFCK first appeared in early July on DF samples. It is unclear whether a DFCK appeared on DF samples in April due to limited sample size (n=2). A DFCK was never seen on samples collected in the NS habitat possibly due to the lack of samples from later in the season. No check or increase in growth was visible on any FW or FRT samples regardless of time of year.

The MIW generally increased as fish moved from FW habitat to tidal delta to NS. The magnitude of the difference in MIW between the tidal delta and DF/NS habitats probably is underestimated and may be an artifact of low sample size (n=3).

There was a corresponding increase in growth rate as the fish migrated from FW to tidal delta to NS habitats. Fish grew 50% faster in the tidal delta than in FW. Fish grew only slightly faster (2%) in the DF compared to the tidal delta, however this estimate was based on a very small sample size (n=3).

Our results suggest that otolith microstructure analysis can be a valuable tool to establish a baseline for use of the Nisqually River estuary habitats by juvenile hatchery Chinook salmon under existing conditions. However, this study provides limited information due to small sample sizes in some months. Collection and analysis of additional hatchery Chinook especially from DF and NS habitat zones later in the season (July – October) 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. Of course, further work should include analysis of hatchery adults because they show the proportions and numbers of adults that reared in the delta as juveniles.

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