Transactions of the American Fisheries Society 145:493–501, 2016

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2016.1143400

ARTICLE

Evaluation of Back-Calculated Size and Timing Estimates for Juvenile Chinook Salmon Using Otolith Structure and Chemistry

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Abstract

Otolith chemistry is often used to reconstruct origin, life history, and migratory pathways in anadromous fishes. Although the accuracy and precision of back-calculated size at a particular life history transition (such as when anadromous fish move from fresh to saline water) is often not estimated. We evaluated back-calculated size and timing estimates based on otolith ratios of strontium to calcium (Sr:Ca) in Chinook Salmon *Oncorhynchus tshawytscha* marked with elevated concentrations of strontium chloride (SrCl). We used a combination of laser-ablation, inductively coupled, plasma mass spectrometry (LA-ICPMS), back-calculation models, and daily increments to evaluate the accuracy of such estimates. Overall, back-calculated size at marking was underestimated by <2 mm using direct and proportional back-calculations of FL based on otolith Sr:Ca. Proportional back-calculations of fish length were underestimated on average by 2.60 mm (SD, 2.09) when somatic growth (%/d) was less than otolith growth (%/d) and overestimated by an average of 4.89 mm (SD, 3.19) when somatic growth was greater than otolith growth. However, when somatic and otolith growth were equal, proportional back-calculations were more accurate than direct estimates. Overall, the number of otolith daily increments since Sr:Ca inflection underestimated the actual days since marking by a median of 1 d (SD, 0.57) and was similar for individuals sampled 8–79 d after marking. Results from this study suggest that life history parameters for Chinook Salmon estimated using LA-ICPMS, back-calculation models, and daily increments are robust estimates suitable for ecological field studies.

Estimating the size at and timing when anadromous fish move from freshwater to estuarine and marine environments is crucial to understanding migration, mortality, and life history expression during this critical transition period. For many species and populations of Pacific salmon *Oncorhynchus* spp., estimating these characteristics may have implications for habitat restoration (Jones et al. 2014) and early marine survival (Woodson et al. 2013). Juvenile outmigration patterns, such as size at and timing of entry into brackish or marine environments can be directly measured by capturing juveniles in the habitat of interest (i.e., tributary, estuary, ocean) or can be reconstructed for juveniles (Campbell 2010; Volk et al. 2010; Tomaro et al. 2012; Claiborne et al. 2014) and adults (Kennedy et al. 2002; Miller et al. 2010; Jones et al. 2014) using otolith microchemistry.

Otolith chemistry may vary depending on factors such as water chemistry, temperature, diet, and metabolic processes (reviewed by Campana 1999). Naturally varying strontium (Sr) is commonly used for reconstructing diadromous fish migrations (Secor 1992; Arai et al. 2005; Zimmerman et al. 2012). Low levels of otolith Sr are related to low levels of water Sr in freshwater habitats and high levels of otolith Sr correspond to high levels of water Sr found in estuarine and ocean environments (Kraus and Secor 2004; Miller et al. 2010). In freshwater and diadromous fishes, Sr is incorporated into the calcium carbonate ($CaCO_3$) matrix that makes up the majority of an otolith in approximate proportion to its abundance in water (Campana 1999; Bath et al. 2000; Kraus and Secor 2004; Brown and Severin 2009, Miller et al. 2010); thus variation in otolith Sr:Ca can be used to estimate the

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Received September 22, 2015; accepted December 29, 2015

approximate location on the otolith where a habitat transition occurred (such as when anadromous fish move from fresh to saline water). In addition, otoliths may also be artificially marked with Sr in order to identify origin or juvenile size when fish are recaptured later in life (Skov et al. 2001; Munro et al. 2008). For example, in freshwater fishes, where water Sr:Ca and subsequently otolith Sr:Ca is low, many juvenile fish may be marked simultaneously by immersion in water enriched with Sr. These samples are easily identified due to the incorporation of this enriched Sr into the otolith $CaCO_3$ matrix. For example, depending on the concentration of Sr in the immersion solution, Sr:Ca levels are typically an order of magnitude higher than in otolith regions before and after marking (Schroder et al. 1995, Lamperth et al. 2013, 2014).

Back-calculated estimates of fish size derived from levels (natural or artificial) of Sr in otoliths rely on an underlying relationship between fish length and otolith length (reviewed by Francis 1990) or size at an ontogenetic transition (Campana 1990). This introduces additional error beyond that related to the time it takes to incorporate Sr into the otoliths, which may be confounded by ambient water temperature and salinity (Campana 1999; Miller 2011). For example, fish with different growth rates may have different relationships between somatic and otolith growth (Panfili and Tomas 2001; Takasuka et al. 2008), which can result in back-calculation error (Campana 1990). In ecological field studies of Chinook Salmon *O. tshawytscha*, several population segments and life history strategies, as well as natural and hatchery production types, may be represented. As such, these groups may show differences between somatic and otolith growth (Zabel et al. 2010; Claiborne et al. 2014). To evaluate the accuracy of back-calculated estimates of size and timing using otolith Sr:Ca, the timing at exposure to increased water Sr must be known. This is most easily manipulated in a laboratory setting; however, to our knowledge few studies have directly evaluated the accuracy of back-calculated estimates.

The primary objective of this study was to validate otolith back-calculation methods commonly used to estimate size and timing of Chinook Salmon at entry into brackish and marine environments. We used a combination of SrCl immersion marking, laser-ablation, inductively coupled, plasma mass spectrometry (LA-ICPMS), otolith structure, and two back-calculation procedures (direct and proportional) to compare known size and timing at marking to back-calculated estimates. Finally, we evaluated the accuracy of back-calculated size and timing estimates in relation to otolith and somatic specific growth rates.

METHODS

Marking and rearing conditions.—Juvenile Chinook Salmon (brood year 2010) were marked and reared at the state of Washington's George Adams Salmon Hatchery on the Skokomish River, Washington. Known size at marking was achieved by selecting fish with a FL of 92 ± 1 mm (mean ± SD). Otoliths of fish were mass-marked by immersion in a 2,000-mg/L solution of SrCl. One hundred and thirty-six individuals were divided among three 19-L buckets containing 15.1 L of water and SrCl. A final group of 22 juvenile Chinook Salmon were placed in a single bucket with water but without SrCl (control). All buckets were placed in the same rearing trough with 8.6°C flowing ambient well water for 5 h. The SrCl-marked individuals were then transferred to four 0.10-m^3 cordoned treatment sections (n = 34 individuals/tank) and control fish were transferred to a fifth identical treatment section. All treatment sections were in the same rearing trough with continuously flowing water at a temperature of 8.6°C. All fish were fed protein pellets (Oregon Moist Pellet) routinely each day and fed to satiation for the duration of the experiment. Over the next ~2.5 months, seven fish per treatment section were removed at 1, 2, 4, 8, and 11 weeks (n ≈ 28 per sampling event) and given a lethal dose of tricaine methanesulfonate (MS 222) (Table 1). Individual fish were measured, weighed, and individually frozen.

Otolith preparation and analysis.—A random sample of 65 fish (11–15 per sampling event) was thawed, and otoliths were extracted, cleaned, and stored dry. The left otolith, when available (i.e.,when the right was not used), was mounted on a glass slide with thermoplastic resin. Otoliths were ground using successive grits of lapping film (30, 12, and 5 µm; 3M) and polished using an aluminum oxide slurry (1 µm; Buehler). We successfully prepared 56 otoliths for microchemical analysis and nine individuals were discarded due to poor preparation. To detect the point of SrCl marking we measured otolith Sr and Ca using a Thermo X series II LA-ICPMS coupled with a Photon Machines G2 193-nm excimer laser at the Keck Collaboratory for Plasma Mass Spectrometry at Oregon State University, Corvallis. Scans were completed from the most posterior primoridium to the otolith edge in the dorsal–posterior quadrant, ~25° off the midline (Campbell 2010) (Figure 1). The laser was set at a pulse rate of 8 Hz and traveled across the sample

TABLE 1. Days elapsed since marking (D), treatment (marked with SrCL or control), replicate (1–3), rearing tanks that fish were selected from (1–5), total number in each trial (n_{total}), and number of Chinook Salmon used to quantify otolith radius at marking using Sr:Ca ($n_{Sr:Ca}$).

D	Treatment	Treatment replicate	Rearing tank	n_{total}	$n_{Sr:Ca}$
8	SrCl	1–3	1–4	28	11
15	SrCl	1–3	1–4	35	9
15	Control		5	7	
36	SrCl	1–3	1–4	28	12
36	Control		5	7	
64	SrCl	1–3	1–4	28	11
81	SrCl	1–3	1–4	23	13
81	Control		5	8	

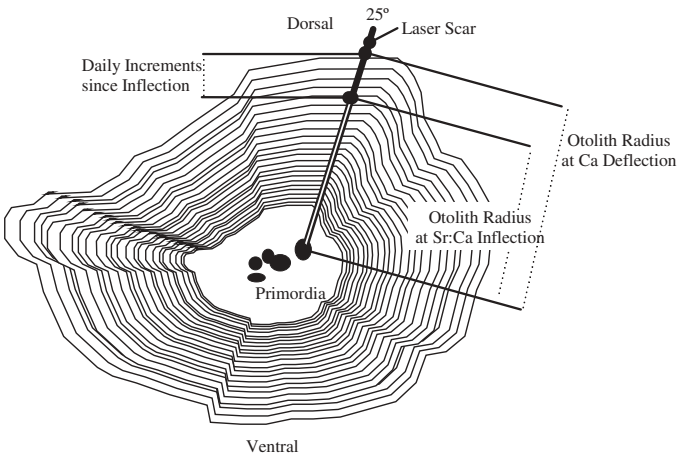

FIGURE 1. Schematic of a Chinook Salmon otolith showing the 25° laser transect from the most posterior primoridium to the otolith edge in the dorsal–posterior quadrant, ~25° off the midline. Also shown are otolith radius to Sr:Ca inflection, Ca deflection, and the daily increments since the point of inflection to the edge of the otolith.

at 5 µm/s with a spot size of 30 µm. Normalized ion ratios were converted to elemental concentrations using a glass standard from the National Institute of Standards and Technology (NIST 610) and finally converted to molar ratios for analysis. The NIST scans were run for every 10 samples to quantify instrument drift.

We determined otolith radius (OR) at the time of marking as the point of inflection in Sr:Ca (Figure 2) Total OR was determined as the point of deflection in Ca (Figure 2). Inflection and deflection points on each otolith were determined graphically as the point at which values of Sr:Ca and Ca continuously increased and decreased, respectively. After elemental analysis, digital images of each otolith were taken using a compound microscope (Leica DM 1000; 200× and 400×) with a mounted camera (Leica DC30). Using 400× images we counted the number and spacing of daily growth increments from the point of Sr:Ca inflection to the otolith edge unaware of the actual days since marking (Figure 1). Daily growth analysis was completed parallel to the laser scar in the dorsal–posterior quadrant (Figure 1).

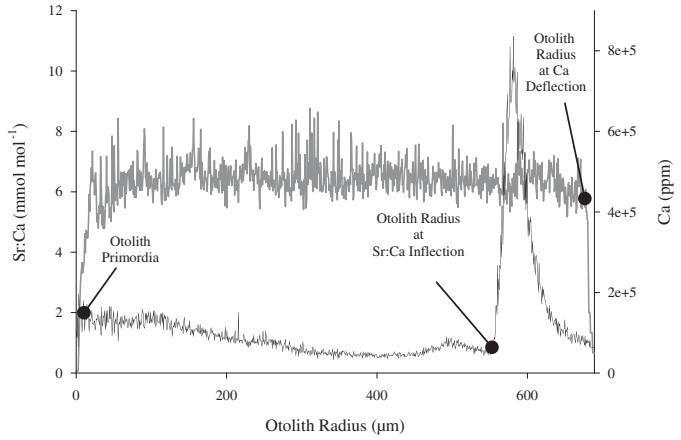

FIGURE 2. Sr:Ca (mmol/mol) and Ca (ppm) from the primordia to the otolith edge for a Chinook Salmon marked using SrCl in this study. Otolith size at marking was determined as otolith radius (µm) at the inflection in Sr:Ca. Similarly, total otolith radius was determined as the radius at Ca deflection.

Fish size at marking and growth.—To estimate fish size from otolith Sr:Ca we first developed a relationship between FL and total OR from visual measurements using individuals in this study ($r^2 = 0.70$, $n = 46$, $P < 0.001$). We estimated FL at marking using a direct regression (Carlander 1981) and proportional method (Francis 1990). We chose the direct method because it is commonly used to back-calculate juvenile size at brackish and marine entry in returning adult salmon (Miller et al. 2010, Jones et al. 2014) and juveniles (Tomaro et al. 2012; Claiborne et al. 2014) and is resistant to changes in the relationship between otolith length and fish length that may occur throughout ontogeny. We chose the proportional procedure because it is most often used to estimate the size of juveniles (Jarrin and Miller 2013; Goertler et al. 2015) and accounts for variation in size among individuals but assumes a single relationship between otolith length and fish length. Direct and proportional back-calculations were made using equations (1) and (2), respectively (as follows). Finally, we estimated specific fish growth and otolith growth rate (%/d) (Ricker 1975) using equations (3) and (4), respectively.

$$\mathrm{FL_{ME}} = \mathrm{OR_{ME}} \times 0.14\ (\pm 0.01\ \mathrm{SE}) + 11.66\ (\pm 9.39\ \mathrm{SE}), \quad (1)$$

$$\mathrm{FL_{ME}} = \{[\mathrm{OR_{ME}} \times 0.14\ (\pm 0.01\ \mathrm{SE}) + 11.66\ (\pm 9.39\ \mathrm{SE})]/[\mathrm{OR_C} \times 0.14(\pm 0.01\ \mathrm{SE}) + 11.66(\pm 9.39\ \mathrm{SE})]\} \times \mathrm{FL_C}, \quad (2)$$

$$G_F(\%/\mathrm{d}) = [\ln(\mathrm{FL_C}) - \ln(\mathrm{FL_{ME}})/D] \times 100, \quad (3)$$

and

$$G_O(\%/\mathrm{d}) = \left[\ln(\mathrm{OR_C}) - \ln(\mathrm{OR_{ME}})/D\right] \times 100, \quad (4)$$

where $\mathrm{FL_{ME}}$ = estimated FL at marking, $\mathrm{FL_C}$ = the FL at conclusion of the trial, $\mathrm{OR_{ME}}$ = the OR at marking as determined from otolith Sr:Ca, $\mathrm{OR_C}$ = the OR at conclusion of the trial, D = the days elapsed since marking, G_F = specific fish growth rate, and G_O = specific otolith growth rate.

Our first hypothesis was that back-calculated size estimates based on inflection in Sr:Ca represent the observed size at marking. For both each back-calculation approach and fish we calculated the difference between FL back-calculated at marking ($\mathrm{FL_{ME}}$) and FL observed at marking ($\mathrm{FL_M}$). We compared the mean direct approach difference to a hypothetical mean equal to

zero using the Student's *t*-test. The proportional approach difference was not normally distributed (Shapiro–Wilk test: $P = 0.01$). Therefore, we compared the median difference to a hypothetical median equal to zero using the Wilcoxon signed-rank test. We also tested whether size estimates were biased by size at the end of each trial (i.e., days 8, 15, 36, 64, and 81) using ANOVA and the Kruskal–Wallis test for direct and proportional differences, respectively. We examined whether there was a linear relationship between somatic growth and back-calculation error for both direct and proportional approaches.

Days since marking.—Our second hypothesis was that otolith daily increments and Sr:Ca can be used to determine the timing of SrCl marking. Otolith increments are formed daily in Chinook Salmon (Neilson and Geen 1982). Therefore, for each fish we estimated days since marking as the number of daily increments from inflection in Sr:Ca to the otolith edge (Figure 1). For each fish we calculated the difference between daily increments (D_{ME}) and the known days since marking (D_M). Differences between daily increments and known days were not normally distributed (Shapiro–Wilk test: $P = 0.01$). Thus, we compared the median difference to a hypothetical median equal to zero using the Wilcoxon signed-rank test. We tested for a median difference among fish reared for 8, 15, 36, 64, and 81 d postmarking using the Kruskal–Wallis test and compared variation in differences for ≤15 d and ≥36 d postmarking using the *F*-test for equal variance. Finally, we used linear regression to evaluate whether there was an effect of somatic and otolith specific growth rate on the differences between daily increments and known days since marking. For all statistical tests the alpha level was set to 0.01.

RESULTS

Size Estimates

We successfully determined otolith radius at Sr:Ca inflection on all samples prepared for chemical analysis ($n = 56$). Mean FL of fish euthanized over the 81-d period ranged from 94 to 114 mm (Table 2). Neither the proportional or direct back-calculation approach was biased to greater error as a function of size at the end of the trial (ANOVA: $P = 0.40$; Kruskal–Wallis test: $P = 0.46$). Neither approach had significant differences in back-calculation error between trial durations (ANOVA: $P = 0.57$; Kruskal–Wallis test: $P = 0.03$).

Size at Sr:Ca inflection using the direct (91.22 ± 4.27 mm [mean ± SD]) and proportional (90.87 ± 3.99 mm) methods of back-calculation provided accurate estimates of the observed size at marking (92 ± 1 mm) (Table 2; Figure 3). There were minor differences between back-calculation approaches. The mean difference in back-calculated size and observed size was not statistically different from zero for either formula (Student's *t*-test: $P = 0.06$; Wilcoxon signed-rank test: $P = 0.03$) and the mean difference was small (0.35 mm) between approaches. Error in the proportional estimate was significantly and positively related to growth rate while error in the direct back-calculation estimate was not related to somatic growth (Table 3).

We found that when otolith growth rate was greater than fish growth rate, size was consistently underestimated using the proportional formula (Figure 4). Conversely, when otolith growth rate was less than fish growth rate, size was consistently overestimated using the proportional estimate (Figure 4). When otolith and fish growth rate were nearly equal, the back-calculation error using the proportional method was near zero (Figure 4). Over- and underestimation of length using the direct method occurred but was not consistently biased by differences in otolith and fish growth rate (Figure 4).

Timing at Marking

We were able to estimate the number of daily increments to the otolith edge in 34 of 56 individuals (Table 2). Otolith daily increments from Sr:Ca inflection to the otolith edge accurately described the timing of SrCl marking and underestimated the actual days since marking by a median of 1 ± 3.13 d (Table 2; Figure 5). The median difference between the number of daily

TABLE 2. Days elapsed since marking (D), treatment (SrCL marked or control), mean and SD of FL of Chinook Salmon at conclusion (FL_C) and at marking (FL_{ME}) estimated using Sr:Ca and using the direct or proportional method of back-calculation. Values are mean (SD). Also shown are growth rate (%/d) of fish length (G_F), otolith radius (G_O), and daily increments since inflection in Sr:Ca (D_{ME}). Sample sizes of individuals used in elemental (n_1) and structural analysis (n_2) are indicated.

D	Treatment	FL_C (mm)	Direct FL_{ME} (mm)	Proportional FL_{ME} (mm)	G_F (%/d)	G_O (%/d)	D_{ME}	n_1, n_2
8	SrCl	94 (1.8)	92 (4.2)	90 (1.5)	0.2 (0.2)	0.6 (0.3)	8 (1.4)	11, 8
15	SrCl	95 (2.8)	89 (4.0)	89 (2.9)	0.2 (0.2)	0.5 (0.2)	14 (0.84)	9, 5
36	SrCl	102 (4.4)	92 (5.1)	91 (4.2)	0.3 (0.1)	0.4 (0.1)	35 (3.6)	12, 9
64	SrCl	116 (5.6)	91 (4.0)	95 (4.4)	0.4 (0.1)	0.3 (0.1)	61 (5.8)	11, 9
81	SrCl	114 (6.7)	90 (3.8)	91 (3.9)	0.3 (0.1)	0.3 (0.1)	79 (2.9)	13, 7
15	Control	97 (1.2)			0.3 (0.1)			7
36	Control	101 (7.4)			0.3 (0.1)			7
81	Control	111 (10.0)			0.2 (0.1)			8

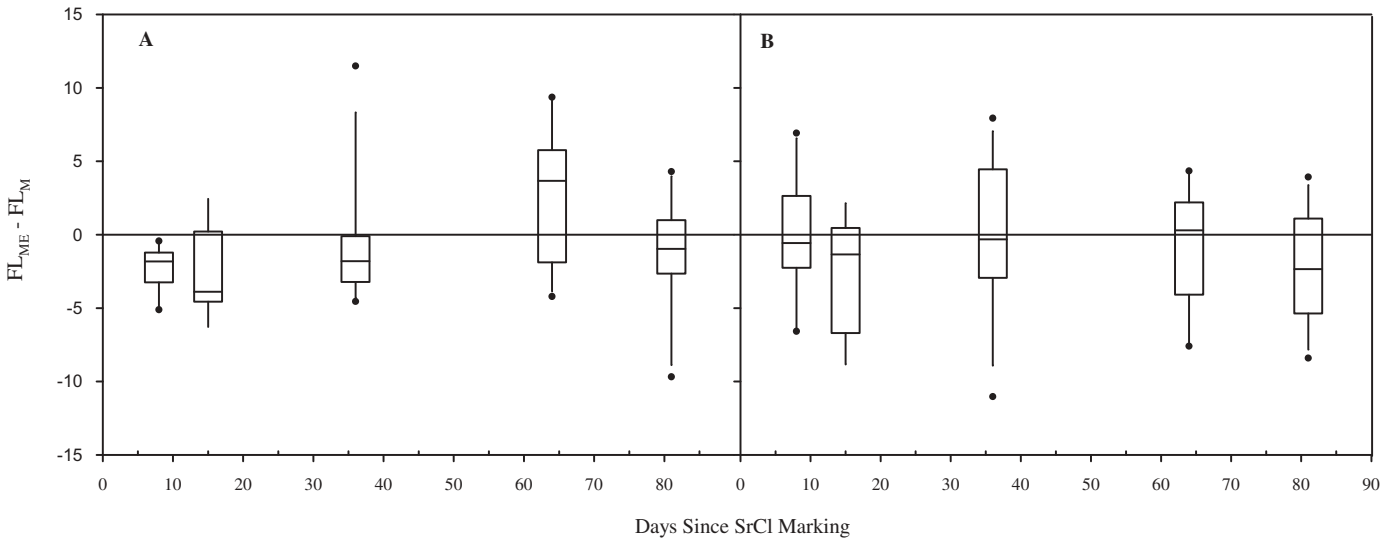

FIGURE 3. Difference between (**A**) the proportional and (**B**) direct back-calculated size using otolith Sr:Ca and known size at SrCl marking ($FL_{ME} - FL_M$) versus days since marked for Chinook Salmon used in this study. The boxes represents the 25th and 75th percentiles and whiskers represent the 10th and 90th percentiles; the solid horizontal line in the boxes represents the median and solid dots indicate outliers.

increments from Sr:Ca inflection to the otolith edge and actual days since SrCl marking was not significantly different from zero (Wilcoxon signed-rank test: $P = 0.08$). There was no evidence that median difference differed among individuals reared for 8, 15, 36, 64, and 81 d postmarking (Kruskal–Wallis test: $P = 0.35$; Figure 5). However, precision in daily increment counts was greater for fish that had been recently marked. Variation (mean SD) in the difference between estimated days since marking and actual days elapsed significantly increased from 1.62 to 5.06 after the second sampling event (16 d) (F-test: $P < 0.01$; Figure 5). There was no evidence that otolith (linear regression: $P = 0.04$) or somatic growth (linear regression: $P = 0.95$) was positively related to the difference between estimated days since marking and actual days elapsed (Table 3).

DISCUSSION

In this study we evaluated back-calculated size and timing estimates for juvenile Chinook Salmon, determined using otolith chemistry and structure. We showed that fish size at and time of exposure to water with increased Sr may be accurately back-calculated using LA-ICPMS, a relationship between fish and otolith length, and daily increment formation. Overall, mean size at marking was accurately back-calculated (<2 mm underestimation) in fish 8–81 d after marking, but differences between somatic and otolith growth rates for individual fish may result in error (±10 mm). Similarly, timing of marking was accurately reconstructed and results from this study suggest that the timing of brackish and marine entry can be accurately back-calculated in field studies between 8 and 81 d after fish have migrated into water with increased Sr.

TABLE 3. Simple linear regressions of the difference between known and back-calculated size at marking ($FL_{ME} - FL_M$) and somatic growth rate (G_F), for both proportional and direct estimates of size at marking. Relationships of somatic and otolith growth rate (G_O) versus the difference between the number of otolith daily increments from Sr:Ca inflection to otolith edge and known days since marking ($D_{ME} - D_M$) are shown. Standard errors for values in each equation are shown in parentheses. Also shown are the degrees of freedom (df), F-value, coefficient of determination (r^2), and the t-value and P-value for the Student's t-test that the slope is equal to zero. The P-value in bold text indicates significance at $\alpha = 0.01$.

Comparison	Regression equation	df	F	r^2	t	P
	Size at marking (mm)					
Proportional	$FL_{ME} - FL_M = G_F \times 12.82\ (\pm 3.08) \pm 4.28\ (\pm 0.97)$	54	17.21	0.24	4.149	**<0.01**
Direct	$FL_{ME} - FL_M = G_F \times -7.82\ (\pm 3.65) + 1.01\ (\pm 1.14)$	54	4.59	0.06	-2.14	0.04
	Growth rate (%/d)					
Somatic	$D_{ME} - D_M = G_F \times 0.22\ (\pm 3.98) - 0.88\ (\pm 1.20)$	31	<0.01	<0.01	0.06	0.95
Otolith	$D_{ME} - D_M = G_O \times 8.49\ (\pm 4.06) - 4.31\ (\pm 1.75)$	31	4.38	0.12	2.09	0.04

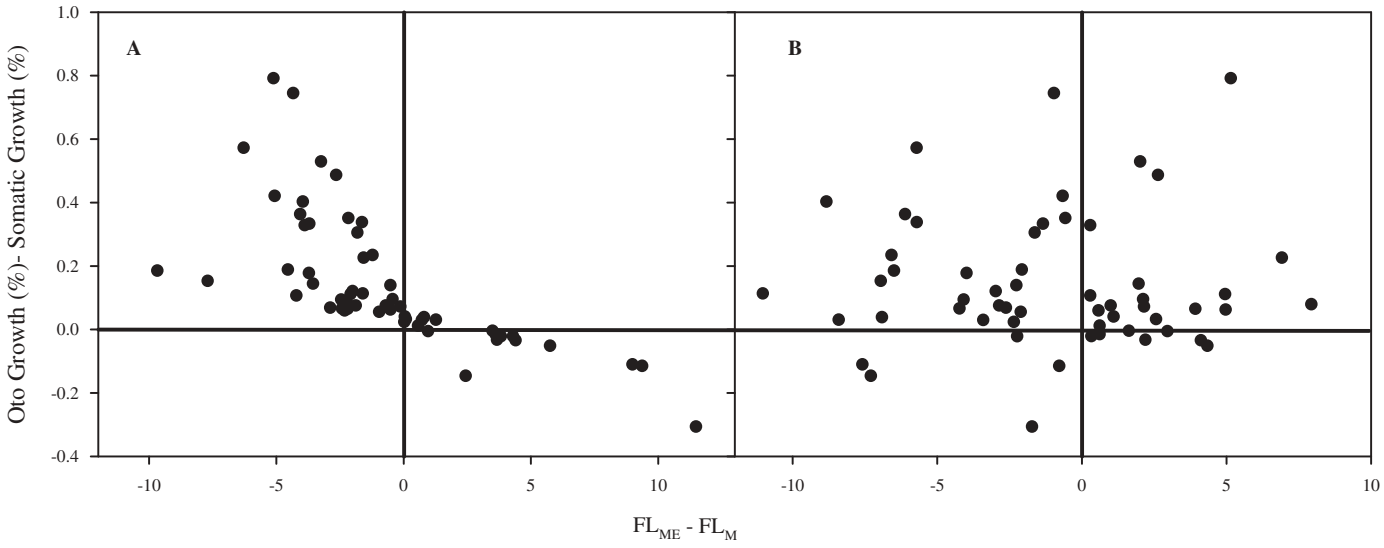

FIGURE 4. Relationships between the difference of the (**A**) proportional and (**B**) direct back-calculated size using Sr:Ca and known size at SrCl marking ($FL_{ME} - FL_M$) and difference between otolith and somatic growth rate (%/d) since marking for Chinook Salmon used in this study.

We identified two sources of potential error in back-calculating fish size from otolith chemistry: (1) effects on the fish size–otolith size relationship, and (2) factors affecting the uptake of the Sr signal into the otolith. The proportional back-calculation formula we used (Francis 1990) utilizes a linear relationship between fish and otolith size, accounts for variation in size, and assumes that fish growth and otolith growth are synchronous. For fish in this study, when this assumption was violated, the magnitude of back-calculation error was positively related to differences between somatic and otolith growth. For example, when otolith growth was greater than somatic growth, size was underestimated, and when otolith growth was less than somatic growth, size tended to be overestimated. Similarly, we observed a positive trend between back-calculation error and somatic growth such that slower- and faster-growing fish were under- and overestimated, respectively. The direct back-calculation formula we used (Carlander 1981) utilizes a linear relationship but does not

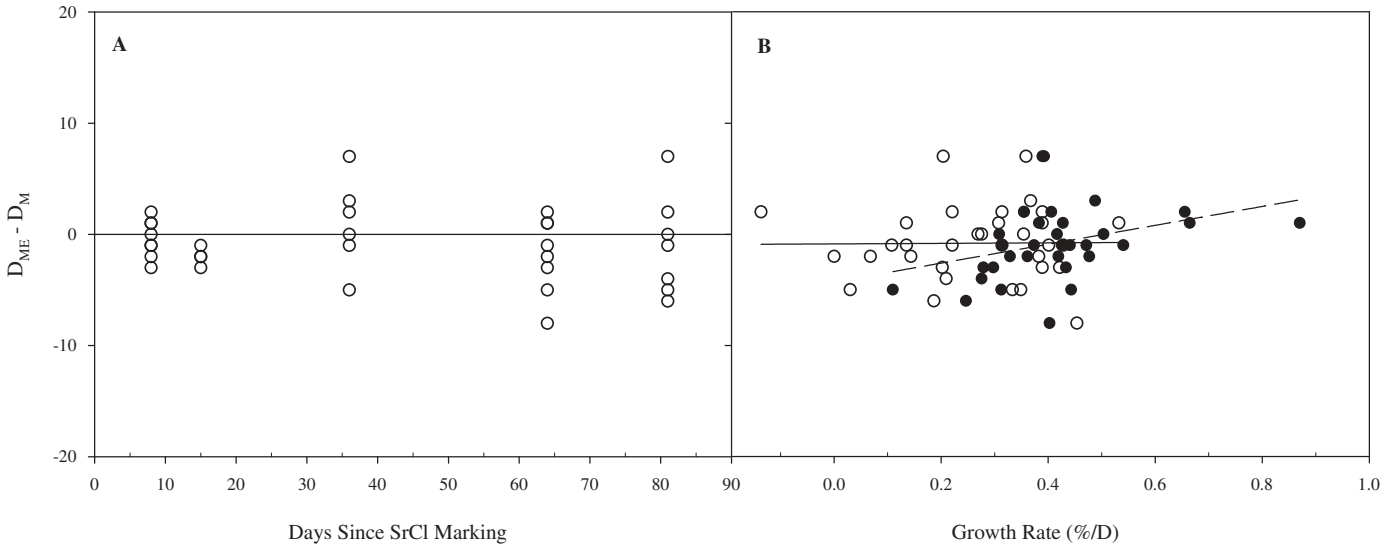

FIGURE 5. (**A**) The difference between the number of otolith daily increments from Sr:Ca inflection to otolith edge and known days since marking ($D_{ME} - D_M$), and (**B**) the relationship between $D_{ME} - D_M$ versus otolith (solid circles, dashed line) and somatic (open circles, solid line) growth rates for Chinook Salmon used in this study.

account for variation in fish size. As such, we observed over- and underestimation of length using the direct method, but it was not consistently biased by differences in otolith and fish growth rates. These results suggest that systematic back-calculation error and misinterpretation of results will occur in field studies if the assumptions and limitations of each back-calculation formula are not considered.

In this experiment we used a subset of fish to create a relationship between total otolith length and fish length and therefore it is not surprising that accuracy was high (<2 mm). However, differences in the relationship between otolith length and fish length may exist between population segments (Zabel et al. 2010), production types, and life history stages (Claiborne et al. 2014) and could lead to greater back-calculation error. For example, using a fish size–otolith size relationship from fall Chinook Salmon in the Salmon River, Oregon (E. Volk, Alaska Department of Fish and Game, unpublished data), the Coweeman River, Washington (Lamperth et al. 2014), and the Columbia River estuary (Campbell 2010), we found the direct approach underestimated the size at marking by an average of 8.9, 7.8, and 8.9 mm, respectively. The proportional method underestimated size at marking on average by 2.0, 3.6, and 3.0 mm, respectively, for those same populations. The consistent underestimation of fish length from this study is likely due to slower average growth rates (0.24 mm/d) compared with higher rates (~0.5 mm/d) reported in the Salmon (Volk et al. 2010) and Columbia (Campbell 2010) rivers. Evidence from this study suggests that error can be minimized for either back-calculation approach by using a robust fish size–otolith size relationship from the population of interest.

We note that careful examination of heteroscedasticity plots and comparisons of relationships of otolith and fish length by the covariates of stock of origin, production type, and life history stage will help structure more robust back-calculated estimates of size. Our results taken into context with previous findings (Francis 1990) suggest that the proportional back-calculation method we used will produce accurate estimates of size when the assumptions of this method are met (otolith size–fish size relationship is relatively consistent). However, when this assumption is violated several investigators have used the direct back-calculation approach for both juvenile (Tomaro et al. 2012; Claiborne et al. 2014) and adult life stages (Miller et al. 2010; Lamperth et al. 2013; Jones et al. 2014). General fish size–otolith size relationships can be used to directly back-calculate size but are strongly affected by differences in growth rate and likely add an error of about ±10 mm, which may or may not be acceptable depending on the study.

The back-calculation approach we used assumes that physical measures of fish size and otolith size are correlated with environmental and otolith chemistries, and that these characteristics respond in a relatively instantaneous manner (1–2 d) and that otolith chemistry and otolith material is not later reworked. The design of this study did not consider the realistic effects of salinity and temperature on otolith elemental incorporation (Elsdon and Gillanders 2002, 2004; Martin and Wuenschell 2006). For example, peak values of otolith Sr:Ca (~12 mmol/mol) in this study were easily identified, but similarity of otolith Sr:Ca in intermediate salinities (~12–19‰) (Zimmerman 2005) and interactive effects of temperature and salinity on otolith Sr:Ca (Miller 2011) may result in misinterpretation and back-calculation error. In addition, somatic growth rate may influence elemental incorporation in otoliths (Walther et al. 2010), and the effect of somatic growth and otolith accretion rate on elemental incorporation are in many cases species-specific (Hoie et al. 2003; DiMaria et al. 2010; Miller 2011). Lastly, a lag period (2–20 d) between encounter and incorporation has been found in several species and elements (Milton and Chenery 2001; Elsdon and Gillanders 2005; Lowe et al. 2009; Miller 2011). Although ions pass through several barriers and pathways before being accreted onto the otolith surface, this lag does not appear to be the most significant source of size back-calculation error in our study. We estimated that Sr:Ca was detected a median of 1 d after marking, which is similar to the 2–3-d time lag in incorporation when Chinook Salmon were exposed to increased salinity as reported by Miller (2011). In addition, our data suggest that for Chinook Salmon, otolith daily increments and otolith Sr:Ca can be used in combination to produce accurate and precise estimates of migration timing in juveniles. However, we do note that the precision of estimates decreased as days since marking increased, likely due to difficulty in enumerating many (~80) otolith daily increments.

The ability to accurately identify fish size and timing at migration points using Sr:Ca and daily increments may have exciting implications for reconstructing the juvenile migration of surviving adult fish. For example, Chinook Salmon may be captured migrating from natal tributaries, marked with SrCl and released to migrate to the sea, then finally recovered as adults. In this case a single LA-ICPMS transect will provide estimates at marking and at entrance into brackish and marine waters and allow estimation of juvenile size, residence, and growth before brackish or marine entry. To our knowledge relatively few studies have used this approach (Lamperth et al. 2013). For salmonid species and life histories that include migration to brackish or marine waters at sizes < 70 mm, natural and artificial elemental markers such as Sr may be an appropriate tool because high-resolution acoustic tags may not be feasible for smaller size-classes (Campbell 2010).

In conclusion, we showed that size and timing estimates for juvenile Chinook Salmon may be accurately back-calculated using the combination of otolith chemistry and structure. This may indicate that studies of life-history diversity, habitat use, and early marine survival can provide accurate estimates of size at and timing of entry into brackish or marine waters.

Similarly, size and timing of SrCl marking may be accurately estimated when physical tags are not feasible, or tagging effects are unwanted. Further laboratory studies should focus on growth rate, size at marking, and their associated effects on back-calculation estimates. In addition, applied research should combine artificial and natural otolith Sr markers to estimate the size, growth, and residence of fish as they migrate from their natal habitats to brackish and marine waters.

ACKNOWLEDGMENTS

This research was supported by the U.S. Army Corps of Engineers, Portland District; the Bonneville Power Administration; National Oceanic and Atmospheric Administration (NOAA) Fisheries, Northwest Fisheries Science Center; and the Washington Department of Fish and Wildlife (WDFW). Special thanks to Steve Schroder for thoughtful advice on experimental design and Eugene Sanborn for fabrication and implementation of the sample chambers and marking methodology. We also thank Lang Nguyen and Dana Anderson for assistance in selecting the samples to be used in this study. We thank Keck Collaboratory for Mass Spectrometry at Oregon State University, and Adam Kent and Andy Ungerer for assistance in microchemistry analysis. Lastly, a special thanks to the Washington Department of Fish and Wildlife's George Adams Hatchery and its staff for their thoughtful advice and effort in caring for and selecting samples to be used in this study.

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