

**SKAGIT CHINOOK LIFE HISTORY STUDY  
PROGRESS REPORT NUMBER 3**

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## **INTRODUCTION**

This report summarizes progress made by the Skagit Chinook Life History Study during 1999. 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 1999.

## **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).

This study focuses on identifying specific ocean-type chinook life history types exhibited by Skagit River chinook. For this study, juvenile chinook otolith samples were collected from the upper Skagit River, middle Skagit River, lower Sauk River, and lower Skagit River near the town of Burlington (Figure 1). Samples were also collected from sites within three different habitat zones in the Skagit estuary: forested riverine tidal (FRT), emergent forested transition (EFT), and the estuarine emergent marsh (EEM) (Figure 2). Together, these samples form a baseline to examine otolith patterns represented by Skagit ocean-type chinook. The baseline samples have enabled us to correlate distinct patterns and regions on chinook otoliths with distinct habitat regions occupied by Skagit chinook. Samples collected from juvenile chinook in the nearshore environment of Skagit Bay represent fish that have successfully completed the freshwater and estuarine rearing part of their juvenile life history. We use baseline samples to interpret otolith microstructural data to juvenile life history types from those juvenile chinook captured in Skagit Bay.

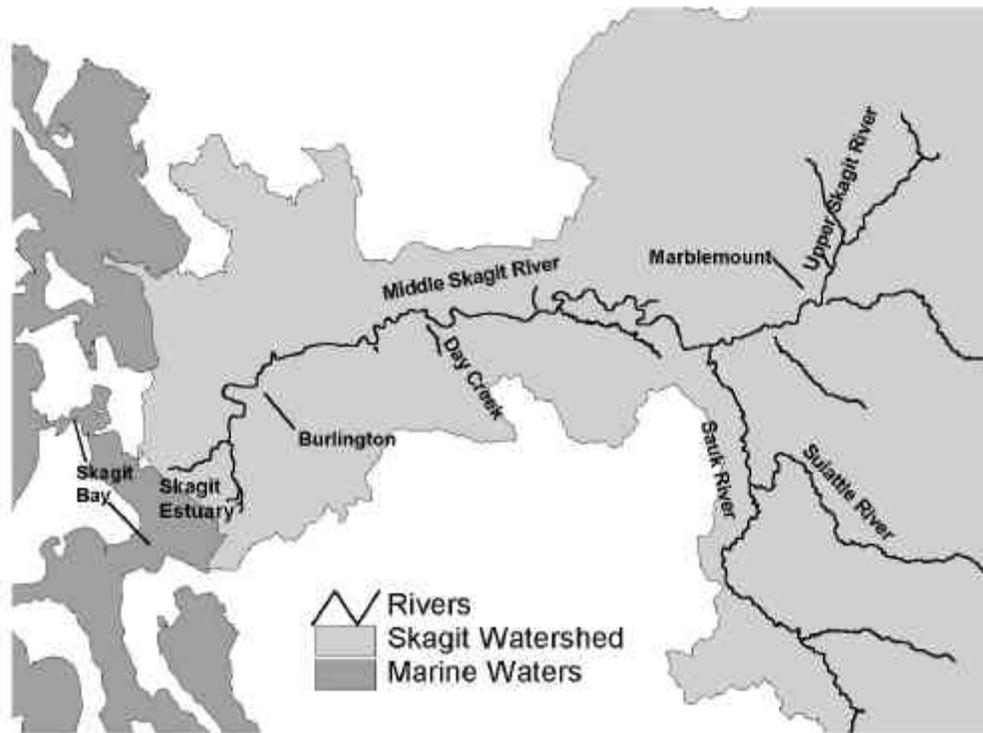


Figure 1. Overview of Skagit River basin, estuary and nearshore environment.

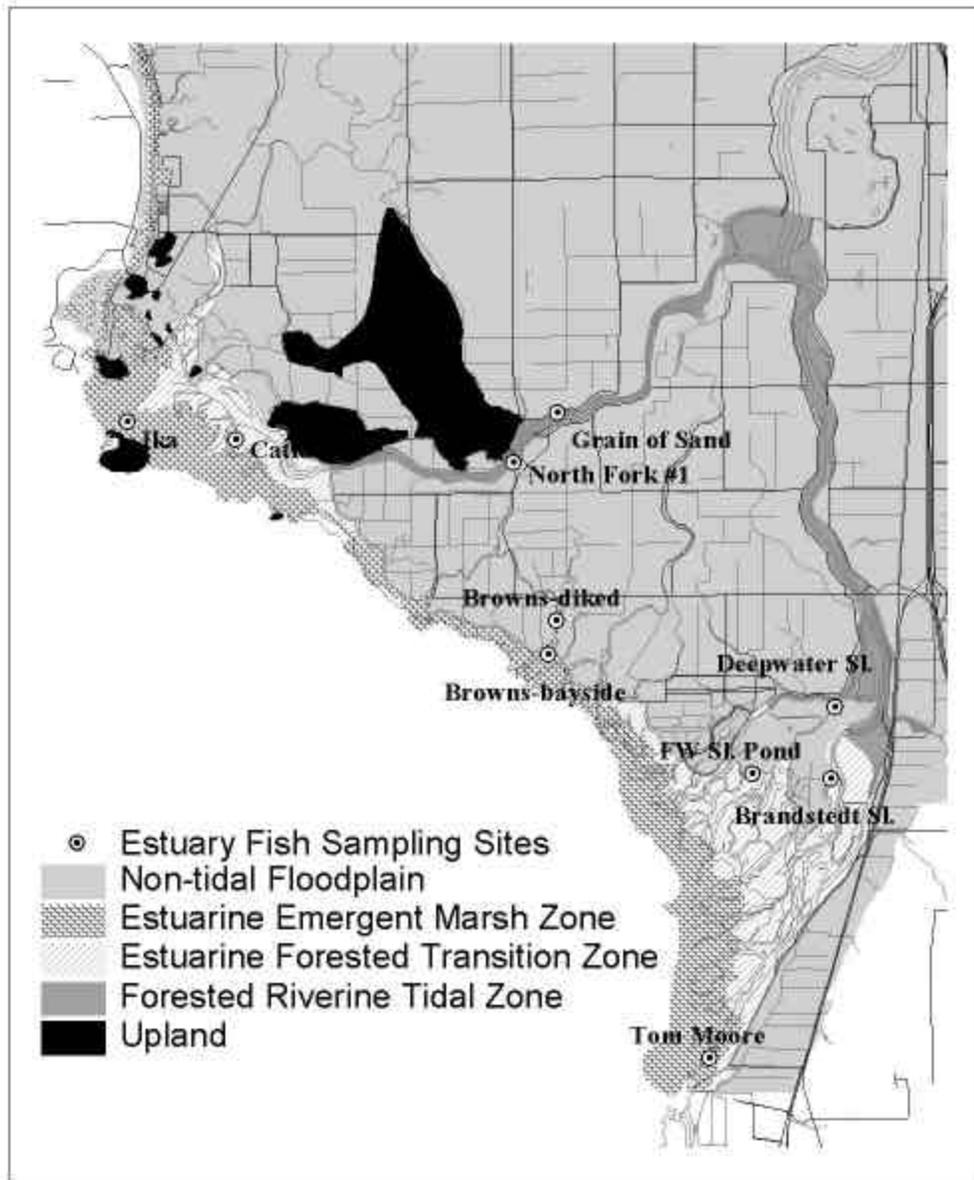


Figure 2. Location of estuarine habitat zones in the Skagit Estuary and juvenile chinook sampling sites.

### Visual Analysis: identification of checkmark patterns

Based on preliminary results (Skagit System Cooperative 1996) and previous otolith work on Skagit chinook (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, 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 points 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 1995 (brood year 1994) from the Lower Skagit River mainstem (near Burlington, Washington), the estuarine emergent marsh (EEM) and estuarine forested transition (EFT) zones of the Skagit estuary were reported in 1998 (Skagit System Cooperative and Western Fisheries Research Center 1998). Results from juvenile chinook otolith samples collected in 1995 (brood year 1994) from emergent fry / freshwater rearing areas, the forested riverine tidal (FRT) zone of the Skagit estuary, and nearshore habitat in Skagit Bay were reported in 1999 (Skagit System Cooperative and Western Fisheries Research Center 1999).

This report presents results from analyses conducted on age 3+ adult chinook (brood year 1994) samples collected from the in-river test fishery and spawning ground areas in 1997 and juvenile samples collected in nearshore habitat of Skagit Bay during 1996 (brood year 1995). The report also summarizes our current knowledge of ocean-type chinook juvenile life history types and estuarine residence period based on the samples collected in nearshore habitat in 1995 and 1996.

## Description of Life History Types

This study focuses on identifying specific ocean-type chinook life history patterns exhibited by Skagit River chinook. 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. From Hayman et al. (1996) we hypothesize at least two logical ocean-type chinook life history types. For otoliths sampled from juvenile chinook in Skagit Bay, we would expect to observe sequential otolith increments originating from a freshwater existence, then increments originating from an estuarine existence, and finally increments originating from a bay existence. When identifying a specific life history type for an individual sample, we then represent freshwater rearing as ‘F’, estuarine rearing as ‘E’, and bay rearing as ‘B’. This life history type (freshwater > estuary > bay) is identified as ‘FEB’. Another logical ocean-type chinook life history type is the ‘FB’. This type represents a fish that rears in freshwater habitat for some time period (may be short or long), then migrates quickly through the estuarine habitat to rear in bay habitat. The ‘FB’ life history type does not spend enough time in estuarine habitat to manifest an “estuarine rearing region” on its otolith.

Our results reported in Skagit System Cooperative and Western Fisheries Research Center (1999), however, revealed that a significant number of the juvenile chinook samples collected in Skagit Bay in 1995 were identified as having one of three “atypical” microstructural patterns (i.e., were not identified as FEB or FB):

1. freshwater to estuary to freshwater to bay (FEFB),
2. freshwater to estuary to freshwater to estuary to bay (FEFEB), and
3. estuary to freshwater to bay (EFB).

Results from otolith samples taken from sub-yearling wild chinook in the lower river in 1995 is the basis of our understanding of the frequency in which juvenile chinook enter estuary habitat and then return to “river” habitat. The 1995 data suggests that this phenomenon does not occur. None of the 204 samples collected from the lower Skagit River (near Burlington – Figure 1) in 1995 exhibited an estuarine check mark (Skagit System Cooperative and Western Fisheries Research Center, 1998). Skagit System Cooperative and Western Fisheries Research Center (1999) showed that juvenile chinook growth within the three estuarine zones was not the same. The slow growth part of the estuary corresponds to the sites in the FRT and EFT zones while the faster growth part of the estuary corresponded to the sites in the EEM zone (Figure 2). By inference, we conclude that the estuary check mark (E) is formed when juvenile chinook are occupying habitat in the EEM zone and that individual fish that move from zone to zone during their estuarine rearing period reflect an otolith pattern of alternating “fast” and “slow” growth – hence, the observation of “atypical” life history patterns. Therefore, our current understanding of the FEFB and FEFEB life history types is that they reflect fish movement within the different estuarine zones following a freshwater rearing period and before movement to habitat in Skagit Bay. The EFB life history type reflects a fish that

has a very brief freshwater existence – so brief that a true freshwater rearing region is not detected on the otolith.

## 1996 Skagit Bay Nearshore Samples

### Visual Analysis

One hundred fifty one juvenile chinook (brood year 1995) were collected between February 29<sup>th</sup> and September 20<sup>th</sup>, 1996 for otolith analysis. All sampling sites were located within Skagit Bay, WA. Sites included: Ala Spit, Hope Island Inlet, Hoypus Point, Hope Island, Lone Tree Island, Strawberry Point, Similk Bay, Skagit Island, Sneeoosh Point, and Yokeko Point. One hundred thirty eight of the 151 otolith samples were visually analyzed. The remaining thirteen samples were not analyzed for the following reasons: 5 samples with annuli<sup>1</sup>, 2 lost samples, and 6 unreadable samples. ‘Unreadable’ represents samples that were prepared poorly or too translucent to identify individual increments in the preferred otolith quadrant. 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.

Samples were examined for checkmarks and microstructural patterning previously identified on juvenile Skagit chinook from the brood year 1994 (refer to Skagit System Cooperative and Western Fisheries Research Center 1999). All samples exhibited a “bay check” as well as other visually identifiable checkmarks (“developmental check”, “reference check”, and one of three “estuarine checks” previously identified). Once again, the “bay check” consisted of two or three wide bright increments within a transitional area. Bay increments appeared consistently wider than estuarine increments and always occupied the area prior to the otolith edge. All five life history types<sup>2</sup> represented on the previous year’s samples were identified in the 1996 samples (Table 1).

*Table 1. Percentage of life history types from wild sub-yearling chinook collected in nearshore habitat of Skagit Bay.*

<b>Life History Type</b>	<b>Sample Year 1995</b>	<b>Sample Year 1996</b>
FEB	70%	56%
EFB	2%	1%
FB	1%	4%
FEFB	16%	20%
FEFEB	11%	20%

<sup>1</sup> This study is currently focused on ocean-type chinook so we are not analyzing stream type chinook samples at this time.

<sup>2</sup> See the description of life history patterns in the Life History Type section of this report for a more complete explanation.

Atypical samples were most represented end of June to beginning of July and end of July to beginning of August due to increased representation of patterns FEFB and FEFEB. The percent ratio of 'typical' to 'atypical' microstructural patterning is 58:42, which is dissimilar to the '95 Skagit bay juvenile collection of 70:30, respectively. Although sample sizes were different, 263 ('95 bay collection) and 138 ('96 bay collection), one may propose that length of sampling season may have impacted the percent ratio for the '96 bay collection. The '95 sampling season was from May 11<sup>th</sup> to August 31<sup>st</sup>, whereas the '96 season was from February 29<sup>th</sup> to September 20<sup>th</sup>. Fifty seven percent of samples collected in February and March of 1996 were identified as 'atypical', as were 83% of samples collected in August and September. Without samples collected within these months, the percent ratio of the patterns (63:37, typical:atypical) would be similar to the '95 Skagit bay collection (70:30), respectively.

All 'developmental' microstructural patterns (A,B,C,D) were represented among the '96 bay collection. Patterns 'A' and 'B' were again the most abundant among the bay samples, 29% and 35% respectively, and peaking mid-June to mid-July. The percentages for patterns 'A' and 'B' are identical to the previous year. Pattern 'D' (17%) was not represented as much as previously (23%), but peaked at the same time as the '95 bay collection (end of June into mid-July). Pattern 'C' was more evident in this bay collection (19%) than in the '95 bay collection (13%), and may also have been influenced by the extended sampling season. Of the nine samples collected on September 20<sup>th</sup>, seven were identified as developmental pattern 'C' (78% of September samples) with two remaining samples identified as 'B'.

### Quantitative Analysis

Quantitative analysis was performed on 112 of the 138 samples used for visual analysis. Twenty six 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.

Linear measurements were recorded for all previously identified checkmarks, habitat regions (including the secondary freshwater and estuarine regions of 'atypical' samples), and individual increments associated with said areas except within the "developmental check". Increments within the "developmental check" area were difficult to consistently locate due to little structural definition. Summarized linear distances, mean incremental widths, and increment counts were recorded in microns and entered in a 'flatfile.xls' (database designed to be implemented in the Skagit Chinook Restoration

Model). The mean incremental width for the freshwater region of the '96 bay samples was 2.47 microns, which is identical to the mean incremental width for the freshwater region of the '95 bay samples (2.47 microns). Mean incremental width for the estuarine region of the '96 bay samples is 5.47 microns, which is comparable to the estuarine region of the '95 bay samples (5.20 microns). The mean incremental width for the bay region of the '96 bay samples is 7.93 microns, which is similar to the bay region of the '95 samples (8.30 microns). Mean incremental width for the second “freshwater”(i.e., the slow growth zones of the estuary) region of the ‘atypical’ samples is 3.69 microns, which is similar to the 3.52 microns recorded for the same region on the '95 bay samples. Also, the mean incremental width for the second estuarine region (5.56 microns) is similar to the 5.19 microns recorded for the estuarine region of the '95 bay samples.

Juvenile chinook residence period within estuarine habitat

Assuming one otolith increment represents one day (from Neilson et al. 1985) and by counting the number of increments within each estuarine rearing region on the otoliths collected from juvenile chinook in nearshore habitat of Skagit Bay, we can estimate estuarine residence period by life history type (Table 2). We use these estimates of estuarine residence period as part of the Skagit Chinook Restoration Model (see Hayman et al. 1996).

*Table 2. Summary of estuarine residence period (in days, assuming one increment = 1 day) by wild sub-yearling chinook in the Skagit Estuary based on otoliths taken from chinook collected in nearshore habitat of Skagit Bay.*

Juvenile Life History Type	1995 Outmigration	1996 Outmigration	Both years combined
	Residence Period in days	Residence Period in days	Residence Period in days
	<b>Mean, (SD), sample size</b>	<b>Mean, (SD), sample size</b>	<b>Mean, (SD), sample size</b>
FEB	<b>25.2</b> , (9.9), 158	<b>35.5</b> , (11.5), 61	<b>28.1</b> , (11.3), 219
EFB	<b>59.0</b> , (32.5), 2	<b>76.5</b> , (34.6), 2	<b>67.8</b> , (29.2), 4
FEFB	<b>44.7</b> , (16.2), 27	<b>45.8</b> , (13.3), 21	<b>45.2</b> , (14.9), 48
FEFEB	<b>52.0</b> , (14.6), 18	<b>50.2</b> , (14.1), 23	<b>51.0</b> , (14.2), 41
All Types	<b>30.4</b> , (15.1), 205	<b>41.4</b> , (15.0), 107	<b>34.2</b> , (16.0), 312

Along with the visual analysis results (which documented the different sequences of F, E, and B regions on otoliths), these results help validate distinctions between the life history types by showing differences in estuarine residence period. For example, the average FEFB and FEFEB life history types in 1995, reared 1.8 (44.7/25.2) and 2.1 (52.0/25.2) times longer in estuarine habitat than the average FEB life history type, respectively.

## **Age 3+ adult chinook Samples (Brood year 1994)**

### Visual Analysis

Visual analysis was performed on twenty six of thirty one 3+ adult (1994 Brood Year) chinook otolith samples collected in 1997. Five samples were unusable due to a lab incident. Samples were collected throughout the Skagit River system from early June to late October and included five areas of interest: Test fishery (Blake Resort), Upper Sauk, Suaittle, Day creek, and Upper Skagit Mainstem (near Marblemount) (Figure 1). 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.

Previously identified landmarks (“developmental check”, “reference check”, “estuarine checks”, bay transition zone, and a “bay check”) on the otoliths of juvenile Skagit chinook were also identified on the adult samples. All radial distances and incremental widths previously recorded on juveniles were recorded on adults except within the bay region. The bay region was excluded from the analysis due to difficulty interpreting where to stop marking within the bay region. Furthermore, equipment constraints did not allow consistent identification of the first annulus at a magnification necessary for our primary analysis purposes (i.e., identifying otolith marks within the early juvenile life history period –generally much younger than 1 year of age).

Early life histories based on microstructural patterns were also identified<sup>3</sup>. The FEB type represented 69% (or 18) of the samples. The remaining 31% (or 8) were identified as having an atypical microstructural pattern of habitat transition. Half of the atypical samples, or 15% of the total sample size, were identified as having a pattern of transition from freshwater to estuary to freshwater to bay (FEFB). The other 50% (also 15% of the total sample size) were comprised of freshwater to estuary to freshwater to estuary to bay (FEFEB). The adult ratio of 69:31 (typical:atypical) is similar to the ratio of 70:30 (typical:atypical) for juvenile samples from Skagit Bay (brood year 1994); although the sample sizes were quite dissimilar (juvenile=263 and adult=26).

Analysis also revealed that developmental check patterns ‘A’ through ‘D’ were represented throughout the samples, and we found the same correlation between specific developmental check patterns and specific geographic ranges within the Skagit River basin as previously identified from juvenile baseline samples, with one exception. All developmental check patterns (A-D) were identified on samples collected at the ‘Test Fishery’ site. The test fishery is conducted in the lower river over the entire river entry curve, so it is logical that all developmental check patterns were detected in this sample.

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<sup>3</sup> See the description of life history patterns in the Life History Type section of this report for a more complete explanation.

Pattern 'A' (19% of total sample size) was observed only on the Sauk and Suiattle samples. Pattern 'A' was what we observed from juvenile baseline samples collected in 1995 in the lower Sauk River (Figure 1). No juvenile samples were collected in the Suiattle River in 1995. However, the Suiattle is a tributary to the Sauk, so all Suiattle origin chinook must pass through the lower Sauk river. Patterns 'B' and 'D' (43% and 19%, respectively, of total sample size) were observed on samples collected from the Upper Skagit mainstem located in the Marblemount area (Figure 1). Again, this pattern is consistent with what we observed from juvenile baseline samples collected in 1995 for the Upper Skagit mainstem. Pattern 'C' (19% of total sample size) was represented on 3 of the 4 samples collected from adult chinook in Day creek (Figure 1). Our hypothesis, based on the results of juvenile samples from this area of the Skagit, suggests that the developmental check pattern 'C' is the norm. The remaining sample was identified by two separate readers as having the developmental check pattern 'A'.

### Quantitative Analysis

Quantitative analysis was performed on 24 of the 26 three-year-old adult samples ('94BY) visually analyzed. Two samples were not used in the analysis due to uneven growth along the axis of measurement. 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.

Previously identified landmarks ("developmental check", "reference check", "estuarine checks", bay transition zone, and a "bay check" ) on the otoliths of juvenile Skagit chinook (brood year 1994) were also identified on the adult samples (brood year 1994). Radial distances, mean incremental widths and incremental counts between the landmarks were recorded on adults, except within the bay region. All measurements were recorded as microns in a database set up for adult chinook otolith samples ('adultff.xls'). The bay region was excluded from the analysis due to difficulty interpreting where to stop marking within the bay region of the otolith. Furthermore, equipment constraints did not allow consistent identification of the first annulus at a magnification necessary for analysis.

## REVISION TO FUTURE SCOPE OF WORK

The following revised scope of work is for chinook otolith analysis funded by the Non-Flow Coordination Committee<sup>4</sup> (NCC) for the year 2000. The original scope of work for year 2000 is shown in Table 3 for comparison purposes. The original scope of work was planned in 1995 at the inception of the Skagit Chinook Life History Study. The changes to the original scope of work were proposed and discussed by NCC members in December 1999 and January 2000. The year 2000 scope of work will follow a list of priorities. Depending on how quickly adult otoliths can be analyzed, it is very likely that only work on priorities 1-3 will be completed in year 2000, and priority 4 will be just be started. Therefore, priorities 4 and 5 will likely run into the year 2001 scope of work.

*Table 3. Originally planned scope of work for year 2000. The following listed priorities replace the NCC funded part of this plan.*

Year	Proposed to be funded by NCC	Funded by Skagit Chinook Restoration Study	Related work: WFRC	Related work: Skagit Chinook Restoration Study
2000	Complete analysis of 1997 bay samples. Complete analysis of 1999 adult test fishery and spawning area samples (5 year old fish only).	For the 1997 juvenile and 1999 adult (5 year olds only) otolith samples: (1) estimate age, growth, and residence in freshwater habitat; (2) estimate age, growth, and residence in estuary habitat; (3) estimate age, growth, and residence (minimum) in bay habitat; and (4) describe the observed juvenile life history patterns.	Further refinement of protocols and validation work as necessary.	Collect otolith samples: 200 bay juveniles, 120 river test fishery adults, and 180 spawning area adults. For the 1996 juvenile population in the bay, estimate the respective percentage of each juvenile life history type. Estimate marine survival by juvenile life history type (BY 94: 4 year old fish only). Annual variability discussion possible.

### Priority 1:

Finish analysis of BY94 four-year-old adults. These were started in 1999, but not finished.

### Priority 2:

Analyze BY94 five-year-old adults. These samples consist of 61 otoliths of which 37 will be used as a “blind test” comparison between two different otolith readers. The blind test

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<sup>4</sup> The NCC authorizes funding under the Chinook Research Program in the Non-flow Mitigation part Seattle City Light’s Skagit Fisheries Settlement Agreement. Federal Energy Regulatory Commission Project Number 553.

will compare between readers, the correlation of identifying otolith check marks (e.g., developmental check, estuarine check, bay check) and check types (e.g., developmental check types: A, B, C, or D). The blind test will also compare the correlation between readers of the specific location of individual check marks on the 37 different otoliths. The readers will know nothing about the sample except its brood year (1994) and overall age (5 yr.).

Priority 3:

Analyze juvenile chinook samples collected from the southern and northern ends of Swinomish Channel. No more than a total of forty samples will be analyzed - twenty from each end of Swinomish Channel. This is part of a feasibility study exploring the potential to improve fish passage from the North Fork Skagit River into the habitat associated with Swinomish Channel and Padilla Bay. The study is partially funded by the Seattle City Light Early Action ESA Program. The reason for inserting this task before priority 4 and 5 is because the principle investigator is a Western Washington University graduate student. His work and funding for the study ends in 2000.

Priority 4:

Analyze age 0+ juvenile samples collected in year 2000 from different developmental check spawning ground areas and WDFW's mainstem trap located in the lower river near Burlington. Approximately 250 samples have been collected from spawning areas representing all four developmental check areas and all six chinook stocks. Ninety of these samples were collected in the upper reaches of the Skagit River. Approximately 200 samples will be collected at WDFW's mainstem trap. Sample collection started in February 2000 at the rate of 20 per month.

There are two important reasons why these samples should be analyzed before the 1997 bay samples (which is listed as priority 5):

1. Some members of the NCC suspect that a significant number of early emerging chinook are exposed to stranding conditions in the upper reaches of the Skagit River. These samples are needed to evaluate the relative contribution of early emerging chinook that are subject to flow conditions conducive to fry stranding in the Upper Skagit River. The sooner the NCC understands the relative contribution of juvenile chinook exposed to stranding conditions, the sooner they can discuss (or dismiss) changes in flow regulation for this part of the river.
2. We only have samples from the lower river in one year (1995) which we are currently basing our understanding of the frequency in which juvenile chinook enter estuary habitat and then return to river habitat. The 1995 data suggests that this does not occur. None of the approximately 200 samples collected in the lower river (Burlington) in 1995 exhibited an estuarine check mark. Data from another sample year will help our understanding on this issue, which is critical when identifying different chinook life history types.

Priority 5:

Analyze juvenile chinook samples collected in nearshore habitat of Skagit Bay during the 1997 season.

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## 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.

## APPENDIX 1. PROTOCOL FOR ADULT CHINOOK OTOLITH PREPARATION

### USGS-Western Fisheries Research Center and Skagit System Cooperative, Chinook Life History Project

#### Removal

##### Frozen Heads

Allow heads to thaw in cool water bath for 3-4 hours; extract otoliths from smaller heads first

Orient fish head with snout facing upward and the flat cut side of head on the cutting surface

Extract sagittal otoliths from head

- Slice off top curved part of head with aid of a hacksaw; cut straight downward from snout to cutting surface approximately 1" above eyes on larger heads and ½" on smaller heads
- Scrape away frozen brain region with scalpel
- Melt away frozen membranous fluid surrounding otoliths with squirt bottle of water; be certain of removing only when thawed, otherwise otoliths will break
- Attempt to remove otoliths without membrane; peel away membrane if attached

Place left and right otoliths in same vial with collection label for measuring and weighing

Store originally with alcohol just covering otoliths to avoid breakage

##### Vials of Membranous Sac in alcohol

Clean membranous tissue from otoliths

- Place otoliths into separate watch glasses of distilled water
- Use a ½ size paint brush to remove membranous tissue

Rinse otoliths with clean water

Place left and right otoliths in same vial with collection label for measuring and weighing

Store originally with alcohol just covering otoliths to avoid breakage

#### Embedding

Measure left otoliths with calipers to the nearest 0.1 mm; omit samples for measurement when tip of rostrum is broken or missing

- Place otoliths in palm of one hand while holding calipers in other
- **Carefully** adjust calipers across otoliths until otolith ends (rostrum and postrostrum) touch the sides of the calipers; lifting otoliths should be possible if calipers are adjusted correctly

Weigh left otoliths to nearest 0.0001 g; omit samples for weighing when tip of rostrum is broken or missing

Record amounts of abnormal crystalline formation, if any

Label larger mold trays (12x16x5 cm wells) with appropriate information

Use Q-tip to apply a thin coat of silicon in each well

Center otoliths lengthwise to long axis in each well (sulcus side down)

Mix resin and catalyst

- For ~ 60 samples, use 2 ½oz of resin and 6 drops of catalyst

Use plastic spoon to add resin to wells without disturbing orientation of otoliths

- Do not leave plastic spoon in resin; will begin to melt
- Distribute resin over postrostrum to eliminate trapped air bubbles
- Fill entire well with resin to completely cover otoliths
- Tap otoliths to bottom of wells with dissection probe to eliminate air bubbles trapped underneath; insert probe at an angle to otoliths, tap down at post rostrum and withdraw probe at an angle without upsetting otolith orientation.

Double check wells for air bubbles and/or incorrect otolith orientation

- Use dissection probe to move air bubbles as far away from otoliths as possible
- Tap down otoliths and fix as needed with probe technique mentioned above

Place molding trays in drying oven for 1 hour at 62° C

## **Slide Preparation**

Label microscope slides

Preheat hot plate (setting 2)

Remove resin blocks from molds (be certain to keep track of the labels to the resin blocks, since the blocks themselves are not labeled)

Prepare a lot of crystal bond chunks for melting

Apply crystalbond to embedded samples

- Visualize core region and identify preferred quadrant of otoliths for analysis
- Attempt to raise resin block so core region of samples will be parallel to slide when blocks mounted on slide; even when samples look fairly parallel
  - Dip short ends of resin blocks into melted crystalbond a few times (~3-4); allow crystalbond to cool and set block on slide to check otolith orientation between dips; number of dips dependent upon original orientation of core region to slide
  - Dip long edge of resin blocks proximal to preferred quadrant into melted crystalbond several times (~5-8), to raise the long edge; allow crystalbond to cool and set block on slide to check otolith orientation between dips; number of dips dependent upon original orientation of core region to slide
  - If too much crystalbond applied to block edges, place block onto slide with melted crystalbond until the excess melts down
  - Keep applying crystalbond to edges if not enough

Place several slides across front edge of hot plate

Apply crystalbond to heated slides with the aid of a dissection probe

- Apply as an outline shape of a square, allowing an airspace or window for the otoliths

- The square of crystalbond should be placed in the upper ¼ length of each slide and oriented across the short width of each slide or perpendicular to slides

Lay flat side of resin blocks over crystalbond

- Make sure otoliths are centered over airspace and all edges of resin blocks are sealed with crystalbond
- Lightly and evenly press down once on blocks and allow to cool; do not ooze crystalbond underneath otoliths

Remove excess resin over top of embedded otoliths with Isomet saw; saw as close to otoliths as possible without hitting them

## **Grinding and Polishing**

### First Side

Place slide in holder of grinding jig

Grind resin blocks with 120 µm silicon carbide and PoliTec 1 polishing cloths

- Wheel speed 5.5-6
- Check progress under dissecting scope 10-63x
- Grind until surface of otoliths barely exposed
- For extremely curved samples grind with 240 µm silicon carbide and Texmet 2000 polishing cloths once rostrum is reached

Grind otoliths further with 600 µm silicon carbide and Texmet 2000 polishing cloths

- Wheel speed 5-6
- Check progress under dissection and compound scopes (250x)
- Grind until core area visible and covered by thin layer of otolith
- Important to check progress regularly since depth from surface of otoliths to core area is slight

Grind even further with 5 µm alumina and Texmet 1000 polishing cloths

- Wheel speed 4-5
- Check progress under compound scope (250x) frequently
- Grind until just inside of outer core increments

### Flipping

Remove resin blocks from slides with razor blade (sharper the better). Start by removing outer crystalbond first, then scrape at sides of blocks

Shave off extra crystalbond built-up on resin blocks from mounting procedure on first side

Scrape all crystalbond off slides with razor blade

Flip and mount resin blocks with cytoseal

- Apply cytoseal to slide in the shape of an “S”, not drops
- Slide resin wedge into middle of “S” shape and let it sink

- Hold on to thick wedge part with forceps and push the resin wedge into place on the slide

Allow a minimum of 48 hours to dry, preferably 72 hours

### Second side

Grind resin blocks with 120  $\mu\text{m}$  silicon carbide and PoliTec 1 polishing cloths

- Wheel speed 5.5-6
- Check progress under dissecting scope 10-63x
- Grind until reach sulcus

Grind otoliths further with 240  $\mu\text{m}$  silicon carbide and Texmet 2000 polishing cloths

- Wheel speed 5-5.5
- Check progress under dissecting scope 10-63x
- Grind until sulcus begins to disappear
- If uncomfortable grinding with 240 may go directly to using the 600  $\mu\text{m}$  silicon carbide

Grind even further with 600  $\mu\text{m}$  silicon carbide and Texmet 2000 polishing cloths

- Wheel speed 5-5.5
- Check progress under dissection and compound scopes (250x) often
- Grind until core region is visible and covered by thin layer of otolith

Grind further with 5  $\mu\text{m}$  alumina and Texmet 1000 polishing cloths

- Wheel speed 4.75-5.25
- Check progress under compound scope (250x) frequently
- Grind until major nuclei in core region and increments to first annulus exposed

Polish samples with 1.0  $\mu\text{m}$  alumina and Mastertex polishing cloth; check progress under compound scope (250x)