

**SKAGIT CHINOOK LIFE HISTORY STUDY
PROGRESS REPORT NUMBER 2**

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TABLE OF CONTENTS

INTRODUCTION.....	1
SYNOPSIS OF METHODS.....	1
Otolith Preparation.....	1
Visual Analysis: identification of checkmark patterns	1
Quantitative Analysis: validation of checkmark patterns	2
RESULTS	2
Mainstem River Samples.....	2
Visual Analysis	2
Quantitative Analysis.....	3
Forested Riverine Tidal (FRT) Samples.....	6
Visual Analysis	6
Quantitative Analysis.....	7
Juvenile chinook growth within estuarine habitat zones	8
Skagit Bay Near-shore Samples	9
Visual Analysis	9
Quantitative Analysis.....	10
LITERATURE CITED	12
GLOSSARY OF OTOLITH AND MICROSTRUCTURE TERMS	13

INTRODUCTION

This report summarizes progress made by the Skagit Chinook Life History Study during 1998. The multi-year workplan includes: (1) preparation of juvenile Skagit chinook otolith samples collected by the Skagit Chinook Restoration Study, (2) visual and quantitative analysis of the otolith samples, (3) identification of naturally induced transition checks or points on otoliths, and (4) identification of juvenile life history types based on the presence or absence of these checks and the time (residence) elapsed between marks. This progress report focuses on results for 1998.

SYNOPSIS OF METHODS

For many years otolith microstructure has been used to determine the age and growth of individual fish. More recently, patterns on juvenile salmonid otoliths have been used to explicitly identify different juvenile life history types or life history events (e.g., Neilson *et al.* 1985; Volk *et al.* 1995; Larsen and Reisenbichler 1993). Marks, referred to as checks or checkmarks on juvenile salmonid otoliths, can be induced naturally when fish migrate from one habitat to another (e.g., freshwater to saltwater), or undergo a specific life history event (e.g., hatch, emergence).

For this study, juvenile chinook otolith samples were collected in upper river, middle river, and lower river mainstem habitat. Samples were also collected from sites within three estuarine habitat zones: forested riverine tidal (FRT), emergent forested transition (EFT), and the estuarine emergent marsh (EEM). Together, these samples form a baseline to examine otolith patterns represented by ocean-type chinook occupying a wide range, but known areas of freshwater and estuarine habitat in the Skagit. This has enabled us to correlate distinct patterns and regions on chinook otoliths with distinct habitat regions within the Skagit Watershed. Samples collected from juvenile chinook in the near-shore environment of Skagit Bay represent fish that have successfully complete the freshwater and estuarine rearing part of their juvenile life history. The baseline samples (i.e., those collected in freshwater and estuarine areas) are used to interpret otolith microstructural results to juvenile life history patterns from those fish captured in Skagit Bay.

Otolith Preparation

Sagittal otoliths from juvenile chinook samples collected under the Skagit Chinook Restoration Study were prepared for analysis per the Western Fisheries Research Center (WFRC) protocol.

Visual Analysis: identification of checkmark patterns

Based on preliminary results (Skagit System Cooperative 1996) and previous work (Larsen and Reisenbichler 1993), we expected three naturally induced otolith marks (developmental, estuarine, and bay) to be detectable on the juvenile chinook otolith

samples used in this study. A “*developmental check*” may be the result of hatching, emergence, first feeding, or other early life history events. In an individual fish's life history, this check represents our earliest reference point to date, capable of validation. An *estuarine checkmark* represents the fish's transition from freshwater habitat to estuarine habitat. A *bay checkmark* represents the fish's change from estuarine habitat to Skagit Bay. Each checkmark was described qualitatively and labeled using Bioscan Optimas System software (version 4.02), a Sony CCD-IRIS color video camera, a Sony Trinitron monitor (super fine pitch), and a Mitsubishi color video printer CP-10U.

Quantitative Analysis: validation of checkmark patterns

Following visual identification, each checkmark was quantitatively analyzed. The analysis process included recording data from each otolith using the same equipment listed above. For each identified checkmark on an otolith sample, the beginning and ending edges are marked, and the distance between these points is measured. For the region between different checkmarks, each increment is marked and its width measured. Descriptive statistics are summarized from these data and entered in an EXCEL spreadsheet (flatfile.xls).

RESULTS

Results from juvenile chinook otolith samples collected in 1994 from the Lower Skagit River mainstem (near Burlington, Washington), the EEM and EFT zones of the Skagit estuary were reported in 1998 (Skagit System Cooperative and Western Fisheries Research Center 1998). This report covers results of analyses conducted on samples collected from upper river mainstem, the FRT zone of the estuary, and near-shore habitat in Skagit Bay.

Mainstem River Samples

Visual Analysis

Visual analysis was completed on various sampling sites in the Skagit and Sauk River mainstem reaches. The variation in “developmental check” patterns was determined for the dates of March 2, 1995 to June 23, 1995. A total of 383 samples were visually analyzed with a few losses generally due to one or a combination of the following circumstances: poor sample preparation, loss of otolith (whole or part), missing field sample, or unreadability from abnormal crystalline formation on the otolith. Of the total number of samples, 331 were collected on the upper Skagit mainstem, 40 on the lower Skagit mainstem, and 12 on the lower Sauk mainstem. Analysis was accomplished through the use of “Bioscan Optimas System” (version 4.02), a Sony CCD-IRIS color video camera, a Sony color Trinitron monitor, and ‘EXCEL 5.0’ software program. Magnification used during analysis was 200x.

Upper Skagit River mainstem sampling sites included: Shovelspur Bank (SSB), Alma Rip Rap (ALR), Copper Bank (CPB), Taylor Bank (TRB), and the Lower Sauk

River mainstem sites [Napoleon Bank (NAB), Sauk Backwater (SAB), and Sauk mainstem (SAM)]. Upper Skagit River sites were all upstream of Marblemount, Washington and within the spawning range of the Upper Skagit Summers. The Lower Sauk sites were within the first 4 miles of the Sauk River near Rockport, Washington and within the spawning range of the Lower Sauk Summers. However, several other chinook stocks are obligated to pass through this area of the Sauk River as they migrate seaward (Upper Sauk Springs and Suiattle Springs).

In the Upper Skagit, the predominant “developmental check” pattern was ‘B’ (230) and was observed on samples from ALR, CPB, and TRB (232) throughout the sampling season. Furthermore, pattern ‘D’ was observed twice on samples of these same sites. However, pattern ‘D’ (101) was the only pattern observed on samples from SSB (99). Shovelspur Bank is the sampling site furthest upstream in the Skagit, leading us to believe that only pattern ‘D’ was present in the Skagit River from Shovelspur Bank upstream to the dams. In the Lower Sauk River, pattern ‘A’ (12) was the only pattern observed. Pattern ‘C’ was never observed from any samples of these upper river sites.

Lower and middle Skagit River sampling sites were pooled into reaches unless otherwise noted. Reach ‘SK070’ (near Hamilton, Washington) included sites Mill Creek Bank (MCB) and Lake Hole Bar (LHB). Reach ‘SK050’ (near Sedro Woolley, Washington) included sites Pipeline Bank (PIB) and Riverfront Park Bank (RPB). An individual sampling site also near Sedro Woolley, included L.O.D. Heaven (LOH). All “developmental check” patterns (A-D) were observed on samples from the lower Skagit reaches throughout the sampling season. A breakdown of check patterns is as follows: pattern ‘A’ (12), pattern ‘B’ (14), pattern ‘C’ (5), and pattern ‘D’ (9) samples. This analysis reveals pattern ‘C’ likely origin as being downstream of the Skagit’s confluence with the Sauk River: the spawning range of Lower Skagit Falls.

Different locations of the Skagit mainstem appear to be specific to a developmental check pattern and these roughly correspond to different Skagit River Basin chinook stock spawning ranges. The Lower Sauk mainstem is within the spawning range of Lower Sauk Summers and is specific to developmental check pattern ‘A’ on juvenile chinook otolith samples. Shovelspur Bank (the uppermost Skagit River mainstem site) is specific to pattern ‘D’ and the other sites upstream of Marblemount were dominated by pattern ‘B’. All the sites sampled upstream of Marblemount lie within the spawning range of the Upper Skagit Summers. By inference, the Lower Skagit mainstem is the source of fish with developmental check pattern ‘C’. This area is the spawning range of Lower Skagit Falls.

Quantitative Analysis

Quantitative analysis was performed on 358 Skagit mainstem samples out of a total of 383. Sample losses were due to one or a combination of the following: poor sample preparation, loss of otolith (whole or part), missing field sample, and /or unreadability from abnormal crystalline formation on the otolith. Analysis was completed through the use of “Bioscan Optimas System” (version 4.02), a Sony CCD-

IRIS color video camera, a Sony color Trinitron monitor and EXCEL 5.0 software program. Magnification used during analysis was 280x. All distances and marked points were recorded in microns along a specific radial axis.

The “developmental check” and individual increments were marked; and their corresponding widths recorded. However, the marking of individual increments across the “developmental check” was not performed. This area has very little structural definition which creates difficulty in locating individual increments and confidently marking them. The linear distances between points of interest were recorded. Of this particular group of otoliths (Skagit mainstem), the points of interest were the following: linear distance from core to beginning of “developmental check”, width of “developmental check”, end of “developmental check” to edge of otolith.

Data on the linear distance from core to beginning of the “developmental check” are used to further validate differences between patterns A-D. Since these are generally the earliest samples collected in the season and the sites are within spawning areas, the fork length of the fish sampled are some of the smallest in the database. This is especially valuable in developing the relationship between fork length and otolith radius (core to edge) which is used for estimating the size and growth of other fish sampled later in their life history (Figures 1- 4).

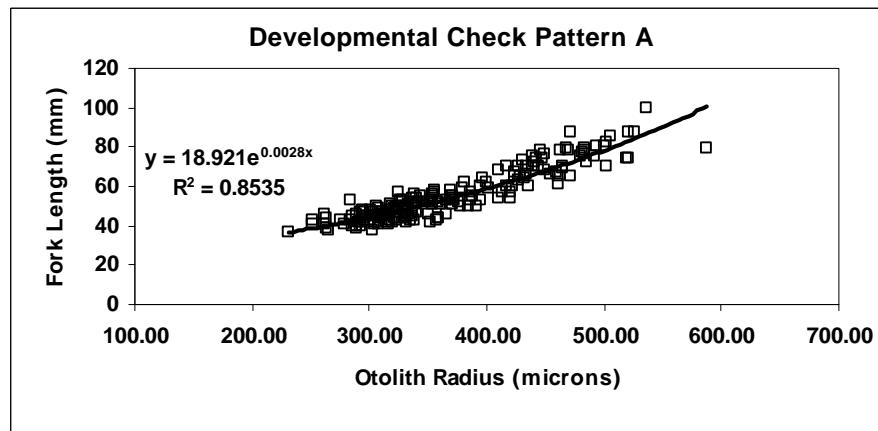


Figure 1. Relationship between otolith radius and fork length of juvenile Skagit chinook for developmental check pattern A.

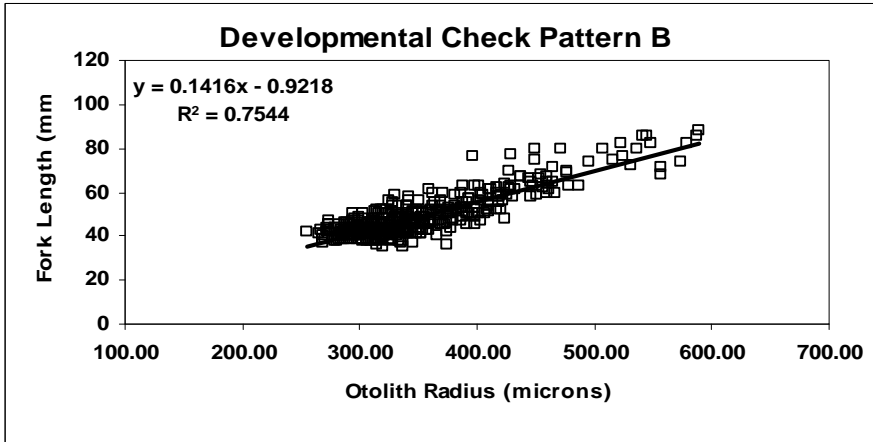


Figure 2. Relationship between otolith radius and fork length of juvenile Skagit chinook for developmental check pattern B.

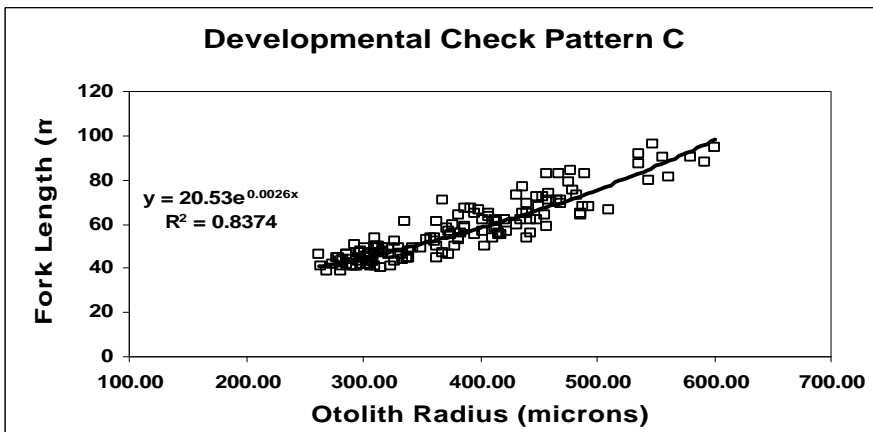


Figure 3. Relationship between otolith radius and fork length of juvenile Skagit chinook for developmental check pattern C.

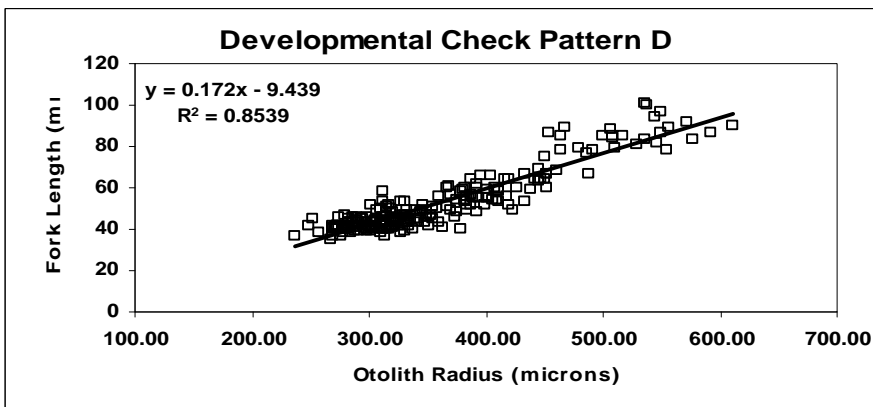


Figure 4. Relationship between otolith radius and fork length of juvenile Skagit chinook for developmental check pattern D.

Forested Riverine Tidal (FRT) Samples

Visual Analysis

A total of 106 fish were collected in the forested/riverine/tidal zone (FRT) of the lower Skagit River between March 6, 1995 and May 15, 1995. Visual analysis was completed on 104 fish with a few losses generally due to one or a combination of the following circumstances: poor sample preparation, loss of otolith (whole or part), missing field sample, or unreadability from abnormal crystalline formation on the otolith.

Visual analysis determines the presence or absence of the “transition area” on samples collected upstream of the previously described habitats (EFT and EEM, see Skagit System Cooperative and Western Fisheries Research Center 1998). Analysis of this upper estuarine zone may aid in determining the exact migratory location of “estuarine check” deposition. Also noted through visual analysis are the “developmental check” patterns and the “reference check”. Visual analysis was accomplished through the use of “Bioscan Optimas System” (version 4.02), a Sony CCD-IRIS color video camera, a Sony color Trinitron monitor, and ‘EXCEL 5.0’ software program. Magnification used during analysis was 200x.

Of the various “estuarine” checks previously identified on EEM samples (Skagit System Cooperative and Western Fisheries Research Center 1998), only one (“transition zone”) appeared on the FRT samples, as was the case for the EFT samples. For FRT samples, the check will be referenced further as “transition area” as noted previously for the EFT samples (Skagit System Cooperative and Western Fisheries Research Center 1998). Samples collected on April 17 displayed a clear incremental pattern for the “transition area”, however the pattern was slightly abbreviated. This was probably due to the fish being collected as samples very shortly following its occupancy of FRT habitat, and prior to otolith increment deposition. The entire incremental pattern of the “transition area”, and a few increments beyond this area, were present on the samples from May 15. Furthermore, the standard change in incremental width beyond the “transition area” is apparent. Samples from March 6 show no disruption in appearance of incremental microstructure and therefore, no type of estuarine check. The April 3 collection shows what could be the beginning few increments to the “transition area”, based on radial distance from the “reference check”. However, there are not enough increments present to clearly define a disruption in the incremental pattern.

Mean increment width beyond the “transition area” appears larger than for those increments prior to the transitional area, but not as wide as incremental widths beyond the “transition area” of EFT samples or the “transition zone” of EEM samples. This change in incremental width leads one to conclude that there is greater growth occurring in the FRT habitat than in the freshwater mainstem habitats; yet not as much as seen in the EFT or EEM habitat areas.

Quantitative Analysis

Quantitative analysis was performed on 96 of 104 FRT samples collected during the 1995 sampling season. Eight samples were unusable due to one or a combination of the following circumstances: poor sample preparation, loss of otolith (whole or part), missing field sample, or unreadability from abnormal crystalline formation on the otolith. Analysis was completed through the use of “Bioscan Optimas System” (version 4.02), a Sony CCD-IRIS color video camera, a Sony color Trinitron monitor, and ‘EXCEL 5.0’ software program. Magnification used during analysis was 280x.

The beginning/end to various checkmarks (“developmental”, “reference”, and “transitional area”) and the linear distance across each mark were recorded in microns for each otolith sample. The type of “developmental” check pattern (A, B, C, and D) was also recorded. Individual increments and their corresponding widths across the otolith radius were measured with the exception of increments found in the “developmental check” area. Increments within this particular area have very little structural definition, creating difficulty in confidently locating individual increments as well as marking them. Therefore, marking across the “developmental check” area was not performed. These analyses quantitatively proved that otolith increments beyond the FRT “transitional area” were larger than increments formed in upper river habitat, but not as large as those increments formed beyond the EFT “transitional area” and the EEM “transitional zone”. This implies that juvenile chinook growth rates are ordered as follows:
freshwater<FRT<EFT<EEM.

Juvenile chinook growth within estuarine habitat zones

Assuming one otolith increment represents one day and estimating the number of increments within the estuarine region of an otolith sample, we can estimate the number of days an individual fish occupied estuarine habitat before it was sacrificed. By estimating the fork length of individual fish samples when they entered estuarine habitat using models shown in Figures 1-4, and subtracting this estimate from the measured fork length at the time of collection, we estimate the amount individual fish grew in length while in estuarine habitat before it was sacrificed. This number, divided by the number of days residing in the estuary yields a growth rate (mm/day) for individual fish sampled in estuary habitat.

Using the above methods, we estimated growth in estuarine habitat for 136 juvenile chinook that had been in the estuary for 7 days or longer. Results indicate that average fish growth rate from samples collected within the estuarine emergent marsh (EEM) sites was over 3 time greater than the average growth rate from forested riverine tidal (FRT) or emergent forested transition (EFT) zone sites (Table 1).

Table 1. Growth rate of juvenile chinook in Skagit River Estuary, 1994.

EEM Sites	Average growth rate (mm/day)	FRT and EFT sites	Average growth rate (mm/day)
Browns Slough Barrow Channel	1.60	Cattail Marsh	0.40
Browns Slough Dike Side	1.80	Deepwater Slough	0.57
Ika Saltmarsh	2.93	Freshwater Pond	0.54
Tom Moore Saltmarsh	1.13	Grain of Sand	0.64
All EEM sites combined:	1.68 S.D. = 0.88 n = 62	All FRT and EFT sites combined:	0.53 S.D. = .24 n = 74

Skagit Bay Near-shore Samples

Visual Analysis

Three hundred and fifteen fish were collected for otoliths in Skagit bay between May 11th and August 31st, 1995. The sampling sites included Ala Spit, Hope Island Inlet, Hoypus Point, Hope Island, Lone Tree Island, Similk, and Skagit Island. Of the 315 samples, only 263 otoliths were used for visual analysis. Thirty two samples were identified as having annuli, 14 were identified as hatchery samples, and six were unreadable. “Unreadable samples” are attributed to poor sample preparation, vaterite formation, or were missing. Visual analysis was accomplished through the use of “Bioscan Optimas System” (version 4.02), a Sony CCD-IRIS color video camera, a Sony Trinitron color monitor and EXCEL 5.0 software program. Magnification used during analysis was 280x, and for video printouts 100x and 200x.

The purpose of this analysis was to identify a checkmark separating estuarine residency from bay residency on the otoliths of juveniles collected within Skagit bay. All samples exhibited a “bay check” consisting of two to four wide bright increments located within a transitional area preceding the bay increments. Bay increments appeared consistently wider than estuarine increments and always occupied the area before the edge of the otolith. Many of the bay samples exhibited thinner, less optically dense increments between the prominent increments within the bay zonation. After personal communication with Kim Larsen of the ‘USGS Biological Resources Division’ and much literature research, it was concluded that these thinner and less optically dense increments may be sub-daily increments (Campana and Neilson, 1985; Neilson, et al., 1985; Stevenson and Campana [eds.], 1992; Volk, et al., 1995). Current validation research on hatchery outmigrants by Kim Larsen may substantiate the sub-daily nature of these increments. Other visually identifiable checkmarks found on bay samples included a “developmental check”, a “reference check”, and one of three “estuarine checks” previously identified.

Typical otolith patterning on bay caught fish consists of sequential increments from freshwater existence (F) to those of estuarine existence (E) to finally bay existence (B). This typical patterning: freshwater/estuary/bay (FEB), was represented by 70% of the samples. The remaining 30% were identified as having one of four “atypical” microstructural patterns of habitat transition: 1. freshwater to estuary to freshwater to bay (FEFB) [54% of atypical samples or 16% of the total sample size], 2. freshwater to estuary to freshwater to estuary to bay (FEFEB) [37% of atypical samples or 11% of the total sample size], 3. estuary to freshwater to bay (EFB) [8% of atypical samples or 2% of the total sample size], and 4. freshwater to bay (FB) [1% of atypical samples or less than 1% of the total sample size].

Pattern “FEFB” peaked at the end of June to mid-July. Pattern “FEFEB” also peaked at the end of June. Pattern “EFB” was collected in extremely low numbers during mid-May and early June and again from mid-July to mid-August. Only one sample exhibiting a “FB” microstructural pattern was ever collected (7-19). The atypical patterns were most represented in total catch by date during mid-May (50%) and the end of June (33%) to mid-July (38%) when patterns “FEFB” and “FEFEB” were more abundant. The highest proportion of atypical patterning was found in the total monthly catches of May (38%) and July (38%). August sampling was minimal (n=5); however, all samples collected were of an atypical pattern type.

Further analysis revealed that developmental patterns ‘A’ through ‘D’ were represented on all 263 samples. Patterns ‘A’ and ‘B’ were most abundant; 29% and 35% respectively; and peaked from mid-June to mid-July. Pattern ‘D’, 23%, peaked at the end of June into mid-July. Least represented was pattern ‘C’, 13%, which steadily decreased in numbers throughout the season.

Quantitative Analysis

Quantitative analysis was performed on 205 samples of the 263 used for visual analysis. Fifty eight samples were not used due to one or more of the following circumstances: unacceptable radial angle, abnormal crystalline formation on otolith, uneven microstructural growth patterns along radial angle, loss of otolith (part or whole), and/or poor sample preparation. Analysis was completed through the use of “Bioscan Optimas System” (version 4.02), a Sony CCD-IRIS color video camera, a Sony color Trinitron monitor, and ‘EXCEL 5.0’ software program. Magnification used during analysis was 280x.

For each sample, measurements of the following regions and of the increments contained within each region were recorded: “bay check”, bay region, “developmental check”, freshwater region, “estuarine check”, and estuarine region. All possible sub-daily increments found within the bay region were not measured. Mean incremental width on the bay samples for freshwater residency was 2.47 microns and the mean incremental width of estuarine residency was 5.20 microns. These values are consistent with incremental width of both freshwater and estuarine residency from mainstem and ‘EEM’ caught fish. Mean incremental width of bay residency was 8.35 microns.

All samples previously classified as “atypical” had a second “freshwater region” to be measured and some a second estuarine region. Quantitative analysis revealed that the second estuarine regions were similar in mean incremental width to the initial ‘EEM’ region (5.20 microns). However, mean incremental width of the second freshwater region was calculated as 3.52 microns. This mean is far greater than the initial freshwater residency mean of 2.47 microns. This difference in mean incremental width indicates that the second “freshwater” region may not be a “true” freshwater region, but may indicate fish residency in the slower growing zones of the upper estuary like the FRT zone (3.46 microns) and/or the EFT zone (3.77 microns) (Also, see table 1). This would

indicate that some estuarine rearing chinook move around within the different zones of the estuary. This is the most likely explanation because none of the 204 samples collected in the Lower River (near Burlington) exhibited any estuarine checkmark or pattern (Skagit System Cooperative 1996, Skagit System Cooperative and Western Fisheries Research Center 1998).

The EFB pattern suggests a life history pattern without significant time spent in freshwater. This may correspond to a late emerging fry and a quick outmigration to the estuary, possibly “pushed” out by a high river discharge.

Analyses planned in the future, correlating the length, residence period, and timing of these “atypical” samples at various life history stages with water flow and temperature may reveal additional findings.

LITERATURE CITED

- Campana, Steven E. and John D. Neilson. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* 42: 1014-1032.
- Larsen K. A. and R. R. Reisenbichler. 1993. The importance of estuarine habitats of the Skagit River, WA, to juvenile chinook salmon, *Oncorhynchus tshawytscha*. Presentation at the International Symposium of Fish Otolith Research and Application January 23-27, 1993, Hilton Head Island, SC.
- Neilson J. D., G. H. Geen, and D. Bottom. 1985. Estuarine growth of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as inferred from otolith microstructure. *Can. J. Fish. Aquat. Sci.* 42: 899-908.
- Skagit System Cooperative. 1996. FY96 Skagit River Chinook Restoration Research Annual Narrative Report. Skagit System Cooperative, LaConner WA. 14 pages.
- Skagit System Cooperative and Western Fisheries Research Center. 1998. Skagit Chinook Life History Study Progress Report Number 1. Skagit System Cooperative, La Conner WA. 8 pages.
- Stevenson, David K. and Steven E. Campana, [ed.]. 1992. Otolith microstructure examination and analysis. *Can. Spec. Publ. Fish. Aquat. Sci.* 117: 126p.
- Volk E. C., D. G. Mortensen, and A. C. Wertheimer. 1995. Nondaily otolith increments and seasonal changes in growth of a pink salmon (*Oncorhynchus gorbuscha*) population in Auke Bay, Alaska. Pages 211-225 in D. H. Secor, J M. Dean, and S. E. Campana. eds. *Recent Developments in Fish Otolith Research*. University of South Carolina Press, Columbia, SC.

GLOSSARY OF OTOLITH AND MICROSTRUCTURE TERMS

“Bay check” (B.C.): An area of otolith that distinguishes a marked transition from estuarine residency to bay residency by a change in incremental width.

Core: Calcified area deposited within the earliest deposited increment; usually made up of a few or several primordia.

“Developmental check” (D.C.): An area of otolith located in close proximity to core that is a distinctive series of increments related to a developmental event in the fishes life.

“Estuarine check” (E.C.): An area of otolith that distinguishes a marked transition from freshwater residency to saltwater residency by a change in incremental width.

Growth axes: Axes within the otolith along which proportionately rapid rates of deposition occur. Otoliths can have more than one growth axis in which case axes are sometimes referred to as major and minor.

Increment (Inc.): Bipartite concentric ring comprised of alternating zones of predominately calcium carbonate accretion zone and predominately organic discontinuous zones.

Increment width: Linear measure of increment, comprised of one accretion zone + one discontinuous zone; usually measured along a major growth axis.

Postrostrum: Posterior most projection of the sagitta.

Primordia: Initial deposition sites of organic matrix and calcium carbonate; usually located in the core. Primordia may fuse or remain separate, forming multiple cores.

Reference angle: A linear distance from a central primordia in core region to edge of otolith along a major growth axis.

“Reference check” (R.C.): A distinct series of increments that references an exact river location for aid in analysis of samples downstream.

Rostrum: Anterior most projection of the sagitta.

Sagitta: Largest otolith located within the saccular vestibule of the semicircular canals. Preferred otolith for analyses.

“Transitional point”: An estuarine check composed of one distinct increment that clearly marks an abrupt transition from freshwater to saltwater residency.

“Transitional zone”: An estuarine check composed of a series of increments with little structural definition that mark a gradual transition between freshwater and saltwater residency.

“Transition area” (T.A.): A region of otolith on ‘emergent/forested transition’ May samples containing one of three types of checkmarks, not unlike the “transitional check” of ‘estuarine/emergent marsh’ samples. Each checkmark contains a series of increments with little structural definition that mark a transition in growth occurring upstream of the true saltmarsh.