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ARTICLE

Correspondence between Scale Morphometrics and Scale and Otolith Chemistry for Interpreting Juvenile Salmon Life Histories

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Abstract

Fish scales have long been used to reconstruct fine-scale habitat transitions such as the movement of juvenile fish from freshwater, estuary, and ocean environments. Despite the importance of life history information to fisheries management and conservation, few studies have validated that scale morphology accurately describes fish movement between these habitats. Therefore, we tested the accuracy of using scale morphometric criteria to identify the movement of juvenile Chinook Salmon *Oncorhynchus tshawytscha* from freshwater to marine portions of the Columbia River estuary by comparing scale morphometric classification, scale chemistry, and otolith chemistry. Nearly one-half of all fish collected in the saline portion of the estuary and approximately one-quarter in the freshwater portion exhibited morphometric patterns (i.e., scale checks and intermediate growth) often associated with periods of estuary rearing. Depending upon the criteria used to define scale checks, otolith chemical results indicated that 33–53% of fish would have been misclassified as estuary residents based solely on their scale patterns. Moreover, many individuals who had resided in strontium-rich estuary water did not form a visible check (37%) on their scales to coincide with estuary entry. We estimated from otolith chemistry that these fish had either entered at or near the size at which scale formation occurs (35–42 mm) or had recently migrated to the saline portion of the estuary (<30 d) before new scale material could be formed and calcified. Scale chemistry alone was a good indicator of entrance into the saline portion of the estuary. Scale chemistry responded to the strontium-enriched salt water, and explained 86% of the variation found in otolith chemistry. Scale morphometric classification did not provide the fine-scale resolution that scale and, even more so, otolith chemistry provided for describing the proportion of juvenile Chinook salmon using the saline portion of the Columbia River estuary.

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For nearly a century, the morphometrics of scales have been used to reconstruct age, growth, and life history parameters of salmon (Dahl 1911; Gilbert 1913; Rich 1920; Shapovalov and Taft 1954; Koo 1962; Connor et al. 2005) and other fish species (Casselman 1983; Carlander 1987). Scales are commonly used because they have three key advantages over other methods for interpreting fish life histories: (1) scales can be collected without sacrificing individuals, an important consideration for studies of small or at-risk populations, (2) scales may be easily prepared and analyzed, and (3) many archival scale collections exist that could provide historical baselines for investigating life history changes in contemporary populations.

Reconstructing fish age and life history using scales relies on the assumption that scale circuli patterns reflect a fish's growth and corresponding rearing and migratory histories. Unfortunately, these scale patterns are seldom validated, and interpretations of life histories based on scale morphology are often subjective, raising questions about the accuracy and reproducibility of results. For example, scale characteristics have been used frequently to interpret the point of estuary and ocean entrance of anadromous salmonids (Rich 1920; Reimers 1973; Schluchter and Lichatowich 1977; Bottom et al. 2005; Burke 2005). Yet few study designs have incorporated independent life history measures to verify the scale interpretations. To test whether the estuarine life history of juvenile Chinook Salmon *Oncorhynchus tshawytscha* can be inferred from scale patterns, we compared scale morphometrics with independent microchemical analyses of scales and otoliths from fish collected within the Columbia River estuary.

Interpreting individual salmonid life histories from scale analysis often relies on changes in circuli spacing or other scale features, such as scale checks, that are believed to coincide with environmental transitions during juvenile salmon migrations. Generally, three types of salmon scale growth and circuli characteristics have been interpreted to reflect the fish's rearing environment: (1) narrow circuli spacing (freshwater growth), (2) intermediate circuli spacing (estuarine growth or reservoir or lake growth), and (3) wide circuli spacing (ocean growth) (Rich 1920; Reimers 1973; Schluchter and Lichatowich 1977; Connor et al. 2005). These interpretations rely on the direct positive relationship between fish size and scale size (Bilton and Robins 1971) and on the assumption that growth increases as salmon enter successively more productive feeding environments in the transition from river to estuary and from estuary to coastal ocean. The time of salmon entry into the estuary is often identified on scales as a region of changing circuli spacing marked by a distinct scale "check" designated by a few narrowly spaced circuli, followed by an increase in circuli spacing (Rich 1920; Bilton 1975).

A lag effect, the lack of validation for observed scale patterns, and the arbitrary criteria often used to identify scale features have led many biologists to question whether scale morphology is a trustworthy tool for reconstructing fish life histories or estimating age (Beamish and MacFarlane 1983; Carlander 1987). Scale checks (Bilton 1975; Boyce 1985) and circuli number and spacing have been produced experimentally with rapid increases and decreases in growth and temperature (Bilton 1975; Boyce 1985; Fisher and Pearcy 1990; Beakes et al. 2013) and are generally apparent after growth has resumed. In these laboratory studies, the authors describe a "lag" between experimental treatments and the addition of scale material and the formation of checks. They estimated that 1 week to a month could lapse before physical evidence of environmental conditions is recorded on the scale (Bilton 1975; Boyce 1985; Fisher and Pearcy 1990; Beakes et al. 2013).

In recent years, otolith microchemistry has provided a more precise method for reconstructing fish life histories (Kalish 1990; Secor et al. 1995; Campana 1999; Volk et al. 2000, 2010; Wells et al. 2003a; Miller et al. 2010) that does not require assumptions about changes in individual growth during environmental transitions or interpretations of scale morphometrics. The element strontium (Sr), which is generally found in low concentrations in freshwater and high concentrations in marine water, has provided a useful marker for designating diadromous fish migrations (Kalish 1990; Volk et al. 2000; Zimmerman 2005; Brown and Severin 2009). Because Sr has a similar valence and ionic radius as calcium (Ca), it can be substituted in the calcium carbonate lattice of the otolith to represent the relative Sr concentrations in the environment (Kalish 1990). Chemical profiles across anadromous fish otoliths are characterized by low strontium : calcium (Sr:Ca) ratios for periods of freshwater rearing and increased Sr:Ca ratios during periods of brackish- and marine-water residency (Kalish 1990; Zimmerman 2005; Brenkman et al. 2007).

The same geochemical record that is found in otoliths may also occur in scales, which would provide a potential method to validate traditional interpretations of scale patterns and analyze juvenile life histories without having to sacrifice individuals for their otoliths (Wells et al. 2000a; Courtemanche et al. 2005). Scale chemistry has reportedly been used successfully to distinguish between resident and anadromous Brown Trout *Salmo trutta* (Bagenal et al. 1973), Brook Trout *Salvelinus fontinalis* (Courtemanche et al. 2005), and Striped Bass *Morone saxatilis* (Coutant and Chen 1993), and to examine geographic variations in scale chemistry for juvenile Weakfish *Cynoscion regalis* (Wells et al. 2000b) and resident Westslope Cutthroat Trout *O. clarkii lewisi* (Wells et al. 2003a). However, we are unaware of any studies that have compared scale and otolith chemistry with scale morphology to validate life history patterns derived from traditional scale-pattern analyses.

Otolith chemical analyses offer an independent method to validate whether juvenile salmon life histories can be determined reliably from measurements of either scale morphology or scale chemistry. We compared results of otolith chemistry, scale chemistry, and scale morphology for reconstructing the juvenile life histories of Columbia River Chinook Salmon sampled within and upstream from the saline portion of the estuary. More specifically, we tested the hypothesis that scale

checks and intermediate growth are accurate indicators of estuarine entry by testing (1) whether scale checks from individual salmon were associated with significant transitions in scale Sr:Ca levels and (2) whether these scale features and chemical transitions corresponded to similar chemical changes in their otoliths.

METHODS

Study Area

The Columbia River headwaters begin in British Columbia, Montana, Wyoming, Idaho, Nevada, Utah, Washington, and Oregon, draining an area of 660,000 km^2 along a total network of 1,932 river kilometers (rkm) before entering the Pacific Ocean near Astoria, Oregon. Maximum saltwater intrusion in the deepest portions of the river channel reaches ~rkm 55, but tidal influence extends >230 rkm from the river mouth to the base of Bonneville Dam on the main stem. To validate whether juvenile Chinook Salmon life histories can be determined reliably from measurements of scale morphology, scale chemistry, or otolith chemistry. Samples were collected monthly at four saline-influenced estuarine sites: Point Adams boat launch (PAB), Clatsop Spit, Point Ellice, and West Sand Island, and one tidal freshwater site, Lower Elochoman Slough (LES) (Figure 1). Sample collection existed as part of a larger study of salmon habitat use in the lower Columbia River estuary (Roegner et al. 2012).

Sample Collection

Fish were sampled at the four saline and one tidal freshwater sites near low slack tide with a 50-m beach seine (1.27-cm mesh in the wings and 0.95-cm mesh in the bunt). As many as 10 samples from three size-classes (fry, 40–60 mm; subyearling, 61–100 mm; and large subyearling and yearling, >100 mm) were retained. However only subyearlings were included in this analysis to minimize the chance that an annulus was counted as a scale check. Paired otolith and scale samples were removed from individual fish in the laboratory, labeled, and stored until analysis.

We determined the frequency of scale checks and intermediate growth in the downstream migrant population from a random sample of fish collected from one tidal freshwater site (LES, $n = 119$) and one high-salinity site (PAB, $n = 108$) in 2003 (Figure 1). We also compared otolith and scale samples from a nonrandom collection of individuals from the saline and tidal freshwater portions of the estuary to evaluate whether morphometric and chemical techniques for analyzing scales and otoliths yield consistent life history interpretations. For the nonrandom samples, we selected fish based on the presence or absence of scale checks in order to test chemically for the presence of elevated Sr levels. The saline samples ($n = 100$) came primarily from PAB, but during months of low sample size, we included individuals from other lower estuary seining sites (Clatsop Spit, Point Ellice, and West Sand Island). All individuals from the tidal freshwater portion of the estuary were collected at LES ($n = 30$). We removed scales and otoliths from frozen samples of subyearling Chinook Salmon collected during the Columbia River estuary study (Roegner et al. 2012). Scales were removed from an area a few scale rows above the lateral line between the anterior insertion of the anal fin and the posterior insertion of the dorsal fin. They were placed between clear acetate, labeled, and later examined for scale checks and intermediate growth (IG) under 48× magnification using a Micron 780 microfiche reader.

FIGURE 1. The lower Columbia estuary, Washington–Oregon, indicating the freshwater site at Lower Elochoman Slough (LES) and marine–brackish water sites at Point Adams boat launch (PAB), Point Ellice (PE), Clatsop Spit (CS), and West Sand Island (WSI) (courtesy of Jen Burke, University of Washington).

Sagittal otoliths were removed, cleaned, and stored in ethanol until preparation.

Sample Analysis

To determine whether Sr levels in juvenile Chinook Salmon scales can be used to indicate time of estuary entrance, we analyzed otolith and scale chemistry from the same individuals. All microchemical analyses were conducted at the Keck Collaboratory for Plasma Mass Spectrometry at Oregon State University, Corvallis. The analysis system consisted of New Wave DUV 193-nm ArF laser coupled with a Thermal Elemental PQ Excell quadropole inductively coupled plasma mass spectrometer (LA-ICP-MS). Helium was used as the carrier gas to transport the ablated material from the laser to the mass spectrometer. Operating conditions for the LA-ICP-MS for both scales and otoliths are described in Table 1 (Campana et al. 1997).

Scales.—We distinguished IG (wider circuli spacing after check formation than before) and scale checks using criteria similar to those of Rich (1920), Reimers (1973), and Bilton (1975). Where checks were identified based on the presence of interrupted circuli formation, crossing over of circuli, and/or the appearance of scale resorption, we classified each scale into one of four types: (1) presence of IG on the scale margin or IG plus a check, (2) a clear check or check with additional growth but no IG, (3) presence of narrow circuli but no clear check or IG, and (4) no check or IG (Figure 2). Scales were carefully removed from the acetate under 10–40× magnification (Leica dissecting scope) and transferred to a petrographic slide prepared with mounting tape. We mounted approximately 4–10 scales per sample with both the distal (osseous, circuli) and proximal (fibrillary, noncirculi) sides facing up. To analyze scale chemistry, we traced a laser transect across each scale from the nucleus to just beyond the scale edge, approximately 20° off the midline, in the anterior plane whenever possible (Figure 3). Of the 4–10 scales mounted from each fish sample, we analyzed both distal and proximal surfaces. However, because no noticeable difference could be

TABLE 1. Operating conditions for the LA-ICP-MS used to analyze otolith chemistry of juvenile Chinook Salmon.

Operating condition	Value	
General conditions		
Cooling gas (L/min)	13.00	
Auxiliary gas (L/min)	0.95	
Helium carrier gas (L/min)	0.75	
Detector mode	Pulse counting and analog	
Sweep mode	Peak hopping	
Dwell time (ms)	10	
Points per peak	1	
Analysis interval (s)	360	
Specific conditions		
	Scales	**Otoliths**
Laser ablation diameter (μm)	40	30
scanning speed (μm/s)	5	5
Pulse rate (Hz)	5	8

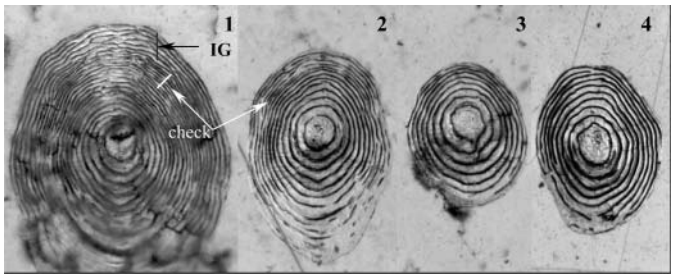

FIGURE 2. Morphometric classification of scales from juvenile Chinook Salmon. From left to right: (1) presence of intermediate growth (IG) on the scale margin or IG plus a check, (2) a clear check or check with additional growth but no IG, (3) presence of narrow circuli but no clear check or IG, and (4) no check or IG. All images captured at 40× magnification. Scales 1, 2, 3, and 4 were collected in October, July, May, and April, respectively.

FIGURE 3. Juvenile Chinook Salmon type 2 scale from the lower Columbia River estuary showing laser scar (transect). Image captured at 100× magnification.

detected and the proximal side was difficult to view, we primarily analyzed the distal (circuli) surface. In most cases, one proximal transect and two or three distal transects were analyzed from each sample.

Scale chemical (SC) profiles were grouped into three categories: (1) SC type 1, with a Sr inflection point (indicating the deposition of scale material in both freshwater and saline water); (2) SC type 2, no scale Sr inflection but an average scale Sr:Ca atomic ratio >0.001 (indicating a transition between freshwater and saline water had occurred, but no clear point of Sr inflection could be identified); (3) SC type 3, no Sr inflection and Sr:Ca atomic ratios < 0.001 (presumably a freshwater signal, since all samples collected at the freshwater site had this pattern). The SC type 2 fish were further split into two groups (<30 d and >30 d residency in saline water) post hoc based on the estimated time taken for new scale material to form and calcify.

Otoliths.—Sagittal otoliths were prepared for chemical analysis by thin sectioning in the sagittal plane. Petrographic slides were heated on a hot plate at approximately 275°C with Crystal Bond 509 added as a bonding medium for the otolith. Otoliths were mounted sulcus side up and ground on a Buehler Ecomet 3 grinder with 1200-grit, silicon carbide paper until the primordia was nearly exposed without sacrificing otolith edge integrity. A fine polish was applied using a 1-μm alumina slurry. The half section was then warmed to melt the Crystal Bond and turned distal side up. We repeated grinding until the primordium was exposed or until there was risk of obliterating the daily growth increments (DGI) in the dorsal posterior region. Again, a fine polish was added using 1-μm alumina paste. Otolith thin sections were rinsed in deionized water and air dried (methodology modified from Volk et al. 2000 and Zimmerman and Reeves 2002). A transect was drawn for laser ablation from the posterior visible primoridium to beyond the otolith edge in the dorsal–posterior quadrant (Figure 4). Daily growth increments were enumerated (Pannella 1971; Neilson and Geen 1982) from the otolith edge inwards along a line parallel to the LA-ICP-MS transect, using light microscopy. Otolith chemistry (OC) was grouped into one of three general patterns: (1) OC type 1, a pronounced rise in Sr:Ca values sustained for >30 d (indicating the deposition of otolith material in freshwater and residence in saline water for >30 d), (2) OC type 2, an intermediate to pronounced increase in Sr:Ca but residence in saline waters for <30 d (indicating the deposition

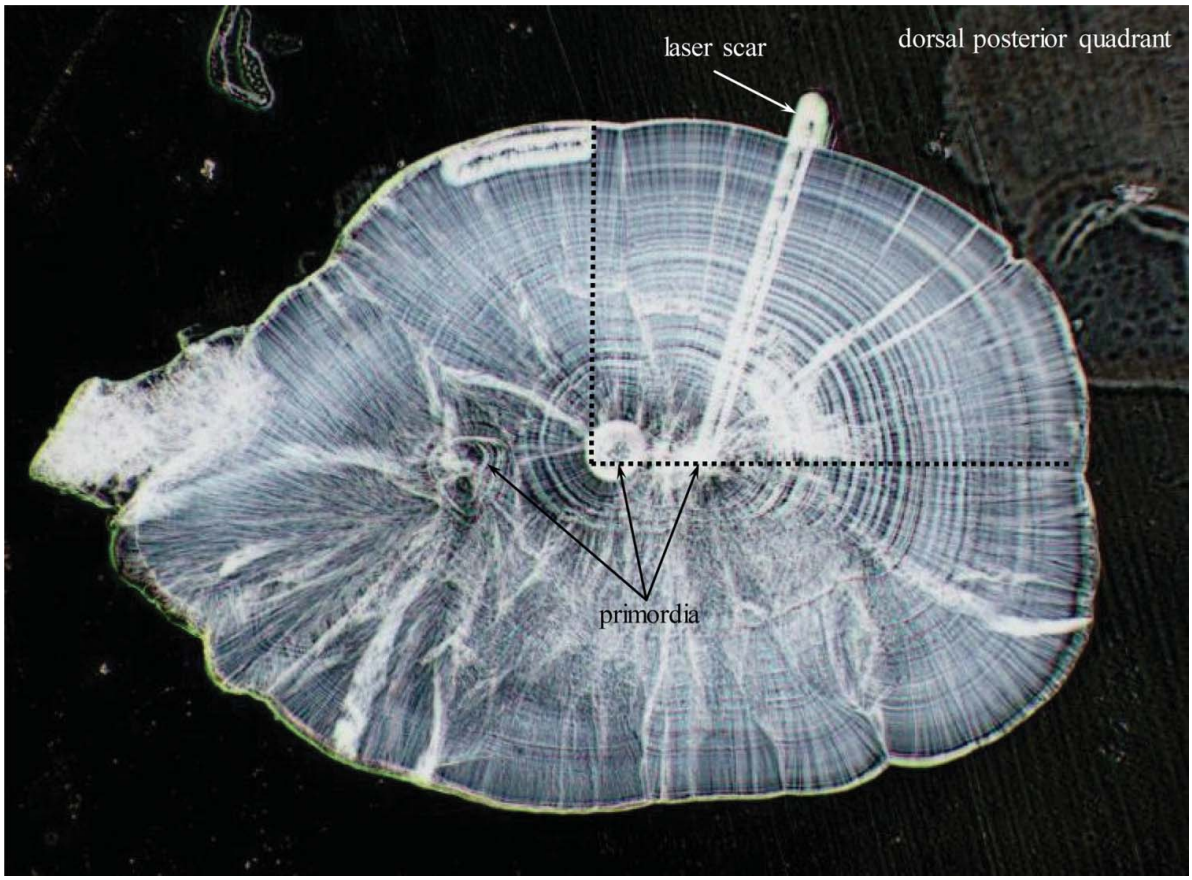

FIGURE 4. Juvenile Chinook Salmon otolith showing laser ablation scar from a primordium to the dorsal posterior edge of the otolith.

of otolith material in freshwater and residence in saline water for <30 d), (3) OC type 3, no appreciable Sr:Ca increase above assumed freshwater levels (indicating the otolith material was formed in a freshwater environment only) (see Figure 7 and Table 3).

Statistical Methods

Each otolith and scale laser transect was coupled with a transect along a polished NIST (610) glass standard with known concentrations of Sr86 and Ca43 for quantifying Sr:Ca atomic ratios. Raw counts of Sr and Ca were plotted, and transect start and end points and the point of Sr inflection were recorded. These points of interest on the chemical output were then related to actual location on the scale or otolith by accounting for laser speed (5 μm/s) (Brenkman et al. 2007; Campbell 2010; Volk et al. 2010).

Strontium inflection points were selected visually and the regions before and after the inflection point were tested for a difference using a two-sample *t*-test. An arcsine-square-root transformation was used to normalize Sr:Ca atomic ratios for one-way ANOVA with a Holm–Sidak multicomparison test. Probability value for significance was <0.05. Statistical analyses were undertaken using the SYSTAT 13 statistical package.

RESULTS

Scale Morphometrics

Morphometric analysis of Chinook Salmon scales suggested that many individuals collected near the river mouth had reared in the estuary before capture. Nearly one-half (49%) of 108 randomly selected individuals collected at PAB exhibited intermediate growth and scale checks compared with 24% of 119 samples collected at a single station (LES) in the tidal fresh zone (Table 2). As the season progressed, the scale-check frequency increased at both the upper and lower sampling sites. Check frequencies between April and June ranged from 15% to 33% at PAB and from 0% to 13% at LES. From July to October, the range in check frequencies at the two sites increased to 52–100% and 21–100%, respectively. By September, nearly all samples at both sites showed evidence of checks, intermediate growth, or both (Table 2).

Scale Chemistry

We selected nonrandomly 100 scale samples with and without checks at the brackish-water site (PAB) and another 30 from the freshwater site (LES) to test whether scales with and without checks had a corresponding Sr signal on their scales and otoliths (Table 3). About one-half of the scale samples we selected from

TABLE 2. Frequency of scale morphometric types: 1, presence of intermediate growth (IG) on the scale margin or IG plus a check; 2, a clear check or check with additional growth but no IG; 3, presence of narrow circuli but no clear check or IG; 4, no check or IG. Scale samples were from juvenile Chinook Salmon captured during beach seining in 2003 from a saline (PAB) and a freshwater (LES) site in the lower Columbia River.

		Scale morphometric classification					
Month	*n*	1	2	3	4	% With scale check	% Without scale check
				Saline site (PAB)			
April	22	5	1	2	14	27	73
May	13	2	0	1	10	15	85
June	21	2	5	6	8	33	67
July	23	8	4	4	7	52	48
August	10	3	4	1	2	70	30
September	10	5	5	0	0	100	0
October	9	7	2	0	0	100	0
Total *n*	108	32	21	14	41	49	51
				Freshwater site (LES)			
April	32	0	0	2	30	0	100
May	23	0	3	6	14	13	87
June	17	0	2	6	9	12	88
July	19	3	1	8	7	21	79
August	10	1	4	3	2	50	50
September	4	1	3	0	0	100	0
October	14	7	3	3	1	71	29
Total *n*	119	12	16	28	63	24	76

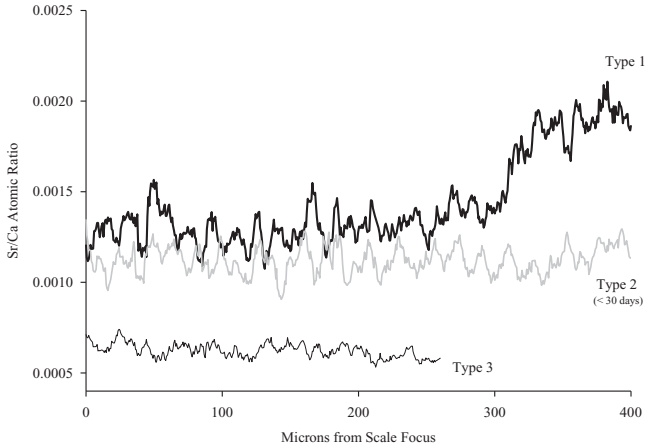

FIGURE 5. Scale Sr:Ca profiles for three juvenile Chinook Salmon individuals exhibiting scale chemistry (SC) types 1–3: SC type 1, Sr inflection; SC type 2, elevated Sr but no inflection; and SC type 3, assumed freshwater patterns.

TABLE 3. Sample sizes from juvenile Chinook Salmon for scale morphometric, scale chemistry, and otolith chemistry types by a saline (PAB) and a freshwater (LES) site in the lower Columbia River.

	Sample size				
Location	Total *n*	1	2	3	4
Scale morphometric type					
PAB	100	19	35	24	22
LES	30	2	12	9	7
Scale chemistry type					
PAB	100	34	32	34	
LES	30	0	0	30	
Otolith chemistry type					
PAB	100	43	31	26	
LES	30	0	0	30	

each site were classified as types 3 or 4 (no scale check). Approximately one-third were type 2 (a clear check), while the remainder showed evidence of checks and intermediate growth (type 1).

Chemical transects across selected Chinook Salmon scale samples ($n = 130$) exhibited a more variable pattern (Figure 5) than their corresponding otolith samples (see otolith chemistry section), most likely due to the uneven surface of the scale circuli as the laser passed through. Scale chemical profiles were grouped into three categories (SC types 1–3) (Figure 6). All scale samples collected at the tidal freshwater site had a SC type 3 pattern, whereas 34% and 32% of the scale samples collected near the river mouth (marine–brackish water) corresponded to SC types 1 and 2, respectively (Table 3). A one-way ANOVA found a statistically significant difference among the four scale chemistry types (including the two variants of SC type 2 as defined by residence times) ($F_{3,\ 122} = 148.5$, $P < 0.001$; Figure 6). To compare between SC types, we used an average value collected in the portion of the scale after the Sr inflection point to the scale edge (SC type 1) and an average value over the entire scale (SC types 2 and 3). All scale chemistry types differed significantly in scale Sr:Ca from one another (Holm–Sidak: $P < 0.001$), except SC types 1 and 2 (>30 d residency, $P = 0.305$) (Figure 6).

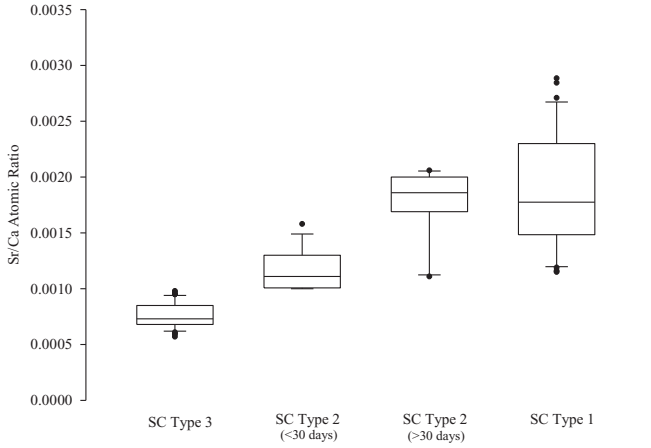

FIGURE 6. Scale Sr:Ca atomic ratios for each of the four juvenile Chinook Salmon scale chemistry (SC) patterns: SC type 3 (assumed freshwater), SC type 2 (<30 d estimated residence time), SC type 2 (>30 d estimated residence time), and SC type 1 (Sr inflection point). The box represents the 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, the solid line represents the median, and dots indicate outliers.

Otolith Chemistry

Chemical transects across selected Chinook Salmon otolith samples ($n = 130$) exhibited patterns similar to those described for other anadromous salmonids (Kalish 1990; Volk

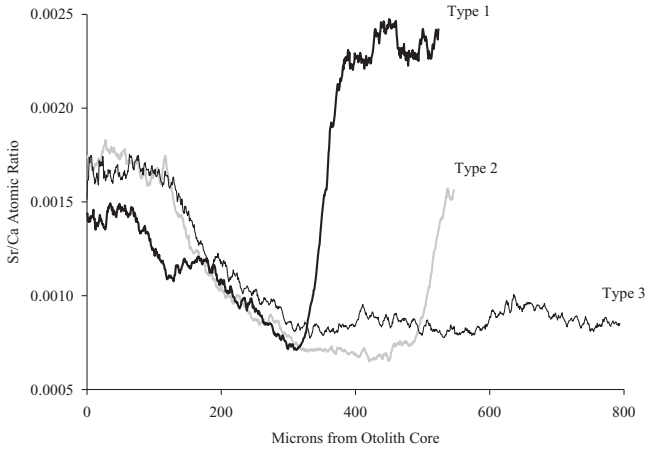

FIGURE 7. Otolith Sr:Ca profiles showing three juvenile Chinook Salmon individuals exhibiting the three otolith chemistry (OC) patterns: OC type 1, extended estuary residence; OC type 2, short estuary residence; and OC type 3, freshwater residence only.

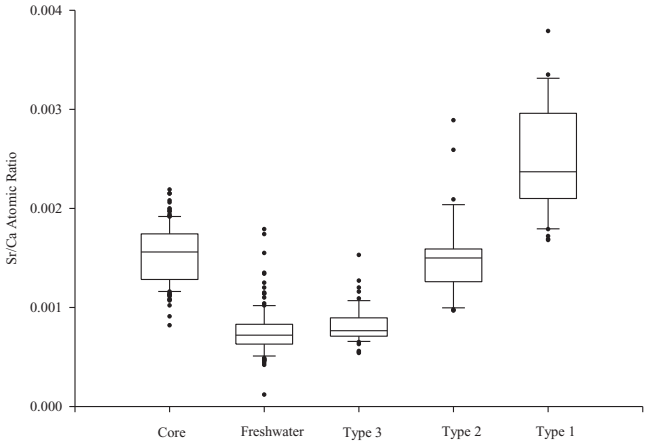

FIGURE 8. Otolith Sr:Ca atomic ratios obtained from juvenile Chinook Salmon for the core, assumed freshwater values, and the otolith edge corresponding to OC types 1 and 2 and near the otolith edge for OC type 3. See Figure 6 for a description of the box plot.

et al. 2000; Zimmerman and Reeves 2002). Generally, otolith Sr:Ca ratios were relatively unchanged from an elevated core signal to hatching, reflecting a maternal signal (i.e., Sr-rich marine water experienced by the mothers and transferred to developing ova). However, as fish came into contact with ambient water and used the remainder of their Sr-rich yolk, Sr levels decreased gradually to low levels corresponding to freshwater residence. Otolith Sr levels then rose again sharply, presumably after the individual entered Sr-rich salt water (Figure 7). We classified OC into one of three general patterns, OC types 1–3 (Figure 7; Table 3). All otolith samples collected at the freshwater site (LES) represented OC type 3 fish, while otolith samples collected at PAB or other sites near the river mouth (marine–brackish water) were 43% and 31% OC types 1 and 2, respectively (Table 3).

Otolith Sr:Ca ratios were averaged from the core (0.00154), an assumed freshwater zone (0.00075), from the region of elevated Sr in OC types 1 and 2 (0.00256 and 0.00152, respectively), and near the otolith edge in OC type 3 (0.00082) (Figure 8). A one-way ANOVA indicated a significant difference among the OC types ($F_{4,\ 385} = 274.8$, $P < 0.001$). A subsequent multiple comparison test found no statistical difference between the freshwater Sr:Ca region in all otolith samples and OC type 3 (Holm–Sidak: $P = 0.657$) (Figure 8). Conversely, all comparisons of OC type 3 (freshwater) to OC types 1 and 2 (elevated Sr) and between OC types 1 and 2 were significant (Holm–Sidak: $P < 0.001$) (Figure 8).

Chemical Validation of Scale Checks

Life history interpretations based on scale morphometric characteristics (checks, IG, or lack thereof) were validated by comparing the microchemistry of paired otoliths and scales from individual fish of the two major morphometric classifications, i.e. with (types 1 and 2) and without checks (types 3 and 4). Sixty-nine scale–otolith pairs from fish showing some form of check and/or IG on their scales (types 1 and 2) were examined, and of these, 47% ($n = 32$) had evidence of a Sr inflection on their scale (Table 4). Using the broadest classification of scale checks (types 1 and 2), we would have misclassified 53% of samples as estuary residents by scale morphology alone, when in fact there was no chemical evidence linking the scale check to a corresponding uptake of Sr. If we only used the most stringent classification (type 1) we would have accurately linked the scale check with an uptake of Sr 67% of the time. However, this would have ignored another 26 samples (nearly double the sample size of type 1) that had some form of Sr signal (either inflection or elevated Sr). Sixty-one pairs of fish showing no recognizable IG or check on their scales

TABLE 4. Comparison of scale morphometric types from juvenile Chinook Salmon (1, IG and scale check; 2, scale check with additional growth, not IG; 3, presence of narrow circuli at scale margin only; 4, no evidence of IG or scale check) with scale chemistry (1, Sr inflection present; 2, high Sr value, but no Sr inflection; 3, low Sr value, assumed freshwater levels) and otolith chemistry (1, high Sr value for prolonged period of time, >30 d; 2, elevated Sr values but for short duration, <30 d; 3, low Sr values, assumed freshwater levels).

	Scale morphometric type							
	1		2		3		4	
Chemistry type	*n*	%	*n*	%	*n*	%	*n*	%
Scale chemistry type								
1	14	67	18	38	1	3	1	4
2	4	19	8	17	11	33	10	36
3	3	14	22	46	21	64	17	61
Otolith chemistry type								
1	18	86	21	44	6	18	7	25
2	0	0	8	17	4	12	7	25
3	3	14	19	40	23	70	14	50
Total paired samples		21		48		33		28

(types 3 and 4) were examined, and of these, 97% ($n = 59$) were found to have no evidence of Sr inflection (Table 4). Of the 61 scale samples that were classified as having no checks or IG, 21 (37%) had Sr levels elevated above typical freshwater levels, but no Sr inflection. In many of the scale samples without a noticeable rise in scale Sr (inflection), but with high average Sr, the otolith-back-calculated size at estuary entrance occurred before or near the time of scale formation (i.e., at 35–40 mm FL).

These scale chemistry and morphometric results indicate that fish entering Sr-rich estuary water do not necessarily lay down scale checks that correspond to estuary entrance. Furthermore, many fish that were residing in the estuary showed no visual signs, in the form of scale checks, that they had entered the estuary.

Comparison of Scale and Otolith Chemistry

Otolith and scale edge chemistry showed a strong positive relationship, explaining over 91% of the variation (Figure 9). All fish with a Sr inflection point on their scale chemical profile also had an inflection point on their otolith chemical profile. Only four fish had elevated otolith Sr (OC types 1 and 2) but not elevated scale Sr. Scale Sr inflection points (SC type 1) were not found on samples from fish that resided in the marine–brackish water portion of the estuary for <30 d. Of the fish that had Sr inflections in their otolith profile but not in their scale profile, all were either present in the estuary for <30 d or had entered the estuary between 35 and 40 mm in size (i.e., at or before scale formation). When samples with scale Sr:Ca inflection points were excluded from analysis and only specimens without inflection points were examined (SC types 2 and 3; Figure 10), we still found a linear relationship between otolith edge chemistry and the average scale chemistry across the entire scale. We found that otolith edge chemistry explained 86% of the variation in the average scale chemistry for fish that did not have any Sr inflection in their scale chemical profile ($P < 0.001$; Figure 10). These results suggest that Sr is incorporated into the scale even before new scale material is deposited and calcified at the scale margin.

DISCUSSION

Scale checks and intermediate scale growth often have been used to identify individual anadromous salmon that have resided in estuaries and to calculate their growth and estuarine residency (Rich 1920; Reimers 1973; Schluchter and Lichatowich 1977; Bottom et al. 2005; Burke 2005). However, the classification of fish life histories from scale morphometrics has been criticized for its subjectivity (Beamish and

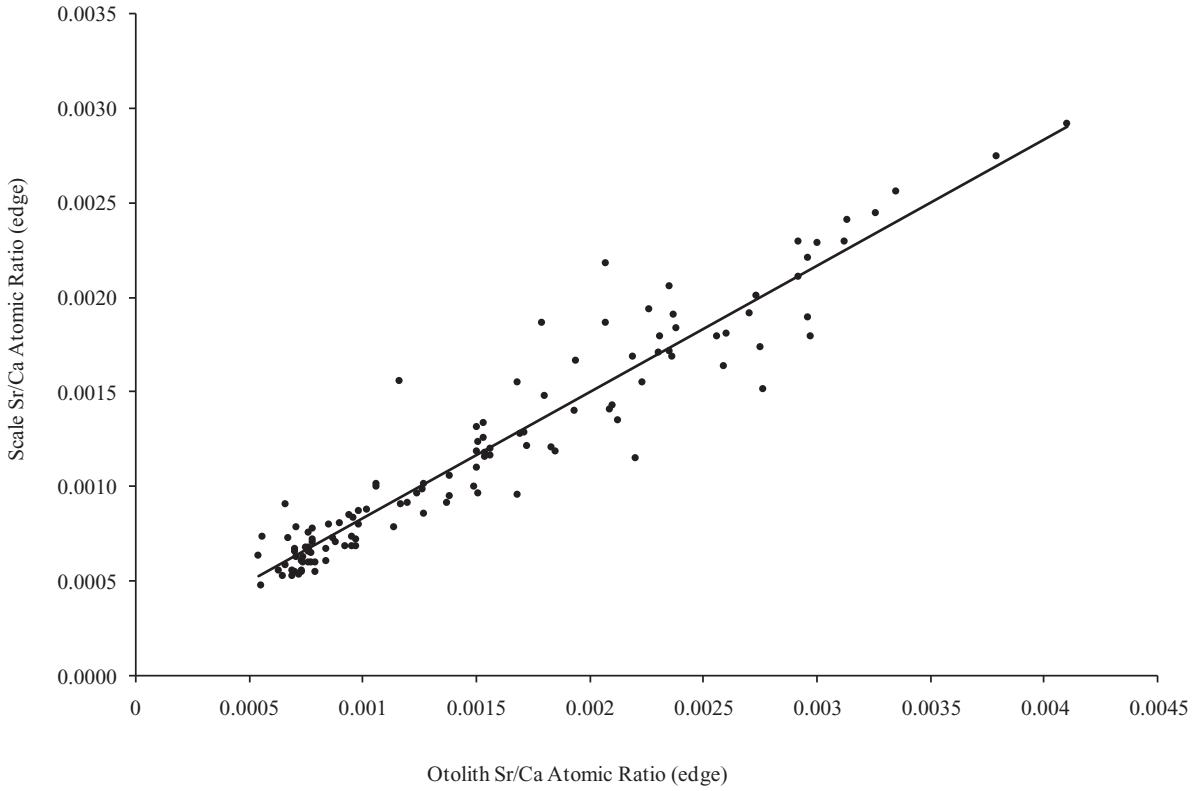

FIGURE 9. Linear relationship between otolith and scale Sr:Ca edge values from juvenile Chinook Salmon within the Columbia River estuary ($y = 0.6674x + 0.0002$, $r^2 = 0.917$, $P < 0.001$).

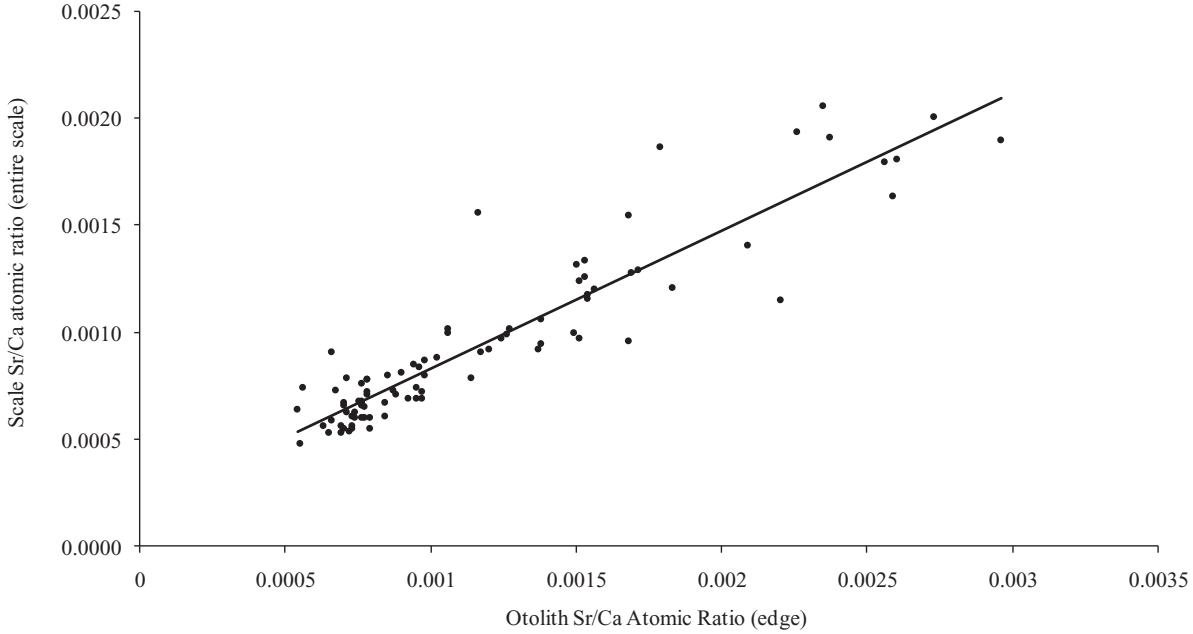

FIGURE 10. Comparison of average scale Sr:Ca with otolith edge Sr:Ca values for individuals classified as SC types 2 and 3 (i.e., no Sr:Ca inflection point in their scale chemical profile) ($y = 0.645x + 0.0002$, $r^2 = 0.855$, $P < 0.001$).

MacFarlane 1983; Chilton and Bilton 1986; Hankin et al. 2005), raising questions about the validity of life history interpretations and the accuracy of these results. Our comparison of scale features and scale and otolith chemistry for Columbia River Chinook Salmon reinforces the concern that scale patterns may not always provide an accurate method for interpreting the life history transitions of migrating fish. We found that scale chemistry, although less sensitive than otolith chemistry, could offer a viable alternative for some life history studies, particularly where lethal sampling of at-risk fish populations is not an option, or only archival scale collections are available to reconstruct historical life histories. Previous studies have linked the presence of scale checks and intermediate scale growth to fish entry into brackish water (Rich 1920; Reimers 1973), and these features also occurred most frequently among the Chinook Salmon we captured in the lower, saline portion of the Columbia River estuary. Nonetheless, we found no consistent correspondence between these features and indicators of estuary entry from scale or otolith chemical analyses. Scale checks underestimated the proportion of estuary residents among Columbia River Chinook Salmon. Otolith Sr:Ca ratios suggested that 37% of the fish with no evidence of a scale check had resided in the saline portion of the estuary for a period of time prior to capture. We conclude that these individuals either (1) entered the estuary at a small size when their scales were just forming (~35–42 mm) or (2) had recently entered the estuary (<30 d) before new scale material could be deposited and calcified.

When considering the factors that contribute to scale-check and circuli formation under experimental conditions, such as moderate to severe temperature and ration change (Bilton 1975; Boyce 1985; Fisher and Pearcy 1990; Beakes et al. 2013), it is likely that any condition that alters growth may produce scale checks in natural populations. For example, among some individual fish in our study, the Sr inflection within the scale coincided directly with the scale check and may have reflected increased growth related to increased estuarine food availability or water temperature changes. In other cases, fish with distinct scale checks showed no chemical evidence of estuary entry either on their scales or otoliths. Here again a variety of growth-altering factors (e.g., prey availability, temperature, smolting, metabolic stress) could have caused scale checks to form as individuals traveled downriver toward the brackish estuary. Of all the samples classified as having some form of scale check (types 1 and 2), less than 50% had evidence of an increase in Sr denoting a transition from a low Sr environment to a high Sr environment.

Our results do not rule out the possibility that scale checks and intermediate growth may be a viable technique for studying salmon populations in other ecosystems. For example, in Sixes River, Oregon, comprehensive sampling throughout the basin and mark–recapture studies verified that scale characteristics and growth transitions during downstream migration were closely linked and that scale checks accurately depicted estuary entrance (Reimers 1973). However, the Sixes River estuary is quite small and tidal influence is limited compared with the Columbia River estuary, where tidal freshwater extends more than 230 km upriver from the mouth. Our findings suggest that scale morphometrics must be applied cautiously because the factors that influence fish growth and scale

formation are likely to vary in each ecosystem. The environmental factors or gradients contributing to growth transitions and scale features therefore must be validated for each population before individual life histories can be inferred from the circuli patterns on fish scales.

Scale chemistry was a good indicator of entry into the saline portion of the lower Columbia River. The majority of fish that had elevated Sr in their otoliths also showed elevated Sr in their scales. We found that the type of Sr signal in scales (SC types 1 and 2) was related to the size of the fish at entry and residency within the estuary. High Sr levels were present in the scales of fish that had encountered salt water at a size near the time of scale formation (35–42 mm), even when the chemical transects showed no significant transition from a low to a high Sr level (the difference between SC types 1 and 2). Scale samples of fish with low saltwater residency (<30 d) showed Sr levels intermediate between SC type 2 (>30 d) and SC type 1 samples. We hypothesize that this intermediate signal could reflect the permeable nature of the scale and the lag time between the time of entry into a new high Sr environment and the time of deposition and calcification of new scale circuli.

The factors that affect Sr uptake and persistence in fish scales are not well known. Unlike otoliths, which are isolated within a semipermeable membrane (Lagler et al. 1962; Campana 1999), scales are in direct contact with water and may be resorbed during periods of starvation or stress, eroding the scale edge and circuli (Wallin 1957; Bilton 1975). Although several studies have examined the stability of trace elements in otoliths (Kalish 1990; Campana 1999), much less is known about scale chemistry and the stability of elements through time within the different regions of the scale. In juvenile Weakfish, Wells et al. (2003b) found that the correlation between scale and otolith Sr:Ca was weak and decreased from age-2 to age-1 fish and hypothesized that scale chemistry could be affected by the deposition and calcification of new scale material in areas other than the scale margin and/or could potentially be reworked given life events (i.e., spawning). Furthermore, in a study of Brook Trout, Courtemanche et al. (2006) concluded that the entire scale was contaminated with elevated Sr once a fish entered a high Sr environment. Consequently, they could distinguish anadromous from resident Brook Trout, but could not reconstruct finer details of juvenile life history.

We hypothesize that our ability to detect a transition from low to high Sr within the scale may depend on the time elapsed since the individual entered brackish water and on the age of the fish and size of its scales. If scale circuli are deposited sequentially in a three-dimensional space, then fine layers of calcified material may override preexisting circuli, and the previous chemical signal would be altered by the current chemical environment. Our results confirmed that total Sr:Ca levels were elevated in the scales of fish that had entered saline waters, even in portions of the scale that we believe were formed during freshwater residence. However, on some samples these regions were still discernable from even higher levels of Sr found near the scale margin. We conclude that this higher Sr region represents scale material that was deposited during fish residency in the saline portion of the estuary (SC type 1; Figure 5).

Otolith samples were a good indicator of entrance into brackish and marine waters. Samples collected at our freshwater site showed no evidence of elevated Sr, unlike the chemical pattern of the samples we collected at mixed marine and brackish water sites. These findings are consistent with laboratory (Kalish 1990; Fowler et al. 1995; Zimmerman and Neilson 2003) and field (Volk et al. 2010) experiments indicating an increase in otolith Sr with an increase in salinity. Overall, the otolith Sr:Ca values were greater than scale values, but the relationship between the two was strongly positive, a result consistent with other scale and otolith chemistry comparisons (Wells et al. 2000a).

Although scale and otolith chemistry were strongly correlated and scale chemistry was an accurate indicator of saltwater entry, scale morphometric characteristics did not coincide consistently with the proportion of individuals entering brackish water or their time of entry. These results raise concerns about traditional scale morphometric methods for reconstructing salmon life histories and reinforce the need to validate interpretations based on scale features. Scale chemistry was strongly and positively related to otolith chemistry and thereby provided an accurate indicator of saline estuary entrance. However, the process by which Sr is incorporated into the scale, as well as the stability of that chemical signal over time, is poorly understood. Further examination of the Sr signal on returning adult fish is needed to determine whether the signal is altered over time. While our results support the hypothesis that past scale material is altered by the fish's current environment, this may be due to the sampling method involved. In our case the laser sampling area (both spot size and sampling depth) may not allow for the precision necessary to accurately test this hypothesis. As spot size and sensitivity of instruments such as LA-ICP-MS improve it may be possible to test a specific region within a circulus (i.e., portion of the circulus forming earliest).

Considering the limitations of scale-pattern analysis and the uncertainties about scale chemistry, we conclude that otolith chemistry offers the most reliable indicator of saline estuary entrance by juvenile salmon. Otolith chemistry is also the most precise and sensitive life history indicator because otolith increments are added with daily periodicity, unlike our scale chemistry results, which support the hypothesis that there is a lag between environmental conditions and scale formation. However, the Sr signal only measures salmon entry into salt water. It does not account for the entire complex of ecosystem gradients—from tidal–fluvial zone to the nearshore ocean—that more broadly defines large-river estuaries (Fairbridge 1980; Day et al. 1989; McLusky and Elliott 2004; Simenstad et al. 2011). In small coastal rivers, where the transition from tidal fresh to marine water occurs over short distances, Sr is an

ideal indicator of salmon entry into the estuary (Volk et al. 2010). However, in large river-dominated ecosystems like the Columbia River, where tidal influence extends far upriver but salinity does not, otolith Sr can provide only a minimum estimate of estuary residency.

Further research should focus on the validation of specific chemical signals and growth patterns in calcified fish tissue that may represent habitat transitions. Natural biological and chemical tags such as the ones discussed in this study (scale checks, Sr as an indication of entrance to saline waters) allow for assessment of fish movement and behavior not biased by application conditions (e.g., minimum tagging size). The value in accurately associating residency and growth in specific habitat zones, such as stream of origin, fresh and saline portions of estuaries, and regions within ocean environments, is critical to understanding life history diversity and guiding habitat restoration efforts.

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