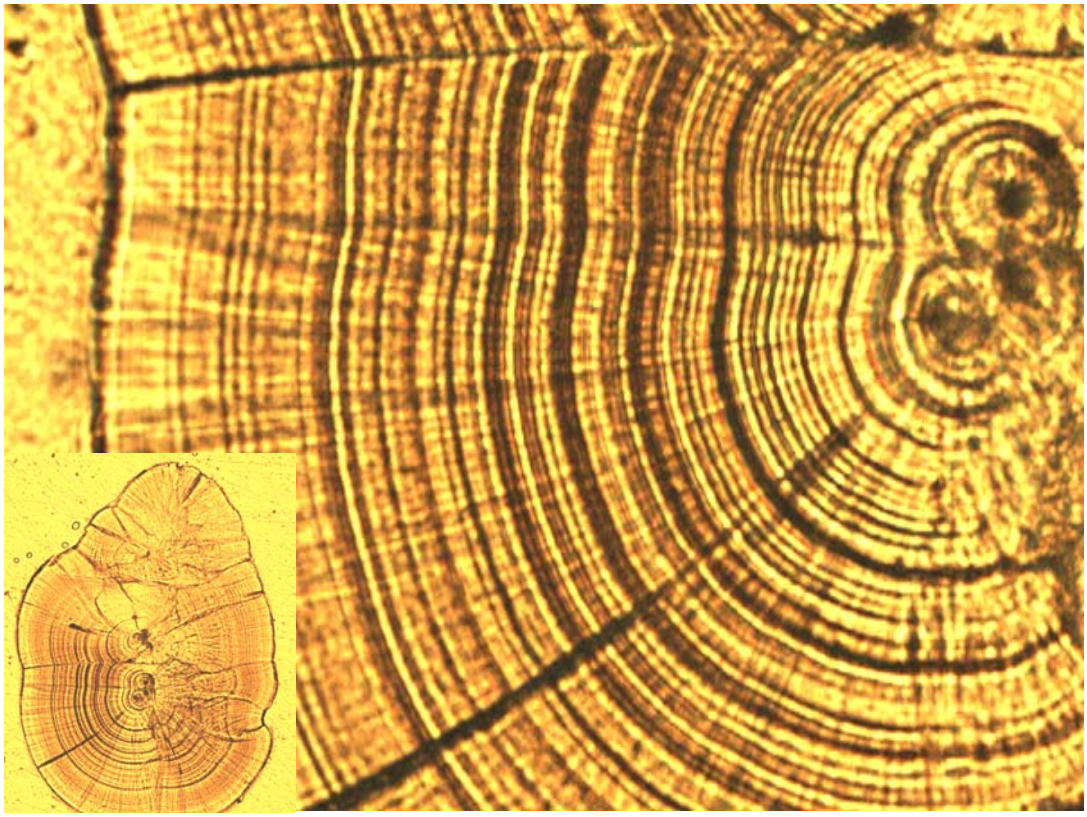
Arcata Fisheries Technical Report TR2005-01

Microstructural Natal Signature of Spring Chinook Salmon Otoliths from the Salmon River Drainage, Northern California



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Abstract. Early life history events in juvenile salmonid development, including eye-up, hatch, emergence, and habitat transitioning, can be linked to otolith microstructural patterns. In recent years, the combination of early life history events and incubation thermal regime has been used to associate stocks with specific incubation sites. Variation in temperature and growth can produce otolith increment patterns that are stock specific, provided the stock incubated under a distinct thermal regime. The resulting otolith increment pattern may be specific to the thermal regime under which embryonic and alevin development occurred, as it is generally accepted that increment deposition occurs prior to hatch. In this study, otoliths were collected from spring Chinook salmon (Oncorhynchus tshawytscha) fry captured from spawning sites on the North and South forks of the Salmon River in northwestern California in spring 2004. Otolith microstructure was analyzed using light microscopy and imaging software and later compared between the two forks and to other samples collected previously in the Klamath Basin. A natal microstructural signature was present on all otoliths collected in the Salmon River Basin (n=91) that was distinctive from other Klamath Basin collections. Natal signatures of fish collected from the North and South forks of the Salmon River were similar, indicating that both sample sites may have comparable thermal regimes during the incubation and intragravel development period for spring Chinook salmon.

Introduction

Otolith microstructure analysis has been used to determine age and growth of individual fish for many years. Recently, otolith microstructural patterns have been used to identify different juvenile life history types or life history events (e.g. Neilson et al. 1985; Larsen and Reisenbichler 1993, Volk et al. 1995) and to differentiate stocks in a system (e.g. Paragamian et al. 1992, Rieman and Myers 1994, Quinn et al. 1999). Transitions in life history such as “eye-up”, hatch, emergence, and migration from one habitat to another (e.g. freshwater to saltwater) are recorded in microstructural patterns of otoliths. These

changes appear as variations in increment deposition on otoliths that alter appearance of the microstructural pattern (Marshall and Parker 1982; Campana and Neilson 1985; Volk et al. 1990, 1996).

Fish otoliths are comprised primarily of calcium carbonate in aragonite mineral form embedded in a collagen-like organic matrix (Degens et al. 1969). Organic and inorganic components of otoliths interact during otolith growth to lay down a series of dark and light bands that reflect the bipartite nature of otolith increments. Incremental patterns may be comprised of a few to many individual increments that reflect previously mentioned life history events or changes in environment such as temperature or salinity.

Variation in temperature and growth can produce otolith increment patterns that are stock specific, provided the stock incubated under a distinctive thermal regime (Zhang et al. 1995, Volk et al. 1996, Quinn et al. 1999). The resulting increment pattern is specific to the thermal regime under which embryonic and alevin development occurred as it is generally accepted that increment deposition initiates prior to hatching (Quinn et al. 1999). The portion of the incremental pattern that exemplifies natal origin is located near the core of the otolith and represents intragravel residency. In this study, the core region of otoliths is referenced as the "developmental check region", encompassing the area between and including the hatch and emergence checkmarks.

The primary objective of this study was to identify a developmental check region and associated microstructural pattern of newly emerged spring Chinook salmon (*Oncorhynchus tshawytscha*) from the North and South forks of the Salmon River through qualitative and quantitative means. Identification of this region and its associated incremental pattern should reflect a natal signature of collection sites. A secondary objective, dependent on the ability to identify a developmental check pattern (objective 1), was to compare the developmental check patterns from samples collected from the North and South forks of the Salmon River to each other; as well as to visually compare the patterns to other recognized patterns.

Study Area

Otoliths were collected from spawning sites on the North and South forks of the Salmon River, located in the Salmon River watershed of the Klamath River Basin in northwestern California (Figure 1). Sample sites were chosen based on knowledge of spring Chinook spawning exclusivity above the two forks of Salmon River (Peter Brucker, Salmon River Restoration Council, personal communication). Sample sites were similar in elevation and hydrology. Spring run Chinook salmon spawn from about mid-September to late October above the forks of the Salmon River (Table 1), whereas fall run Chinook salmon spawn later in the year in the mainstem Salmon River below the forks. Spring Chinook salmon fry incubate and emerge from redds in January to May (Table 1). Otoliths were collected from fish captured directly below Idlewild Campground (elevation ~783 m) on the North Fork Salmon River and about 1.6 km below Blind Horse Creek (elevation ~823 m) on South Fork Salmon River (Figure 1).

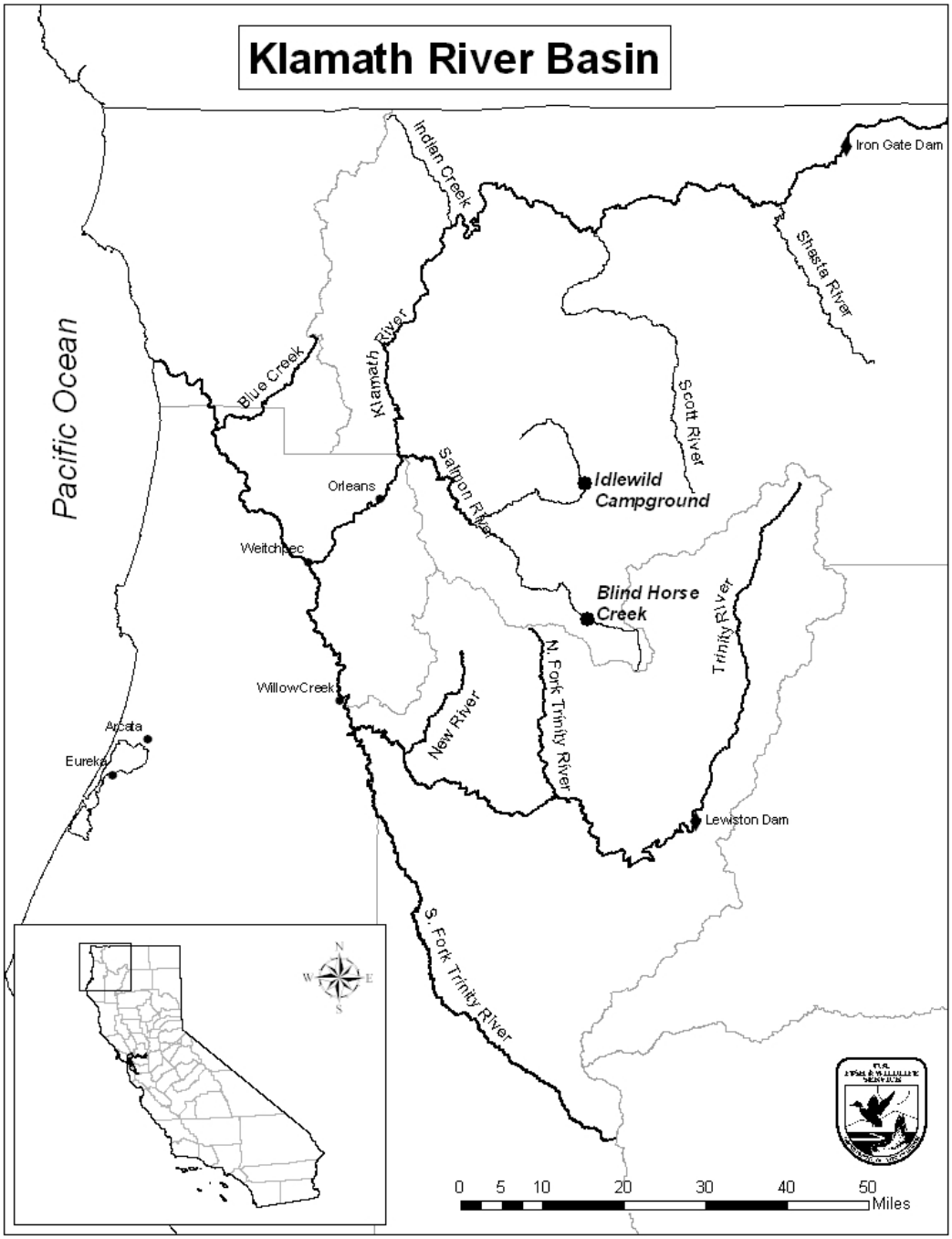

Figure 1. Map of Salmon River showing collection sites.

Table 1. Salmon River Chinook salmon early life stage periodicity (courtesy of Karuk Tribal Fisheries Program).

		Month											
Life Stage	Run	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Redd construction	Spr									X	X		
	Fall										X	X	
Egg incubation and fry emergence	Spr	X	X	X	X	X							
	Fall	X	X	X	X	X							
Fry rearing (up to 50mm)	Spr				X	X	X	X					
	Fall				X	X	X	X					

Methods

Spring Chinook salmon fry were captured from Idlewild Campground and Blind Horse Creek spawning areas using an 18.5 x 14.5 cm dipnet having a mesh size of about 1 mm. A targeted sample size of about 70 spring Chinook salmon fry from each fork of Salmon River was established prior to field collection. Sample size was based on three criteria: (1) type of study (i.e. lethal or non-lethal), (2) life stage of the fish, (3) adequate numbers to establish variance and reliability, and (4) experience of researcher (UFR Committee 2004). Fish fork lengths were measured to nearest millimeter (mm) before fish were frozen prior to laboratory preparation. Otoliths were removed, prepared, and analyzed in the Otolith Laboratory of the Arcata Fish and Wildlife Office in Arcata, California. Otoliths were extracted, cleaned of membranous tissue, and allowed to dry before length (nearest 0.1mm) and weight (nearest 0.00001g) were measured. Extracted otoliths were then embedded in a resin block that was lapped on both sides until the primordial region of the otolith core was exposed. This procedure allowed the pre-and post-hatch area incremental patterns to be viewed by light microscopy.

Prepared samples were visually examined for incremental patterns and “checkmarks” in the region between the core and edge of the otolith. A particular checkmark associated with hatching was noted. ‘Hatch checkmarks’ of salmonids usually appear in the form of a very dark band or structural discontinuity from previous increments. An ‘emergence checkmark’ occurs prior to a transitional area from broad or indistinct increments of the post-hatch alevin to the well-defined daily incremental banding of emergent fry (Volk et al. 1995). For the purpose of this study, a developmental check was defined as a region of increments located between a hatch check and an emergence check, including the two checks. The natal signature was defined as the increments located between the otolith core to the end of the developmental check region since increment deposition in this region occur during intragravel development.

Descriptions of visually examined incremental patterns of specific otolith regions were noted and recorded for each sample. Analyzed otolith regions included the following areas: (1) core to beginning of developmental check, (2) developmental check, and (3) end of developmental check to otolith edge. These areas are referred to as 'regions' for the purpose of this study. All samples were visually analyzed twice with a lapse of three weeks between analyses to avoid reader bias as the same reader conducted both analyses.

Quantitative analyses of otolith regions were completed using light microscopy, an image analysis software system, and a statistical software package. Linear distances of radii (core to specific checkmarks and to otolith edge), incremental widths, and distances between specific checks (hatch and emergence) were measured, recorded, and entered into a database.

Determination of significant difference between developmental check regions of the North and South forks of Salmon River was established using an independent *t*-test (Murphy and Willis 1996), as shown by the equation:

$$t = \left(\bar{y}_1 - \bar{y}_2\right)\sqrt{\frac{n_1 n_2}{n_1 + n_2}} \Big/ \sqrt{\frac{\left(n_1 - 1\right)s_1^{\,2} + \left(n_2 - 1\right)s_2^{\,2}}{n_1 + n_2 - 2}}$$

Where t = Test of significant difference between two sample means, $\bar{y}$ = sample means, s^2 = sample variances, and n = sample sizes.

Results

Collection of spring Chinook salmon fry at Idlewild Campground (North Fork) was completed on two separate dates because sampling on the first date of March 25, 2004, resulted in a total of five samples. A second visit to the campground on April 6 yielded 28 additional samples. Collection of fish near Blind Horse Creek on the South Fork of the Salmon River yielded 74 samples on March 5, 2004. Mean fork lengths of North Fork and South Fork samples were 36.3 and 35.8 mm, respectively (Appendix A).

Of the 107 samples collected (North Fork=33, South Fork=74), only 91 samples (North Fork=28, South Fork=63) were used in the visual and quantitative analysis. Sixteen 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.

Visual analysis of North Fork and South Fork samples resulted in a consistently identifiable developmental check pattern located in the same region of the otoliths. Both sample groups exhibited a developmental check pattern that began with a hatch check composed of two dark bands separated by one light band and ending with an emergence check composed of three dark bands separated by two light bands (Figure 2). These distinctive increment pattern segments of the developmental check were apparent for all samples, even as deposition of increments varied in color intensity (Figure 3). The only subtle difference observed was a color intensity change of a few increments (about 2-4) in the area directly preceding the hatch checkmark sequence on the North Fork samples (Figure 2). This small number of increments did not appear larger or smaller in width

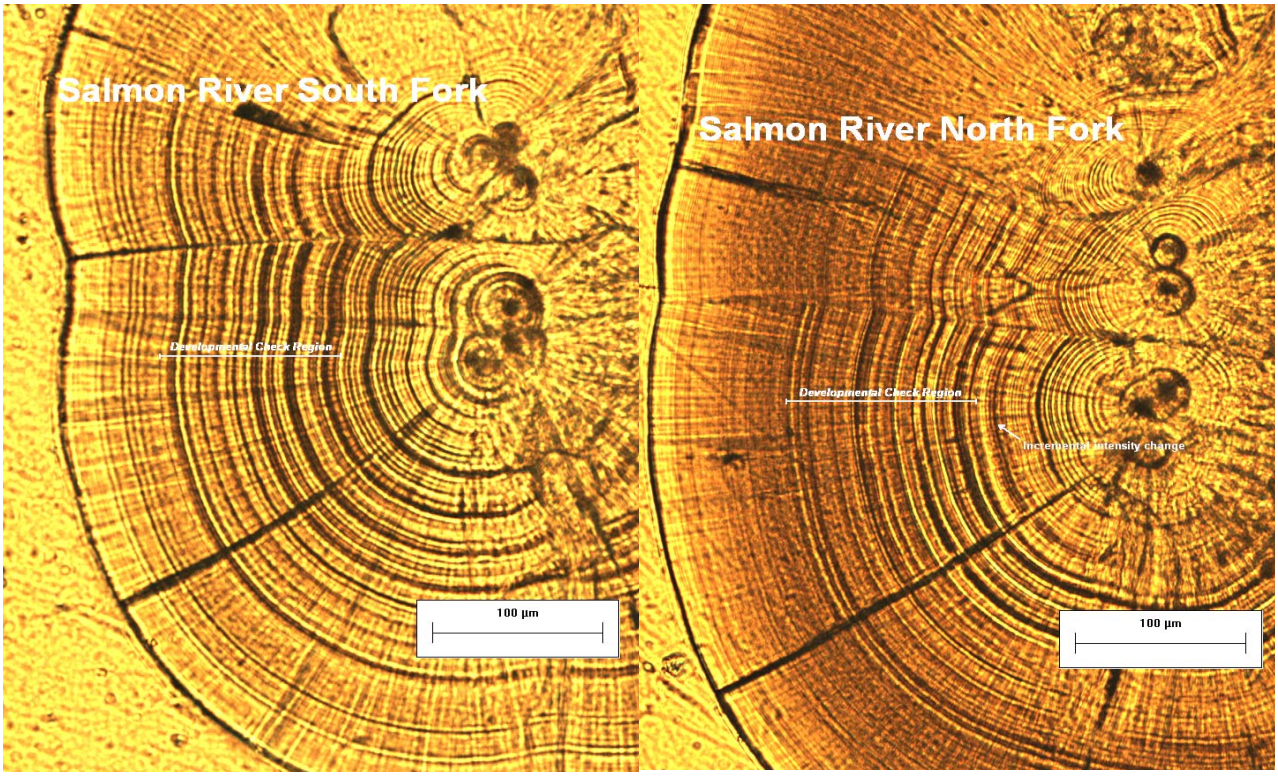

Figure 2. Photographs of representative otoliths from spring Chinook salmon fry collected from the North and South forks of the Salmon River, Klamath River Basin, northwestern California in 2004. Note the similar 'developmental check' regions.

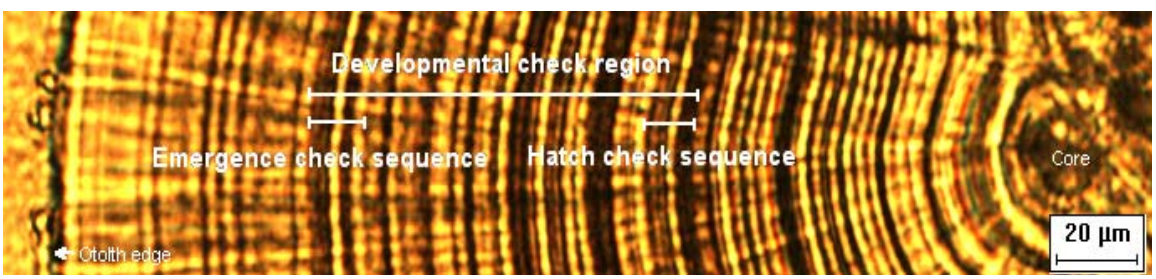

Figure 3. Developmental check sequence on an otolith extracted from a spring Chinook salmon fry collected in the Salmon River drainage in the Klamath River Basin, northwestern California in 2004.

from other increments prior to the change of intensity and did not yield a transitional appearance of increments beyond this point as would a hatch check.

Based on this subtle visual differentiation of the North Fork Salmon River samples and the South Fork Salmon River samples, a blind examination of 30 mixed samples was conducted. This test resulted in 26 samples or 86% correctly identified to associated collection site (Appendix B).

The developmental check width and linear distance from the core region did not differ significantly (p=0.7 and 0.1 at $\alpha = 0.05$, respectively) between the two sample groups (Table 2). Mean widths of the developmental check region for the North Fork Salmon

Table 2. Summary of a two sample t-test ($\alpha = 0.05$. df=89) comparing mean linear widths of specific otolith regions between spring Chinook salmon collected on the North and South forks of the Salmon River in 2004.

		Core to Developmental Check Region					Developmental Check Region					Post Developmental Check Region				
Site	n	Mean (µm)	SD (µm)	SE (µm)	t value	p value	Mean (µm)	SD (µm)	SE (µm)	t value	p value	Mean (µm)	SD (µm)	SE (µm)	t value	p value
North Fork	28	98.8	15.5	2.9			88.6	1.7	0.3			72.1	11.6	2.2		
					-1.6	0.1				-0.4	0.7				11.1	0.0
South Fork	63	103.2	10.4	1.3			88.7	1.4	0.2			44.0	11.0	1.4		

River samples (mean =88.6, s=1.7, n=28) and the South Fork Salmon River samples (mean =88.7, s=1.4, n=63) were similar (t=-0.4, df=89, p=0.7) (Table 2). Mean distance of developmental check from core area for North Fork Salmon River samples (mean =98.8, s=15.5, n=28) and the South Fork Salmon River samples (mean =103.2, s=10.4, n=63) were also similar (t=-1.6, df=89, p=0.1) (Appendix C). The developmental check region contained similar increment counts with a mean of 27 increments (s=1.9, n=28) for the North Fork Salmon River group and a mean of 28 increments (s=2.0, n=63) for the South Fork Salmon River group (t=-1.4, df=89, p=0.2) (Table 3) (Figure 4).

Mean width of post-developmental check area for North Fork Salmon River samples (mean=72.06, s=11.59, n=28) and South Fork Salmon River samples (mean=43.99, s=10.98, n=63) were found to be significantly different (t=11.06, df=89, p<0.001) (Table 2). This difference is a direct result of the number of increments within this region for each sample group. Spring Chinook salmon fry from the North Fork had an average of 18 increments post emergence, which differed significantly from South Fork samples that had an average of 29 increments for the same region (t=11.13, df=89, p<0.001) (Table 3).

Table 3. Summary of a two sample t-test ($\alpha = 0.05$. df=89) comparing mean increment counts of specific otolith regions between spring Chinook salmon collected on the North and South forks of the Salmon River in 2004.

		Core to Developmental Check Region					Developmental Check Region					Post Developmental Check Region				
Site	n	Mean	SD	SE	t value	p value	Mean	SD	SE	t value	p value	Mean	SD	SE	t value	p value
North Fork	28	15	3.5	0.7			27	1.9	0.4			29	4.4	0.8		
					-2.7	0.0				-1.4	0.2				11.1	0.0
South Fork	63	17	2.5	0.3			28	2.0	0.3			18	4.2	0.5		

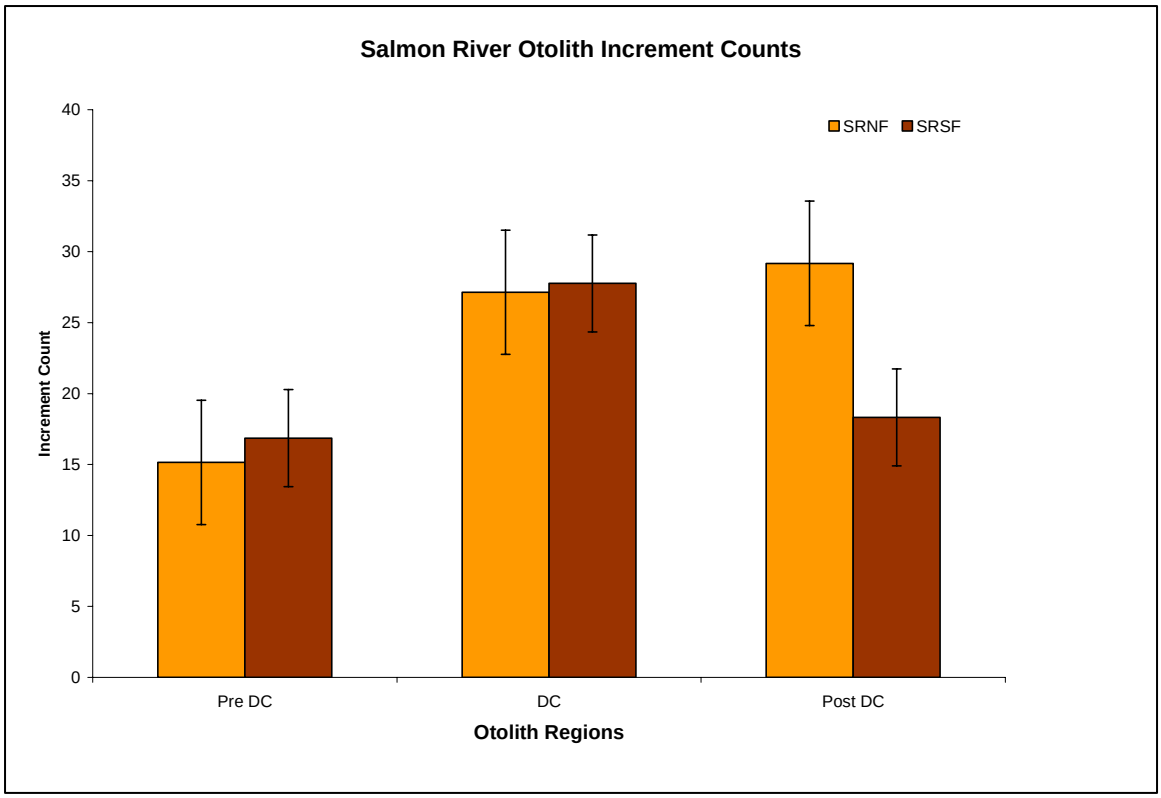

Figure 4. Mean incremental counts of otolith regions from spring Chinook salmon fry collected from North (SRNF) and South (SRSF) forks of the Salmon River in 2004. Vertical lines represent standard error at 95% confidence interval (DC = developmental check).

Discussion

Otoliths collected from Salmon River spring Chinook salmon fry exhibited a particular pattern of contrasting variation in incremental definition of the pre- and post-hatch regions regardless of sampling site. This particular increment pattern was consistently apparent even when sample quality was flawed, indicating similar incubational thermal regimes between the Salmon River sampling sites.

Visual examination of otoliths from the North and South forks of the Salmon River indicated that spring Chinook salmon fry incubated under similar thermal regimes. Both groups exhibited the same hatch check incremental sequence and the same emergence check incremental sequence with some variation of increment deposition between the two checks. The only visual difference between the two groups was that the North Fork samples had a few increments that appeared visibly darker prior to the developmental check region than the South Fork samples. Unfortunately, overwintering water temperature data were not available; only temperatures at the time of the collections were recorded by field crews. Water temperature at North Fork sample site was 8°C on April 6, 2004, and 9°C at the South Fork sample site on March 5, 2004. Although a blind test on this subtle difference yielded an 86% correct pattern:site identification ratio, the test was

time consuming, even when conducted by a proficient reader and should be performed with care. If time and/or reader experience is a concern, genetic analyses may provide a more efficient means to assess stock origin, assuming spring Chinook salmon in the North and South forks of the Salmon River differ genetically.

Comparative visual analysis of the Salmon River developmental checkmark was distinct from microstructural patterns associated with Chinook salmon from the Iron Gate hatchery, Trinity River hatchery, and samples collected from the upper Trinity River mainstem (Figures 5-7). This visual difference suggests that the developmental thermal regimes varied between collection sites.

Linear measurements across the developmental check pattern and distance of the developmental check region from the core area did not differ between the North and South forks of the Salmon River. However, there was a significant difference ($p<0.001$) in the width of the post-emergence region of otoliths between the two streams. The mean width of the post-emergence region from otoliths collected on the North Fork was 72.06 µm compared to 43.99 µm for the South Fork samples. As expected, a larger area encompasses a greater number of increments. This is important as teleost fish otoliths deposit increments with a daily periodicity (Campana 1992). More increments beyond the emergence check constitutes more post-emergent days at liberty. The North Fork Salmon River group contained an average of 29 increments within this region, whereas the South Fork Salmon River group contained an average of 18 increments within this region (Figure4). Date of collection appears to support the theory of increment depositional rate

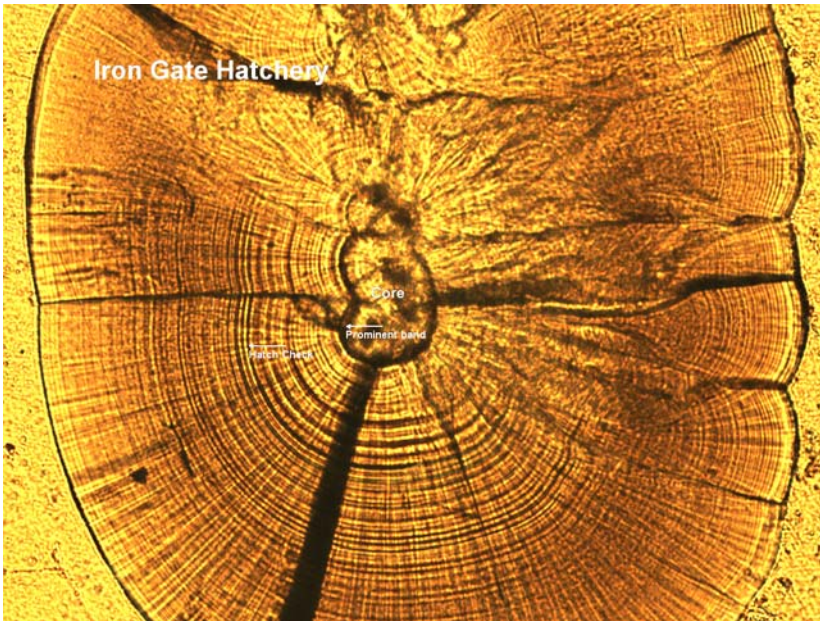

Figure 5. Photograph of otolith microstructural pattern representative of fall Chinook Salmon from Iron Gate Hatchery located in Siskiyou County, California. Note the prominent band surrounding the core region which differentiates this particular pattern from other Klamath Basin patterns sampled thus far.

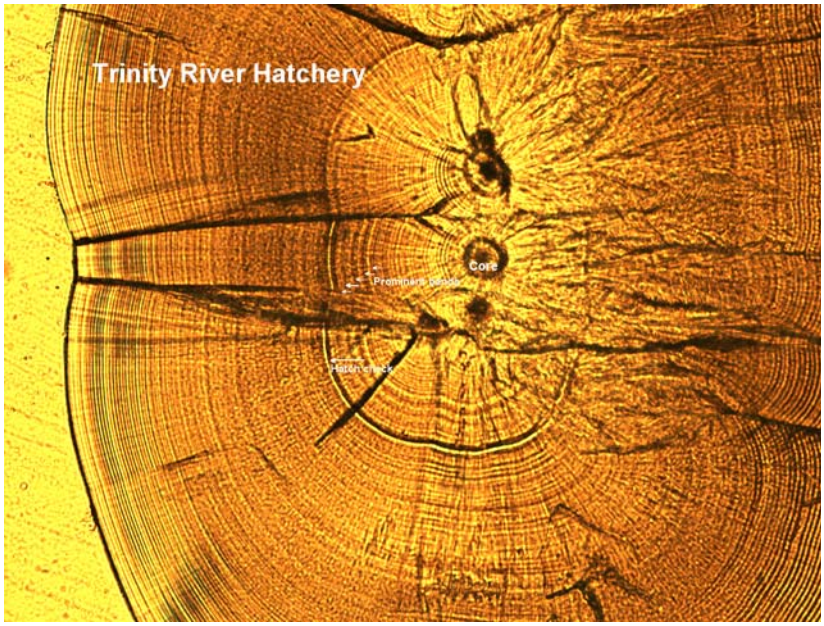

Figure 6. Photograph of otolith microstructural pattern representative of fall Chinook salmon from the Trinity River Hatchery located in Trinity County, California. Note the five prominent bands occurring prior to hatch check. This banding pattern differentiates this particular pattern from other Klamath Basin patterns sampled thus far.

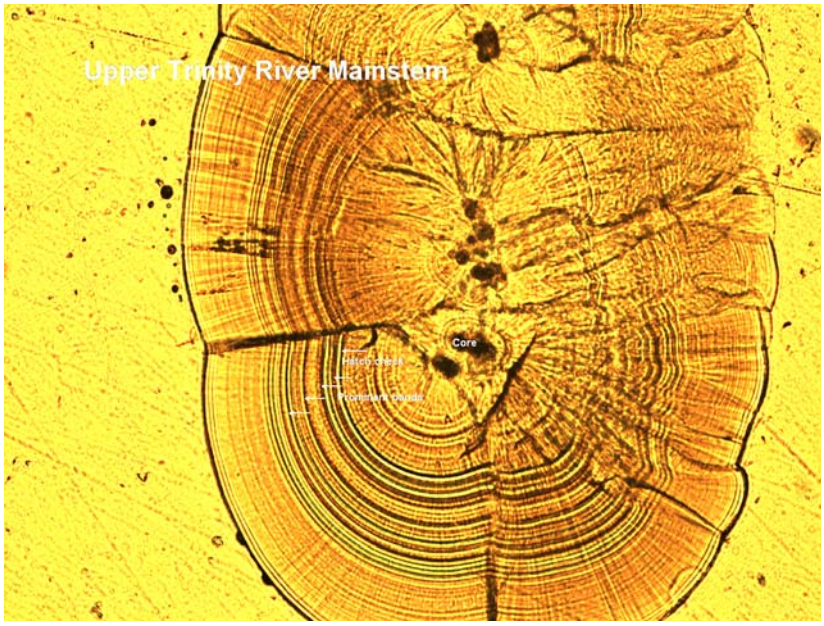

Figure 7. Photograph of an otolith microstructural pattern of a Chinook salmon sampled from upper Trinity River mainstem located in Trinity County, California. Note the four prominent bands post hatch check.

as the North Fork was sampled near the end of March 2004 and again in early April 2004. South Fork was sampled in early March 2004.

This study identified a distinct microstructural pattern in spring Chinook salmon spawned in 2003 in the North and South forks of the Salmon River that differed from patterns documented for other collections in the Klamath Basin. However, results of this study are limited due to small sample sizes, sample size inequality, and its reliance on a single brood year, stream, and race. Additional studies are needed to clarify the specific natal microstructural signature of spring Chinook salmon stocks in the Salmon River, including: (1) otolith study of spring Chinook salmon adults returning to the Salmon River, (2) continuing the otolith study of spring Chinook salmon fry in the Salmon River drainage with increased sample sizes and collection of overwintering water temperatures, (3) a comparative study between fall and spring Chinook salmon fry otoliths in the Salmon River drainage, and (4) a comparative study of spring Chinook salmon fry otolith microstructure from the Salmon River to microstructure of other river systems outside the Klamath Basin.

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The author would like to thank Nat Pennington and crew of the Salmon River Restoration Council; as well as Toz Soto and crew from the Karuk Tribe for their collection efforts on behalf of this study. The author would also like to acknowledge USFWS personnel Joe Polos, Nicholas Hetrick, Michael Cunanan, David Hines, and James Bettaso.for their assistance in preparation of this report. Funding for this study was provided by the Klamath River Basin Conservation Area Restoration Program.

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Appendices

Appendix A Summary of two sample t-test ($\alpha = 0.05$; df=89) used to compare mean fork lengths of spring Chinook salmon collected on the North and South forks of the Salmon River in spring 2004.

Site	n	Mean (mm)	SD (mm)	SE (mm)	t value	p value
North Fork Salmon River	28	36.3	1.8	0.4	1.0	0.3
South Fork Salmon River	63	35.8	2.0	0.3		

Appendix B. Summary of the developmental check blind test of spring Chinook salmon fry samples from the North (NF) and South(SF) forks of the Salmon River mixed. .

Sample #	NF	SF	Correct?	Comments
1		X	Yes	
2		X	Yes	
3		X	Yes	
4		X	Yes	
5	X		Yes	
6		X	Yes	
7	X		Yes	
8		X	No	Poor Preparation
9	X		Yes	
10	X		Yes	
11		X	No	Poor Preparation
12		X	Yes	
13	X		No	Broken slide
14		X	Yes	
15	X		Yes	
16		X	Yes	
17	X		Yes	
18	X		Yes	
19	X		Yes	
20	X		Yes	
21	X		Yes	
22		X	Yes	
23		X	Yes	
24		X	Yes	
25	X		Yes	
26	X		Yes	
27		X	Yes	
28		X	Yes	
29		X	No	Poor Preparation
30		X	Yes	