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Final Report Presented to the Nisqually Indian Tribe

Pre-Restoration Habitat Use by Chinook Salmon in the Nisqually Estuary Using Otolith Analysis: An Additional Year

Open-File Report 2009-1106

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
millimeter (mm)	0.03937	inch (in.)
millimeter per day (mm/d)	0.03937	foot per day (ft/d)

Abbreviations and Acronyms

ANOVA	Analysis of variance
DF	Delta flats
DFCK	Delta-flats check
EEM	Estuarine Emergent Marsh
EFT	Emergent Forested Transition
ESA	Endangered Species Act
FRT	Forested Riverine Tidal
FW	Freshwater
MIW	Mean Increment Width
NS	Nearshore
PE	Pocket estuary
TDCK	Tidal delta check

Pre-Restoration Habitat Use by Chinook Salmon in the Nisqually Estuary Using Otolith Analysis: An Additional Year

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Abstract

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 is currently taking place to assist in recovery of the stock as juvenile Fall Chinook salmon are dependent upon the estuary. A pre-restoration baseline that includes characterization of life history types, estuary residence times, growth rates, and habitat use is needed to evaluate the potential response of hatchery and natural origin Chinook salmon to restoration efforts and determine restoration success. Otolith analysis was selected to examine Chinook salmon life history, growth, and residence in the Nisqually Estuary. Previously funded work on wild samples collected in 2004 established the growth rate and length of residence associated with various habitats. The purpose of the current study is to build on the previous work by incorporating otolith microstructure analysis from 2005 (second sampling year), to verify findings from 2004, and to evaluate between-year variation in otolith microstructure. Our results from this second year of analysis indicated no inter-annual variation in the appearance of the tidal delta check (TDCK) and delta-flats check (DFCK). However, a new life history type (fry migrant) was observed on samples collected in 2005. Fish caught in the tidal delta regardless of capture date spent an average of 17 days in the tidal delta. There was a corresponding increase in growth rate as the fish migrated from freshwater (FW) to tidal delta to nearshore (NS) habitats. Fish grew 33 percent faster in the tidal delta than in FW habitat and slightly faster (14 percent) in the delta flats (DF) habitat compared to the tidal delta.

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) (Nisqually Chinook Recovery Team, 2001). Preservation and extensive restoration of the Nisqually delta ecosystem is currently taking place to assist in recovery of the stock. A pre-restoration baseline that includes the characterization of life history types, estuary residence time, growth rates, and habitat use is needed to evaluate the potential response of wild and hatchery Chinook salmon to restoration.

Otolith analysis was selected to examine Chinook salmon life history, growth, and residence in the Nisqually Estuary. Analysis of otolith microstructure typically is superior to traditional mark-recapture methods (Brothers, 1990). Mark-recapture methods are extremely expensive or inadequate in estuary habitats, typically biased, substantially underestimate use, and do not directly reveal the importance or contribution to adult recruitment. For example, other methods 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 and hatchery Chinook stocks as well as a similar study that USGS and partners are conducting in the Skagit River Estuary located in northern Puget Sound. This work is based on research by Neilson and others (1985). We expect to use the Skagit River as a reference for the pre- and post-restoration comparison in the Nisqually River.

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 and counted to back-calculate fish size and estimate timing of various habitat transitions. 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 rate of growth within the estuary (Neilson and others, 1985). Juvenile Chinook salmon can exhibit a variety of life history strategies – some enter the sea (or Puget Sound) as fry (fry migrants), some rear in the estuary before entering the sea (delta users), and some rear in the river and then move rapidly through the estuary into the sea as smolts (parr migrants) (Healey, 1991; Beamer and Larsen, 2004).

The long-term goals of this study are to compare differences in habitat use between wild and hatchery Chinook to further protect ESA listed stocks, determine if estuary restoration actions cause changes to the population structure (i.e., frequency of the different life history strategies), compare pre- and post-restoration residence times and growth rates, and suggest whether estuary restoration yields substantial benefits for Chinook salmon.

Previously funded work on samples collected in 2004 established the growth rate and length of residence associated with various habitats. The purpose of the current study is to build on the previous work by incorporating otolith microstructure analysis from 2005 (second sampling year), to verify findings from 2004, and to evaluate between-year variation in otolith microstructure.

Objectives

1. Characterize the importance of the Nisqually Estuary to unmarked Chinook salmon in 2005 by (1) estimating growth rates, (2) residence times, and (3) size at entry to the tidal delta and nearshore habitats.
2. Evaluate between-year variation in otolith microstructure patterns by comparing the 2004 and 2005 collections.

Methods

Unmarked and marked juvenile Chinook salmon were sampled by the Nisqually Tribe and the U.S. Fish and Wildlife Service – Nisqually National Wildlife Refuge from February through October 2005 at several 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 subtidal and intertidal areas (accessible by beach seine; hereafter referred to as nearshore) outside of the Nisqually delta complex (fig. 1). Most fish were collected by beach seining in the following distinct habitat zones (Cowardin and others, 1979; fig. 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 subtidal 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. Fyke net trapping ended in August.

Subsamples of juvenile Chinook were collected for otolith extraction. Each fish was euthanized and measured for length and weight. The fish were preserved in alcohol and sent to the U.S. Geological Survey where the sagittal otoliths of unmarked fish were extracted. A total of 333 pairs of otoliths were collected from unmarked fish in 2005 (table 1). All fish otoliths (one from each pair) were processed according to the Western Fisheries Research Center's standard protocols, excluding PE (n = 11) and previously analyzed samples from the Animal fyke trap site (n = 44) and NS (n = 8). The total sample size for Animal fyke trap was supplemented with seven complementary right otoliths of equal length. Three additional samples were not processed due to misplacement of samples or vaterite (a calcium carbonate morph), for a total of 263 samples available for analysis. Sixty juvenile Chinook salmon also were collected in 2005 from the Clear Creek and Kalama hatcheries less than two weeks prior to hatchery release for determination of unique patterns specific to each hatchery. An average of 15 fish per hatchery group was sacrificed and the otoliths of at least eight fish per hatchery group were processed. This number was selected because of the consistent incremental otolith pattern of fish under constant rearing conditions and to minimize the number of sacrificed samples.

After processing, all samples were sorted as Clear Creek, Kalama, or wild Chinook (table 2). Samples identified as Clear Creek or Kalama origin were not analyzed. Samples not distinguishable as hatchery or wild were categorized as "unknown origin" and not analyzed further. A total of 15 fish were identified as Clear Creek or Kalama hatchery origin, 232 as wild, and none as unknown origin. Sixteen additional samples were not suitable for analysis because of uneven microstructural growth along the radial axis or processing error. In total, 232 samples were analyzed out of the 263 available (table 3).

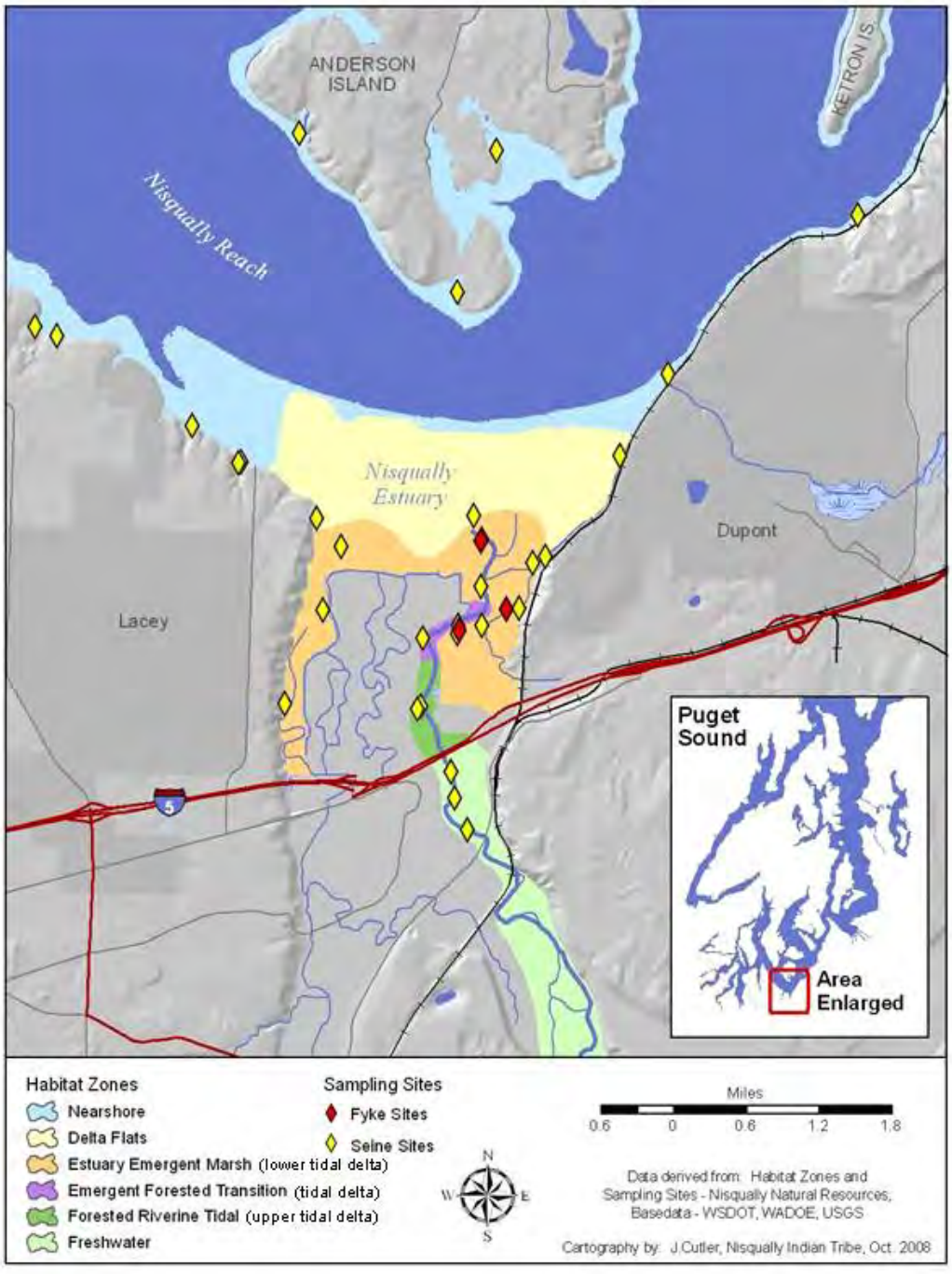


Figure 1. Nisqually River, tidal delta (2005) and nearshore (NS; 2004–2006) field sampling sites.

Table 1. Number of unmarked juvenile Chinook salmon collected for otolith analysis, Nisqually Basin, Washington, 2005. Total number includes samples previously processed from 2005 Animal fyke trap site and nearshore (NS) sites.

Habitat Type	February	March	April	May	June	July	August	September	October	TOTAL
Freshwater (FW)	8	10	6	6	3	0	0	0	2	35
Forested Riverine Tidal (FRT, tidal delta)	6	4	3	6	9	3	8	0	0	39
Emergent Forested Transition (EFT, tidal delta)	4	0	3	9	6	3	7	0	0	32
Estuarine Emergent Marsh (EEM, tidal delta)	11	13	32	59	37	19	13	0	0	184
Delta Flats (DF)	1	1	9	2	10	1	0	0	0	24
Nearshore (NS)	0	0	0	2	5	1	0	0	0	8
Pocket Estuary (PE)	6	0	1	4	0	0	0	0	0	11
TOTAL	36	28	54	88	70	27	28	0	2	333

Table 2. Number of unmarked hatchery samples separated from the dataset.

Habitat Type	February	March	April	May	June	July	August	September	October	TOTAL
Freshwater (FW)	0	0	0	2	0	0	0	0	1	3
Forested Riverine Tidal (FRT, tidal delta)	0	0	0	0	0	0	0	0	0	0
Emergent Forested Transition (EFT, tidal delta)	0	0	0	2	0	0	0	0	0	2
Estuarine Emergent Marsh (EEM, tidal delta)	0	0	1	7	1	0	0	0	0	9
Delta Flats (DF)	0	0	0	1	0	0	0	0	0	1
TOTAL	0	0	1	12	1	0	0	0	1	15

Table 3. Number of otoliths (one per fish) analyzed/processed. The number analyzed does not include unmarked hatchery fish separated from the dataset after processing.

Habitat Type	February	March	April	May	June	July	August	September	October	TOTAL
Freshwater (FW)	6 / 8	10 / 10	6 / 6	4 / 6	3 / 3	0 / 0	0 / 0	0 / 0	1 / 2	30 / 35
Forested Riverine Tidal (FRT, tidal delta)	5 / 6	4 / 4	3 / 3	6 / 6	8 / 9	3 / 3	5 / 6	0 / 0	0 / 0	34 / 37
Emergent Forested Transition (EFT, tidal delta)	4 / 4	0 / 0	3 / 3	6 / 9	6 / 6	3 / 3	7 / 7	0 / 0	0 / 0	29 / 32
Estuarine Emergent Marsh (EEM, tidal delta)	9 / 11	11 / 12	21 / 22	36 / 47	22 / 25	9 / 9	8 / 9	0 / 0	0 / 0	116 / 135
Delta Flats (DF)	1 / 1	1 / 1	9 / 9	1 / 2	10 / 10	1 / 1	0 / 0	0 / 0	0 / 0	23 / 24
TOTAL	25 / 30	26 / 27	42 / 43	53 / 70	49 / 53	16 / 16	20 / 22	0 / 0	1 / 2	232 / 263

Fish collected from FW habitat showed a consistently recognizable pattern that was used as a reference for all fish otoliths collected downstream of FW habitat. This reference pattern had no checks beyond the recognizable checks associated with emergence and first feed. A check is a consistently prominent mark or pattern on the otolith that 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 zones 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 a digital imaging system, Image-Pro. A standardized radial axis was selected for measurements at 85 ±5 degrees ventral of the longitudinal axis passing through an identifiable and preferred nucleus. Distances and individual increment widths between checks or an increase in growth assumed to represent change in habitat were recorded for each fish along the radial axis.

Growth rates in the tidal delta and DF/NS habitats were calculated using millimeters per day (mm/d) from lengths based on the Fraser-Lee method (DeVries and Frie, 1996):

$$L_i = \frac{L_c - a}{S_c} S_i + a \qquad (1)$$

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 (check presence or increase growth), and

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

Average growth rate and mean increment widths (MIW) in microns (μm) were determined for all habitat zones. Residence time and fork lengths upon entry to the tidal delta and DF/NS habitat zones also were calculated.

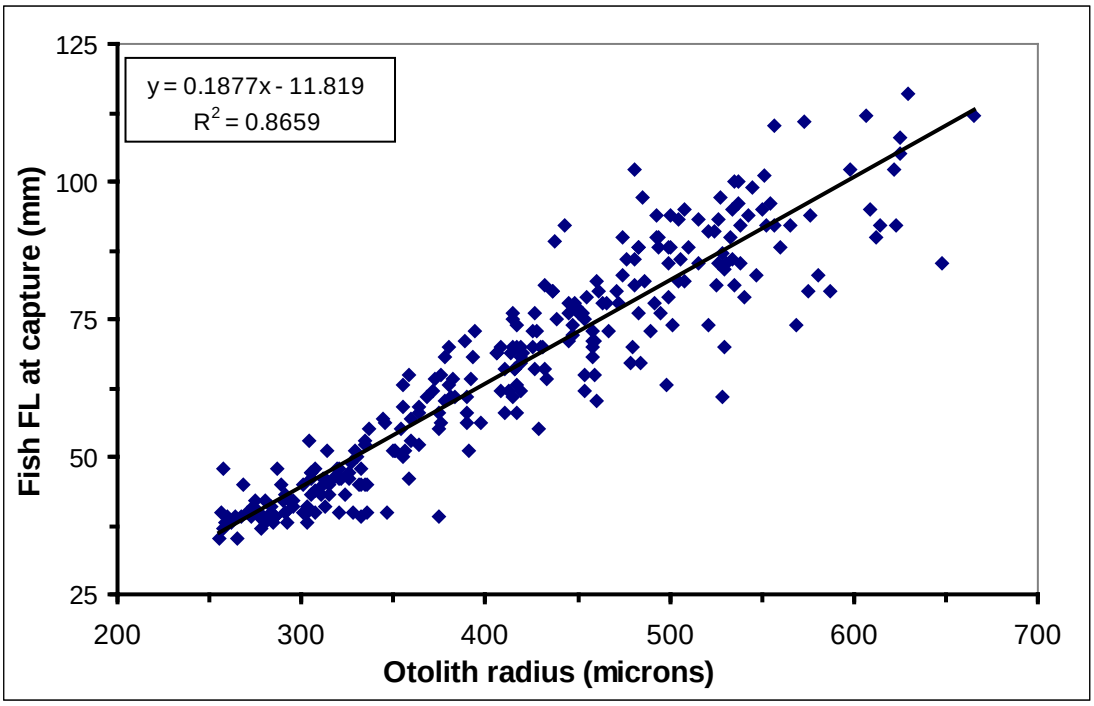
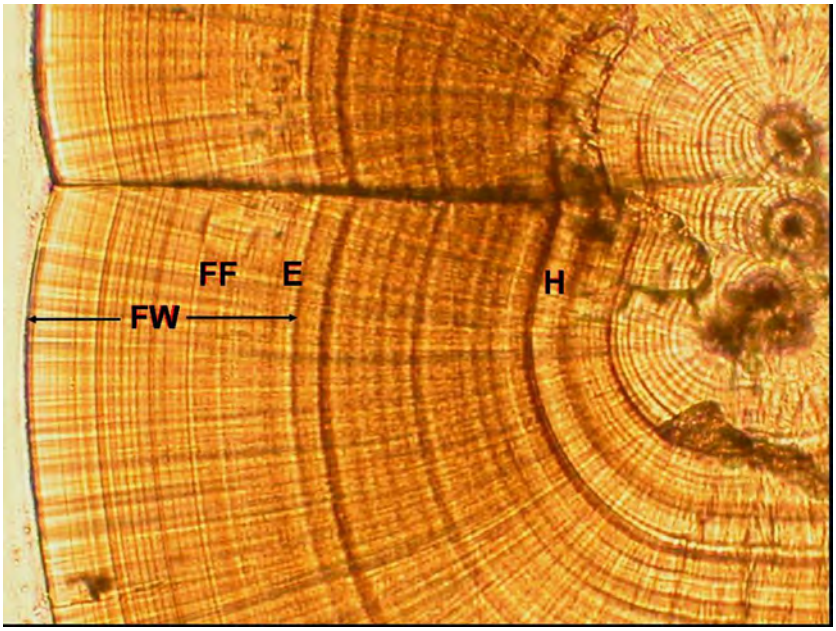


Figure 2. Relation between fish fork length (in millimeters) and otolith radial distance (in microns). The data represents samples collected in the Nisqually Basin, Washington, 2005 from all habitats and includes samples from nearshore (NS) in 2004 and 2006.

Results

The increments on all otoliths became more legible and consistent across the radial axis beyond the emergence check (fig. 3). An interruption in the microstructure pattern, designated as a tidal delta check (TDCK), was detected on samples collected within the EFT and EEM habitats indicating a transition to tidal delta habitat (table 4). Increments were consistently thin with narrow spacing across the radial axis until the TDCK was observed. 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 (fig. 4). No TDCK or increase in growth was seen on otoliths from fish collected in FW or FRT habitats. Hereafter, EFT and EEM habitats are referenced as tidal delta.

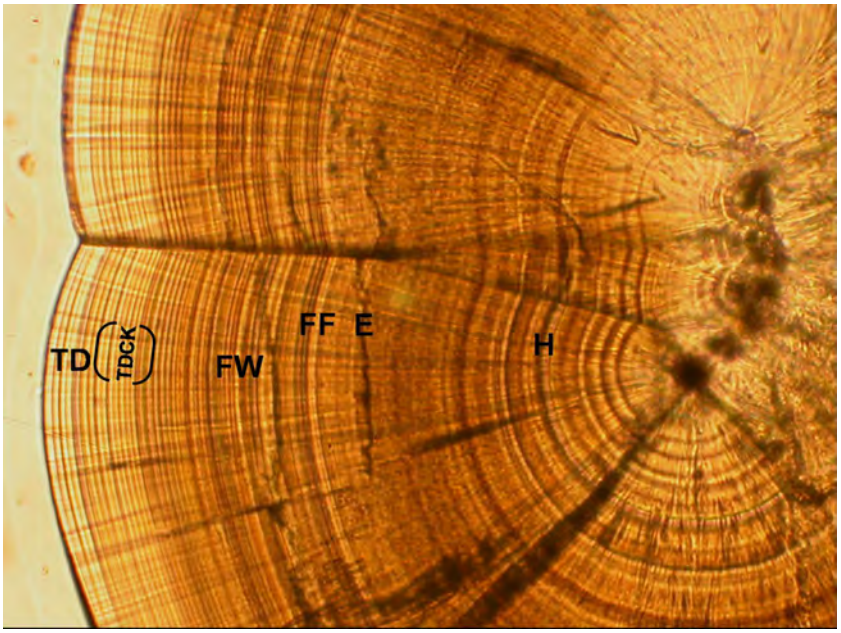


26x objective

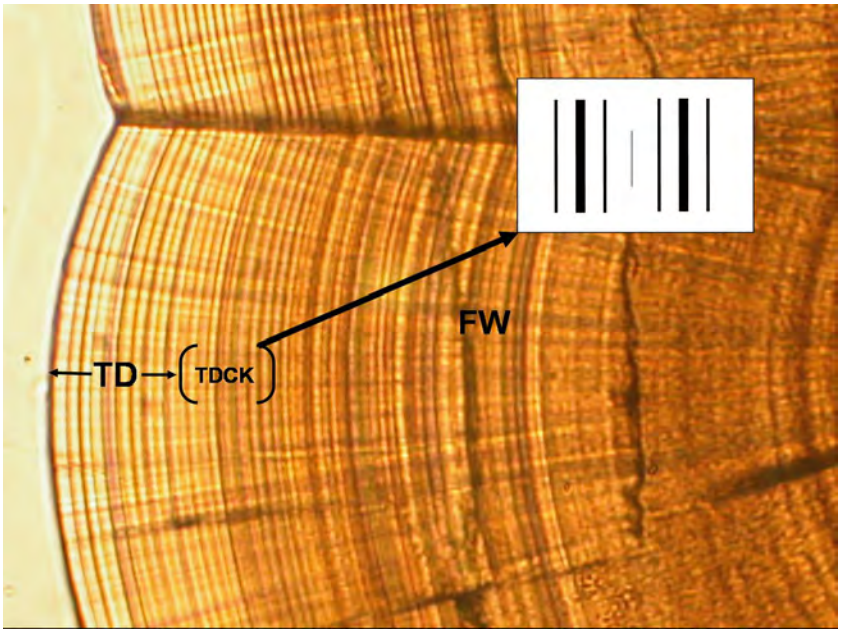
Figure 3. Representative otolith sample of freshwater (FW) growth from Nisqually Basin, Washington, 2005. Abbreviations: H = hatch, E = emergence, FF = first feed, and FW = freshwater residence.

Table 4. 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.

	February		March		April		May		June		July		August	
Habitat Type	TDCK	DFCK	TDCK	DFCK	TDCK	DFCK	TDCK	DFCK	TDCK	DFCK	TDCK	DFCK	TDCK	DFCK
Forested Riverine Tidal (FRT, tidal delta)	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Emergent Forested Transition (EFT, tidal delta)	0	-	0	-	0	-	2	-	6	-	3	-	7	-
Estuarine Emergent Marsh (EEM, tidal delta)	0	-	0	-	11	-	10	-	28	-	14	-	12	-
Delta Flats (DF)	0	0	0	1	1	5	1	1	9	6	1	1	0	0
TOTAL	0	0	0	1	12	5	13	1	43	6	18	2	19	0



18x objective



32x objective

Figure 4. Representative otolith sample of the tidal delta check (TDCK) seen on samples collected in the emergent forested transition (EFT) habitat beginning in mid-May 2005 and the estuarine emergent marsh (EEM) habitat beginning in early April 2005, Nisqually Basin, Washington. 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 after approximately one increment. Beyond the TDCK, increments were consistently wider indicating increased growth. Abbreviations: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, TDCK = tidal delta check, and TD = tidal delta residence.

The TDCK was first observed on samples collected in mid-May from EFT habitat even though samples were collected in February and April. The TDCK also was initially observed on samples collected in late April from EEM habitat even though samples were collected in February through mid-April. These samples from late April were from only one location within the EEM (Red Salmon Slough). By early June, all samples regardless of habitat zone had a TDCK. The presence and absence of a TDCK in the EEM habitat from early May through early June is represented in figure 5.

In addition to the TDCK, an additional check was seen on some otoliths collected in the DF and NS habitats. As referenced in previous reports, we called this check a delta-flats check (DFCK) due to classification of sites. The DFCK indicated the fish's transition from tidal delta habitat to the DF/NS habitat (fig. 6). The DFCK was visible on samples from the DF habitat beginning in March (n = 1). The presence and absence of a DFCK in the DF habitat from early April through mid-June is represented in figure 7. For fish collected in the DF habitat early in the season (early March through mid-April, n = 6), we observed FW residence followed directly by a DFCK and DF/NS residence indicating the presence of a fry migrant life history (fig. 8). These particular fish reside in the FW habitat for an extremely short period after emergence, quickly migrate downstream bypassing the tidal delta habitat, and then move directly into the DF/NS habitat.

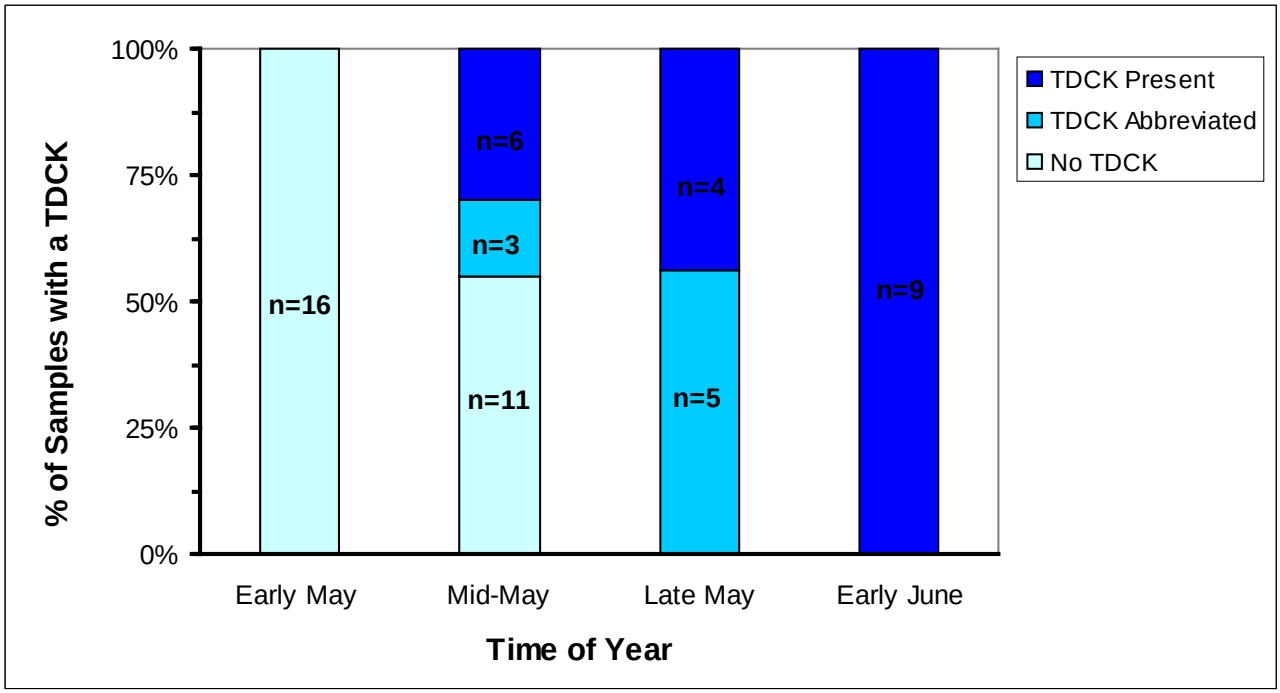
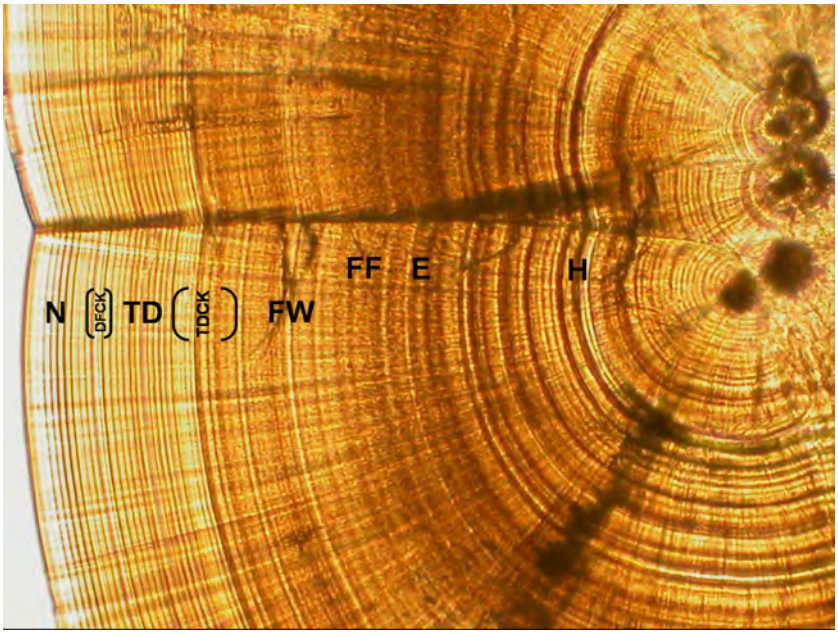
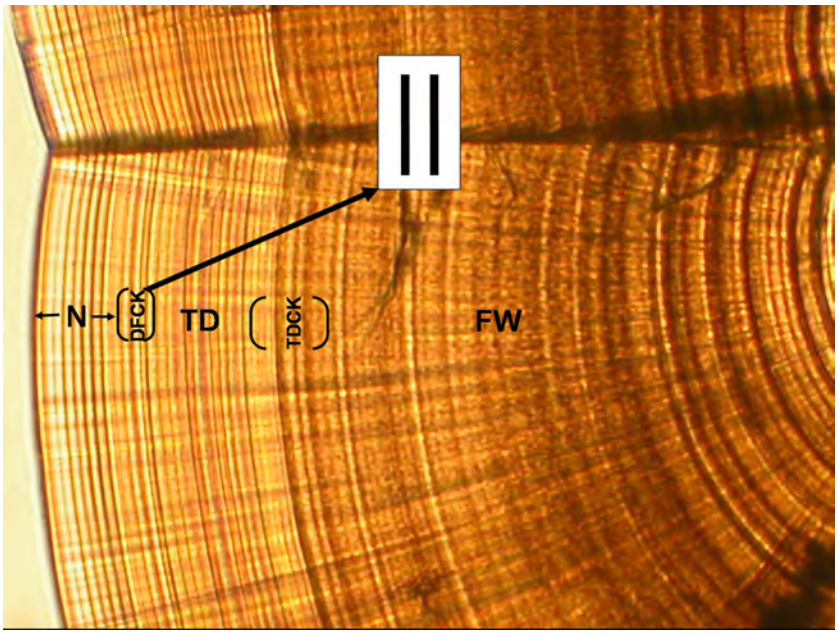


Figure 5. Presence and absence of a tidal delta check (TDCK) on samples collected in the estuarine emergent marsh (EEM) habitat, Nisqually Basin, Washington, 2005.



24x objective



34x objective

Figure 6. Representative otolith sample of the delta-flats check (DFCK) seen on samples collected in the delta flats (DF) habitat beginning in March 2005 and the nearshore (NS) habitat beginning in early June 2005, Nisqually Basin, Washington. The check was bold and prominent consisting of two wide dark bands with a wide bright band in between. Beyond the DFCK, increments were consistently wider indicating increased growth. Abbreviations: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, TDCK = tidal delta check, TD = tidal delta residence, DFCK = delta-flats check, and N = delta flats/nearshore residence.

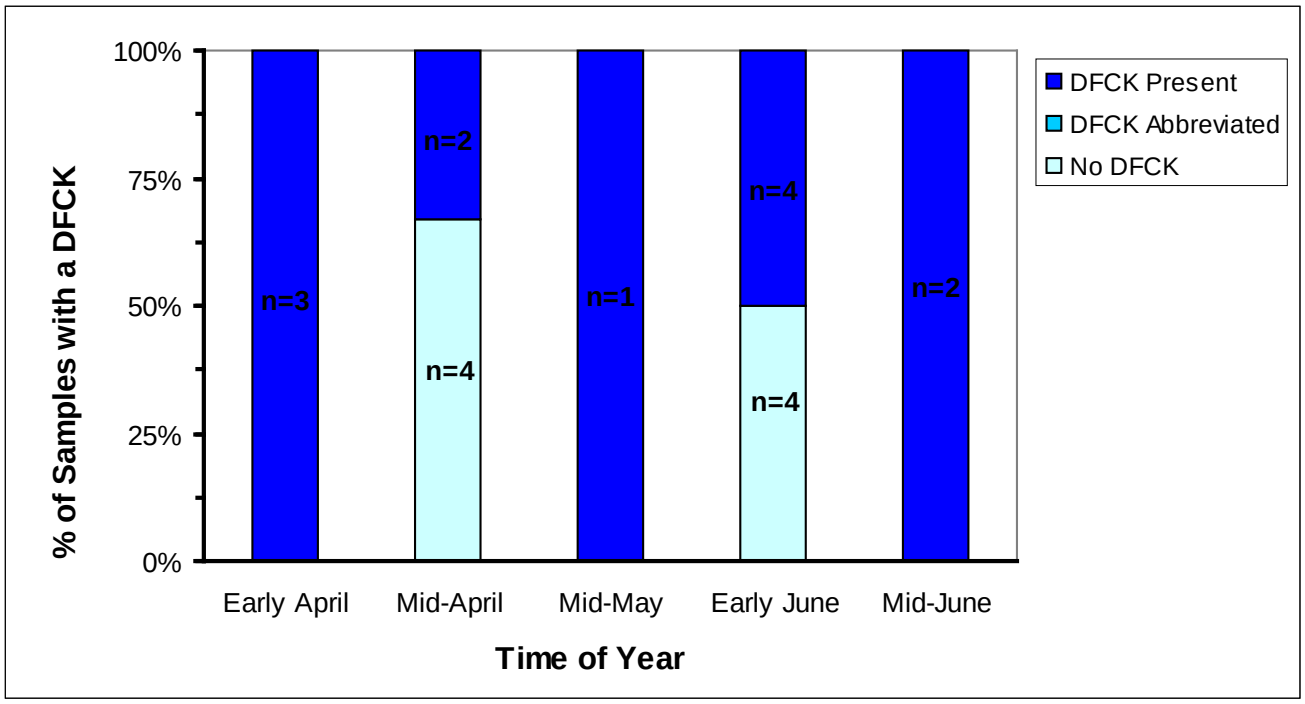
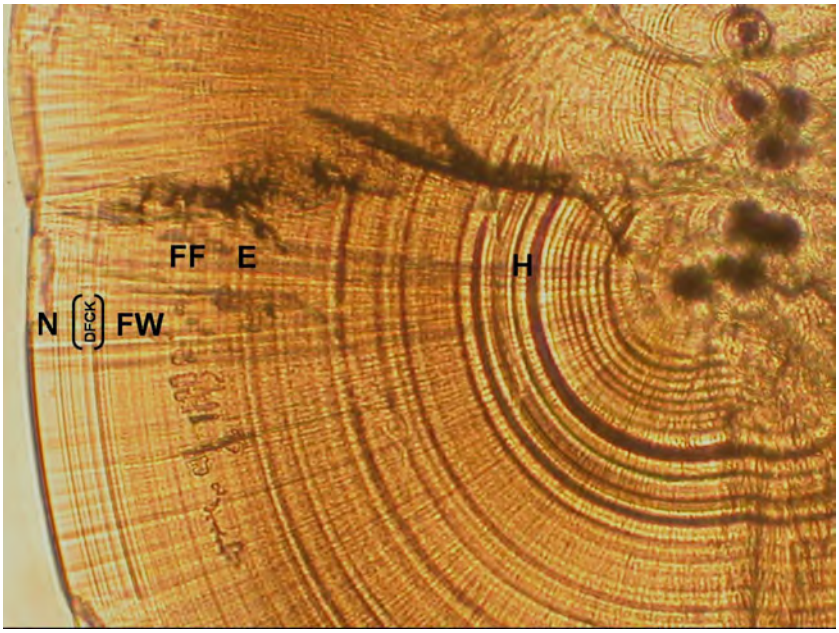


Figure 7. Presence and absence of a delta-flats check (DFCK) on samples collected in the delta flats (DF) habitat, Nisqually Basin, Washington, 2005.

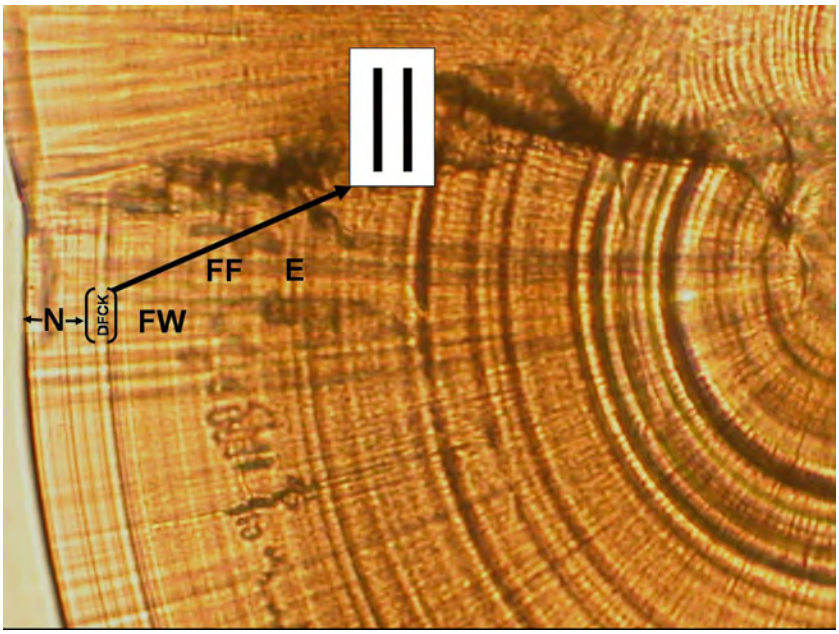
The DFCK was observed on samples in the NS habitat beginning in June; however, the number of samples containing a DFCK was only one out of eight. An insufficient number of samples was available to determine whether a DFCK was visible on samples collected in the NS habitat in March, April, or May.

No difference was visually observed in the microstructure pattern between EFT and EEM habitats. To further validate this observation, a one-way ANOVA was run to test for significant differences between EFT and EEM habitats. No significant differences occurred in growth rate or MIW ($P \geq 0.22$); therefore, the data were combined and classified as "tidal delta." FRT habitat was not included as part of the tidal delta habitat for analysis because the microstructure pattern did not visually differ from FW samples nor was an additional check or increased growth ever observed.

The NS habitat collection was supplemented with previously processed samples collected in 2004 (n = 2) and 2006 (n = 19) because of the small sample size in 2005 (n = 8). With samples analyzed from multiple years in the NS habitat, a one-way ANOVA was run to test for differences between years. There was no significant difference between years among tidal delta and FW MIW and growth rates ($P \geq 0.22$).



28x objective



36x objective

Figure 8. Representative otolith sample of fry migrants seen in the delta flats (DF) habitat beginning in March 2005, Nisqually Basin, Washington. These fish displayed freshwater (FW) residence followed directly by a delta-flats check (DFCK) and delta flats/nearshore (DF/NS) residence. Abbreviations: H = hatch, E = emergence, FF = first feed, FW = freshwater residence, DFCK = delta-flats check, and N = delta flats/nearshore residence.

On average, fish from the NS habitat had the lowest FW (2.73 µm) and tidal delta (3.63 µm) MIW. The FW portion MIW (3.08 µm) of all otolith samples generally was smallest followed by the tidal delta (3.92 µm) and the DF/NS (4.27 µm) portions, respectively. We tested for differences in MIW in the FW, tidal delta, and DF/NS portions of the otoliths within habitats (fig. 9). The one-way ANOVA showed a significant difference ($P \leq 0.01$). Additionally, we tested for differences in MIW in the FW, tidal delta, and DF/NS portions of the otolith across habitats. The one-way ANOVA showed a significant difference ($P \leq 0.01$).

The equivalent results for growth rate were that the FW growth rates (mean = 0.54 mm/d) were lower compared to the growth rates of the tidal delta portion for fish residing in the tidal delta (mean = 0.71 mm/d), DF (mean = 0.62 mm/d), and NS (mean = 0.60 mm/d) habitats, with a 33 percent increase in growth from FW habitat to tidal delta habitat. The DF/NS growth rate for fish caught in the DF (mean = 0.82 mm/d) was higher and in the NS (mean = 0.68 mm/d, n = 1) lower than the tidal delta growth rate. A significant difference was found between tidal delta and DF/NS growth rates (one-way ANOVA, ($P \leq 0.01$). The increase in growth from the tidal delta habitat to the DF habitat was only 14 percent; however, sample size was small (n = 9).

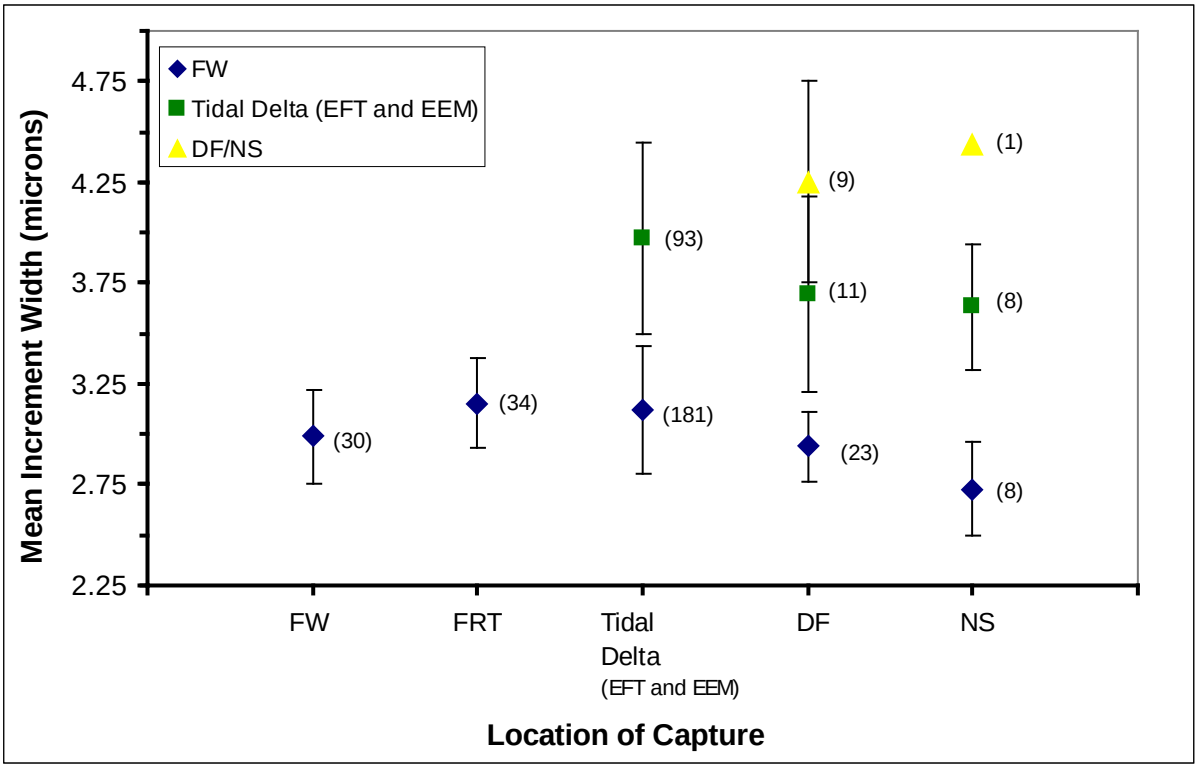


Figure 9. Mean increment width (MIW; in microns) for fish residing in freshwater (FW), tidal delta (EFT and EEM), and delta flats/nearshore (DF/NS) habitat zones, Nisqually Basin, Washington. One sample collected in the tidal delta was excluded from the tidal delta portion and five samples collected in the delta flats (DF) were excluded from the DF/NS portion of the MIW analysis because residence time was only one day. The number of samples is represented in parentheses. Error bars represent ±1 standard deviation.

Fish caught in the tidal delta regardless of capture date had an average fork length of 69.8 millimeters (mm) upon entry to the tidal delta and spent an average of 17 days in the tidal delta with a minimum residence time of 10 days and a maximum of 35 days. The majority of fish resided for 3 weeks or less (fig. 10). These fish provided a minimum estimate of residence because they were sacrificed prior to entering the DF/NS habitat. Evaluation of those fish caught in the DF and NS habitats indicated an average residence time of 15 days in the tidal delta (n = 9). This value represented a truer estimate of residence time in the tidal delta; however, the sample size was quite small. Fish caught in the DF habitat were on average 70.9 mm upon entrance into the tidal delta and 74.9 mm upon exit, whereas fish caught in the NS habitat were 71.4 mm upon entrance into the tidal delta. Fry migrants collected in the DF habitat early in the season (early March to mid-April) were on average 38 mm when they entered the DF/NS habitat.

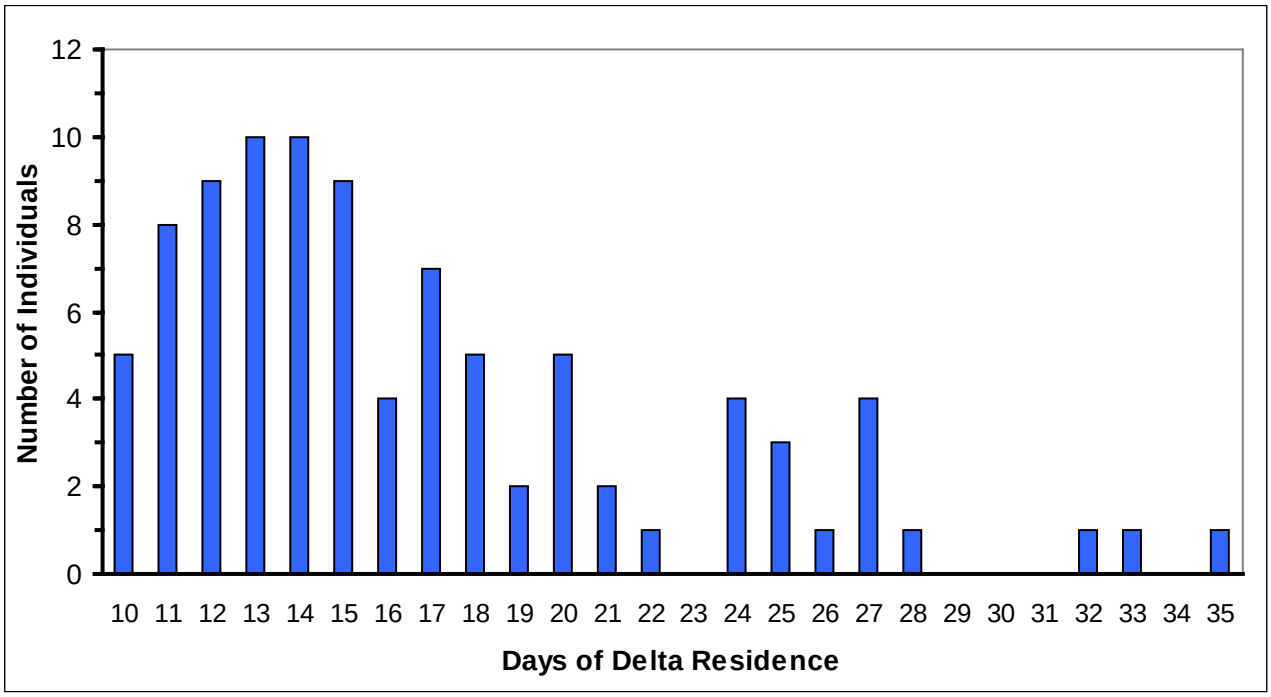


Figure 10. Residence time (days) for individual fish caught in the tidal delta, Nisqually Basin, Washington, 2005.

Discussion

Hatchery Chinook salmon outnumber wild Chinook in the Nisqually River; however, distinct microstructural patterns unique to the Clear Creek and Kalama hatcheries allowed us to recognize and separate the collection into unmarked hatchery and wild fish. The majority of unmarked hatchery fish were seen in natural habitats during May subsequent to hatchery release. A single hatchery fish was caught in April prior to intended release.

As with the 2004 collection, we were able to characterize a Nisqually-specific signature of otolith microstructure growth patterns and checks for wild Chinook that allowed us to distinguish entry into the tidal delta and DF/NS habitats. The TDCK and DFCK varied slightly in appearance from that seen on samples collected in 2004. The DFCK looked similar except for one fewer increment; therefore, no sign of inter-annual variation in check appearance was recorded. However, a new life history type (fry migrant) was observed on samples collected in 2005. Because of the low number of DF and NS samples collected, it is unknown whether the small number of fry migrants is a true representation of the population. Therefore, it may be of interest to analyze the samples collected from the PE habitat that are assumed to be fry migrants based on time of collection.

Presence or absence of checks was once again seen on samples throughout the migration season, but did not vary from that seen in 2004. We conclude that these results are not reflective of the otoliths deposition rate of expression or incorporation to changes in environmental conditions. Rather, the results can be explained by the movement of cohorts into and out of specific habitats as it relates to time of catch. For example, the otoliths from fish migrating in March and caught in tidal delta habitat did not show much growth (about 7 increments) beyond our referenced pattern for FW growth. These particular fish, therefore, were caught almost immediately upon entering the habitat and may not have had sufficient time to develop a visible check. The otoliths of fish caught in May showed the greatest diversity of check presence or absence, correlating with peak timing in juvenile Chinook migration. The fish ranged in size from 40 to 94 mm fork length. Those fish that showed no TDCK had a similar pattern as those caught in March-April without a TDCK. Once again, these fish were caught immediately upon entrance to the habitat. By early June, when migration into the tidal delta had slowed, all samples displayed a TDCK. The DF/NS samples showing no DFCK had a radial distance to the edge corresponding to the radial distance of the longest residing fish having a TDCK and inhabiting in the tidal delta before capture.

Mean increment widths generally increased as the fish moved from FW to the DF/NS habitats. There was a corresponding increase in growth rate as the fish migrated from FW to tidal delta to NS habitats. Fish grew 33 percent faster in the tidal delta than in FW habitat. Fish grew slightly faster (14 percent) in the DF habitat compared to the tidal delta; however, this estimate was based on a small sample size (n = 10). The magnitude of the difference in MIW and growth rate between the tidal delta and DF/NS habitats probably is underestimated and may be an artifact of the low sample size.

Our results from this second year of analysis suggest even further that otolith microstructure can be a valuable tool to establish a baseline for use of the Nisqually River estuary habitats by juvenile Chinook salmon under existing conditions. Restoration efforts may provide additional rearing habitat favorable to juvenile Chinook. This response after restoration may be reflected on the otoliths through higher growth rates and longer residence times. However, this study provided limited information because of small sample sizes in some habitats (DF and NS). These low sample sizes contributed to the significant differences in MIW across habitat types. For instance, the significant difference in FW MIW may be explained by the much lower sample size for NS habitat as well as the majority of those samples (five out of eight) had been collected in 2006, a year not yet completely analyzed. Even though we tested for differences between years in NS samples and were able to pool the data, samples from 2005 have shown a higher FW MIW when analyzed compared to 2004. Perhaps samples from 2006 have a

lower FW MIW contributing further to the significant difference. The low sample sizes from the DF/NS habitats also may have influenced tidal delta MIW across habitats, especially given the large sample size collected from the tidal delta (n = 93) that most accurately reflects tidal delta MIW for 2005.

Analysis of additional fish from DF and NS habitat zones, especially from multiple collection years, should be addressed. This would allow for evaluation of inter-annual variation, which also may reveal additional life history types as seen by the presence of fry migrants in this 2005 collection. Analysis of otolith microchemistry in conjunction with microstructure may provide an additional tool for identifying entry into the DF/NS if multiple years of adequate sampling do not address the issue. Future funding for the analysis of DF/NS samples collected in 2006–08 has been addressed. Further work also should include validation of habitat entry with otolith microchemistry and analysis of the adult population because they show the proportions and numbers of juveniles that reared in the estuary and successfully returned to spawn.

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