Variability in fish size/otolith radius relationships among populations of Chinook salmon

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Abstract Back-calculation of growth trajectories from otolith microstructure is a valuable tool for understanding mechanisms underlying variability in growth among fish populations. We analyzed fish length/otolith radius relationships for Snake River spring/summer Chinook and Snake River fall Chinook salmon, listed as separate "Evolutionarily Significant Units" (ESUs) under the US Endangered Species Act, to determine whether these ESUs shared relationships. In addition, we analyzed otoliths from seven separate populations within the Snake River spring/summer Chinook ESU to assess the variability in relationships among populations, which are much more closely related than ESUs. We also examined several potential functional forms for the equations. We found that the separate ESUs had significantly different fish length/otolith radius relationships, but that variability in otolith growth rate could not explain the difference. Relationships among populations within the spring/summer Chinook ESU did not vary nearly as much as those between ESUs. The quadratic model and the power model fit the data equally well, and constraining these models to pass through a biological intercept (estimated fish length and otolith radius at hatching) resulted in only a slight decrease in model fit. To test the ability of the models to back-calculate fish lengths, we predicted the length at tagging for 17 PIT-tagged fall Chinook that were measured at release and at recapture. The back-calculation demonstrated little bias (<1 mm FL, on average) and relatively small standard deviation (~3.5 mm) for the best model. When we repeated the back-calculation with data from both ESUs combined, bias increased substantially (to 15 mm FL), demonstrating the importance of determining the proper taxonomic level at which to combine data within a species.

Keywords Otolith · Back-calculation · Growth · Chinook salmon

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Introduction

A rich history exists of relating size of fish to the size of their hard parts, particularly scales and otoliths (Lee 1920, Pannella 1971). These types of relationships serve many roles, particularly the ability to back-calculate growth trajectories of sampled individuals (Francis 1990; Morita and Matsuishi 2001). It is clear that these relationships are species-specific (Fey 2006), but it is not clear that the species-specific relationships apply to all populations that comprise a species. An important tool for understanding growth processes is to compare how growth varies across populations under variable physical and environmen-

tal conditions. To apply this tool, we need to know how fish size/otolith size relationships vary across populations, but few studies have examined this issue. Notable exceptions are Radtke et al. (1996), who observed different fish size/otolith size relationships between North American and Asian populations of Dolly Varden (*Salvelinus malma*), and Strelcheck et al. (2003), who observed differences in relationships in populations of gag (*Mycteroperca microlepis*) in the Gulf of Mexico. The latter study is particularly important because the authors determined that if they applied a species-specific relationship to distinct populations, they arrived at misleading conclusions about how growth varied among populations. In this study we focus on how relationships between fish length and otolith radius vary across populations of Chinook salmon in the Columbia River basin in the northwestern US.

Fish size, particularly that of juveniles, plays a major role in the population dynamics of Chinook salmon. Zabel and Williams (2002) observed strong selection on juvenile length at migration for survival to adulthood. Furthermore, migration timing is positively related to juvenile length (Zabel and Achord 2004; Achord et al. 2007), and migration timing influences adult return rate (Scheuerell et al. 2009), with early migrants typically returning at greater rates. Thus understanding juvenile growth processes is fundamental for recovering populations of Chinook salmon, listed as threatened under the US Endangered Species Act (ESA). However, patterns of growth in natural populations vary in complex ways across time and space depending on ecological conditions. For instance, for Snake River spring/summer Chinook, the relationship between juvenile length and temperature shifts from positive to negative, depending on density of juveniles (Crozier et al. 2010). The ability to back-calculate growth trajectories of juvenile fish sampled from a variety of natural populations will provide a powerful tool to begin understanding this complexity.

We focused on Snake River fall Chinook salmon and spring/summer Chinook salmon, which are distinguished as separate Evolutionarily Significant Units (ESUs), the units of conservation in the ESA. This distinction is based on differences in adult run timing (which is the basis for the designation of fall versus spring/summer), geographical range, genetics, and life history variability. In fact, based on genetic

analyses, Waples et al. (2004) determined that these two ESUs function as separate, but closely related species, separated for at least 12 000–15 000 years. Within the spring/summer Chinook ESU, 31 separate populations have been identified (IC-TRT 2003). In this study we address whether fish length/otolith radius relationships vary between the two ESUs and among populations within the spring/summer Chinook ESU. In addition, we evaluate which are the most appropriate models of fish length/otolith radius relationships for juvenile Chinook salmon.

Many researchers have emphasized the limitations of back-calculation methods (Francis 1990). In particular, several studies have demonstrated a decoupling between somatic and otolith growth, with otolith size related to factors such as growth rate and temperature in addition to fish size (Mosegaard et al. 1988; Secor and Dean 1989). Consequently, we also addressed whether growth rate and temperature are important in determining variability about fish length/otolith radius relationships, both within and among ESUs.

Our study was based solely on fish collected from natural populations that had experienced ambient conditions, while most other studies examining variability in fish length/otolith size relationships have been conducted in a laboratory. Specifically, we developed a series of alternative models that related fish length to otolith radius, representing several different functional forms, and including or excluding terms. We used model selection criteria to determine which models were most appropriate, and in doing so, determined which factors were important to the relationships. Finally, we performed a validation of back-calculation methods by examining individuals that were measured, PIT tagged, released, and then recaptured and re-measured. Based on several back-calculation equations, we predicted size at tagging based on size at recapture, otolith size at recapture, and number and width of otolith increments.

Methods

Study species, otolith collection and preparation

Snake River Fall Chinook are designated as "ocean-type" fish (Healey 1991), which typically migrates to seawater as subyearlings several months after hatch-

ing. However, they have recently exhibited increasing propensity to migrate as yearlings, with the change likely due to a shift in predominant spawning sites due to the construction of impassible dams in Hells Canyon in the 1950s (Williams et al. 2008). Adults return to spawn in the fall after 1–4 years in the ocean and spawn primarily in the lower reaches of the Snake and Clearwater rivers (Fig. 1a). Snake River spring/ summer Chinook are designated as "stream-type" fish (Healey 1991), which spends a full year in freshwater prior to ocean migration. The adults migrate upstream during the spring and summer after 1–4 years in the ocean, but typically delay spawning until the fall. Relative to Snake River fall Chinook, they spawn in higher elevation (approximately 1,200 to 2,200 m compared to approximately 300 m) and narrower tributaries, typically less than 15 m wide (Fig. 1b).

Fall Chinook were captured at Lower Granite Dam on the Snake River in 1993, 1994, 2007, and 2008. The fish were previously PIT tagged upstream from the dam in the Snake River, and then diverted using a sort-by-code facility at the dam. Fork lengths were recorded at the time of capture, which occurred from June to August. The 1993 and 1994 samples were archived by the US Geological Survey and stored in resin. A total of 61 fish was included in the fish length/ otolith radius relationships, with fish selected to represent the range of fork lengths in the sample. Because we were restricted to the small set of fish that were captured, we could not select lengths to evenly represent the range of lengths as recommended by DeVries and Frie (1996).

Spring/summer Chinook otoliths were collected from age 0 parr collected in their rearing areas in the Salmon River basin in central Idaho, USA. We relied on fish that were collected for other studies (e.g., Achord et al. 2007). Fish were collected over three years (2004–2006) in July, August and September. Fish were sampled from seven separate populations: 5 populations in the Middle Fork Salmon subbasin (Bear Valley Creek, lower Big Creek, Elk Creek, Marsh Creek), two populations in the South Fork Salmon River subbasin (South Fork Salmon River and Lake Creek), and one population from a tributary to the main fork of the Salmon River Valley. A total of 296 fish was collected, and fork lengths were recorded at capture, with fish sampled during July, August, or September. We did not have control of the distribution of fish lengths sampled, so we could not produce an even distribution of lengths across the

range. For each spring Chinook population, we also recorded daily average stream temperatures in their rearing areas using a Tidbit in-stream data logger that records °C at five-minute intervals. We removed sagittal otoliths from each individual, removed adhering tissue, and stored dry. Left sagittal otoliths were mounted to a microscope slide with Crystal Bond© (http://www.crystalbond.com/). Each otolith was polished on both sides in a sagittal plane, using slurries (grit sizes of 1 and 5 alumina micropolish) and a grinding wheel with Buehler© 1500 and micro-polishing pads. Polishing ceased when the core of the otolith was exposed and daily increments were visible under a light microscope.

We photographed polished otoliths using a digital camera (Cybernetics©) mounted on a compound microscope (Zeiss©; set at 20× magnification). Using Image Pro software (MediaCybernetics©), we first measured total otolith radius (i.e, distance from the otolith core to its margin) along a transect perpendicular to the longitudinal axis on the ventral side of the left otolith. This transect was selected for its reliable clarity. When possible, we also identified each otolith's hatch check, which was identified by a dark band and a secondary primordium (Zhang et al. 1995) and correspond to the transition in the fish's life from embryo to fry. We measured the distance from the primordium to the hatch checks along a transect perpendicular to the longest axis, as above.

For the growth rate analysis (see below), we measured otolith increments over a variable period prior to collection (28 to 84 d) for a subsample of fall Chinook with discernable daily increments collected in 1993, 1994 and 2007 ($n=38$). For spring Chinook, we measured daily otolith increments over the 40 days prior to collection for the subsample of fish collected during September in 2005 and 2006 ($n=86$). We measured these increments along a transect perpendicular to the longest axis on the ventral side, as above.

Analyses

Our approach was to develop relationships, both linear and nonlinear, between fish length and otolith radius. By comparing alternative models using Akaike's Information Criterion (AIC; Burnham and Anderson 2002), we could determine which factors explained the greatest amount of variability in these relationships. The model with the lowest AIC value

Fig. 1 Maps of spawning areas for Snake River fall Chinook (**a**) and Snake River spring/summer Chinook (**b**). The current extent of spawning by fall Chinook in the Snake River is shaded (**a**). Specific spawning sites of spring/summer used in this study are indicated by fish symbols (**b**)
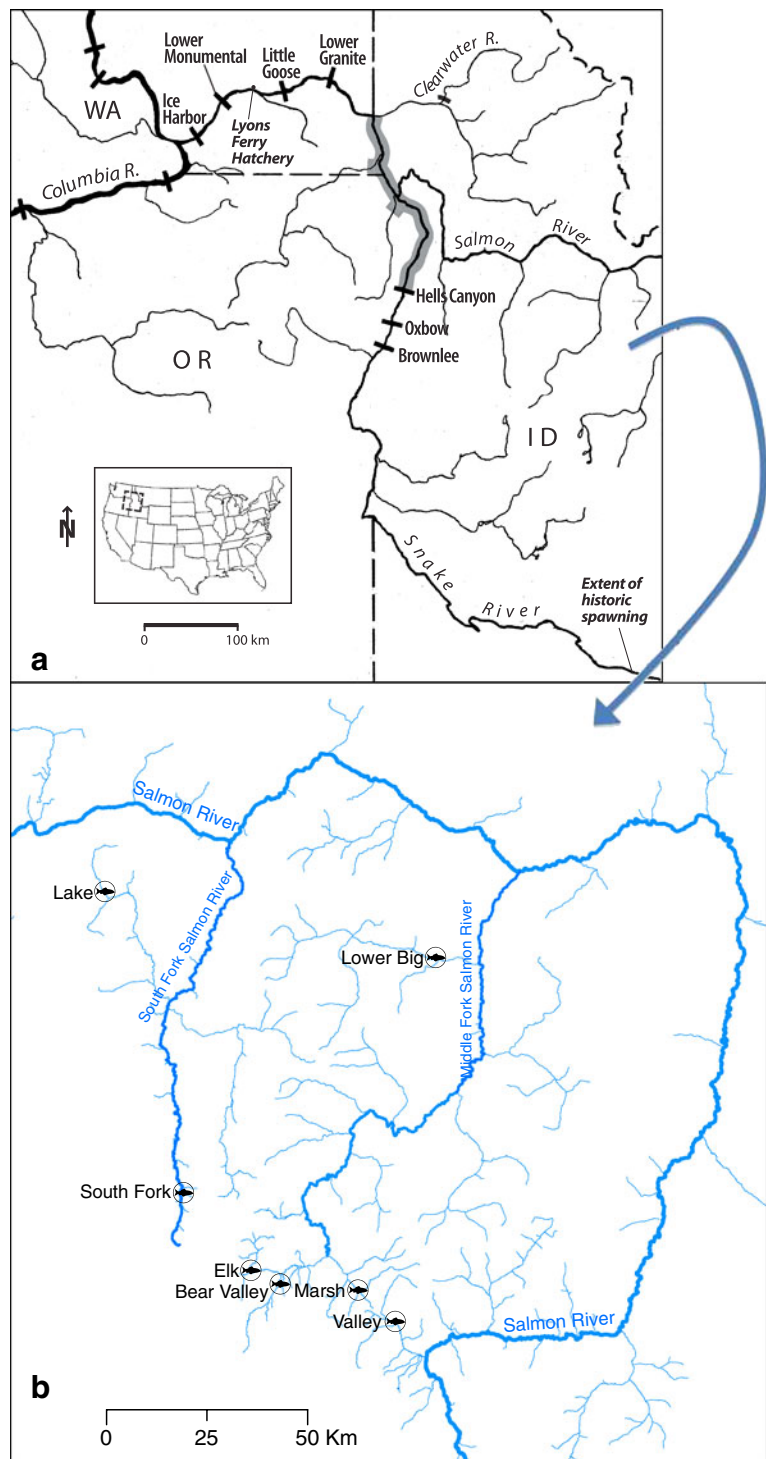
had the best fit. Differences between models of less than 2 indicated that models performed similarly, whereas differences of greater than 10 were strong support for the model with the lower AIC (Burnham and Anderson 2002).

We first examined whether difference existed between ESUs, using models of the form

$$L_i = a + b \cdot O_i + c \cdot O_i^2 + c \cdot ESU_i \qquad (1)$$
$$+ d \cdot ESU \cdot O_i + e \cdot ESU_i \cdot O_i^2 + \varepsilon_i$$

where L_i is the fork length, and O_i is the otolith radius for the ith individual. ESU_i is a dummy variable representing whether a fish was from the fall Chinook or spring/summer Chinook ESU. In addition, ε_i is the error term, which was assumed normally distributed with mean 0 and variance σ^2. The lower case letters are coefficients. We included interactions between O and ESU to represent separate slopes for the separate ESUs. Eq. 1 can be abbreviated as $L = I + ESU*(O + O^2)$, indicating that the model contains an intercept term (I) and an interaction between ESU and O and O^2. The "*" term indicates that the model includes both interactions and all main effects. By constructing simpler models nested within Eq. 1, we tested for the importance of model terms, particularly that of ESU. Note that although we used a quadratic equation for this part of the analysis, we tested other forms.

Based on the results of the ESU analysis, we treated the ESUs separately when we tested different model forms. We considered the following abbreviated equations:

$$\text{Linear}: L = I + O \qquad (2)$$

$$\text{Log/Exponential}: L = \exp(I + O) \qquad (3)$$

$$\text{Quadratic}: L = I + O + O^2 \qquad (4)$$

$$\text{Power}: L = I + O^c \qquad (5)$$

Similar to Eq. 1, each of these models contains a normally distributed error term. Note that we expressed the log model in the exponential form so that it would have the same response variable and error structure as the other models, allowing for possible model comparisons.

We also constrained the four models in Eqs. 2–5 such that they passed through a biological intercept. We defined this intercept to represent otolith radius and fish length at the time of hatching. We estimated separate intercepts for each ESU by first estimating otolith radius at the hatch check marks for the subset of individuals where a hatch check mark was readily identifiable, and then taking means across ESUs (mean = 110.4 microns, cv=0.164, n=57 for fall Chinook, and mean = 95.8 microns, cv=0.180, n= 162 for spring/summer Chinook). These means were highly significantly different (t test, $P<0.001$), justifying a separate value for each ESU. We estimated fish length at hatching using mean egg mass for stream- and ocean-type Chinook (Healey 2001) applied to published hatch length/egg mass relationships (Beacham and Murray 1990) (mean = 22.4 mm for fall Chinook and 21.6 for spring/summer Chinook). To constrain the models to pass through these intercepts, we first subtracted the intercept from each individual's fork length and otolith radius, designating these terms as L_B and O_B. We then constructed models that did not contain intercept terms. For instance, a quadratic model constrained to pass through the biological intercept is designated as $L_B = O_B + O_B^2$.

To test whether individual populations within the Snake River spring/summer Chinook ESU have distinct fish length/otolith radius relationships, we examined a series of models with and without population effects. The fullest model had the form

$$L = I + O*POP + O^2 \qquad (6)$$

where POP is a factor with levels represented by each of the seven populations, meaning each population had a separate intercept and slope coefficient. We did not consider an interaction between POP and O^2 because this model would have required too many parameters. In addition, when we considered models constrained to pass through the biological intercept, we also had to drop the POP main effects because these would have produced curves that did not pass through the biological intercept. We designated the quadratic form of this model as $L_B = O_B + POP : O_B + O_B^2$, where the ":" term designates an interaction without the main effect.

Finally, we tested for the effects of growth rate and temperature. We measured daily otolith increments for a subsample of the individuals analyzed. We then calculated mean increment width per individual to

represent otolith growth rate. We used two approaches to test whether a "decoupling" existed between somatic growth rate and otolith growth rate. First, we examined the performance of models that contained interactions between otolith growth rate and otolith radius, reasoning that the growth rate effect would be expressed as differences in slope between fish length and otolith radius. We did not examine models that contained otolith growth rate as a main effect because although we expected a positive relationship between fish size and otolith growth rate, we were really testing for an effect of growth rate that changed the slope of relationship. To further test for this effect, similar to Fey (2006) and Wilson et al. (2009), we related residuals from models that contained O and O^2 to growth rate. If the growth-rate effect existed, we would expect to see residuals above the predicted relationship to have greater growth rates than those below. We tested these models both within and between ESUs to determine if differences in growth rate could explain observed differences between ESUs. We had rearing temperature data for spring/summer Chinook populations, which were sampled in their respective rearing areas, but not for the fall Chinook because they migrated through river segments with different temperature regimes. Temperature was measured as the mean stream temperature over the 40 days prior to collection.

Temperature was introduced to the models additively and as an interaction with otolith radius. Models that included temperature and otolith growth rate were not constrained to pass through the biological intercept because we were initially concerned with the contribution of these effects to the more general model.

Back calculations and validations

We used 17 fall Chinook juveniles with discernable increments from 2007 that were PIT tagged at a known FL, released, and then recaptured approximately 1 month later to perform a validation of back-calculations based on the equations described above. We measured daily increments so that we could estimate otolith size at the time the fish were initially PIT tagged. We used the linear and quadratic equations, with unconstrained and biological intercepts. For models with unconstrained intercepts, we used the "regression approach" (Campana 1990), where the back-calculated trajectory is parallel to the regression-based relation-ship. For the models with biological intercepts, we used the "biological intercept approach" (Campana 1990), where back-calculated trajectories were constrained to pass through the biological intercept.

The first step was to use the radius of the otolith measured at recapture, along with a parameterized equation to predict fish length at recapture. We then calculated the deviation of observed fish length at recapture from the predicted length. In cases where the intercept was unconstrained, we maintained this deviation through the entire back-calculated trajectory. If the equation was constrained to pass through an intercept, we linearly decreased the deviation with respect to otolith radius such that the deviation was zero at the biological intercept. We knew the dates of recapture and tagging, so we counted otolith increments to estimate the otolith increment corresponding to the date of tagging. Based on the estimated size of the otolith at the time of tagging, we estimated fish length at time of tagging by applying the regression equation predictively and then applying the appropriate deviation from the predicted line. Finally, we compared our predicted length at tagging to the observed length at tagging and calculated this deviation.

Results

We measured fish lengths and otolith radii for 296 spring/summer Chinook distributed across years and populations (Table 1) and 61 fall Chinook distributed

Table 1 Sample sizes by year for the fish length/otolith radius regressions

Year	BVA	ELK	LAK	LBG	MAR	SFS	VAL
Snake River spring/summer Chinook salmon populations							
2004	10	8	13	10	11	14	16
2005	13	15	20	18	16	15	21
2006	20	15	20	–	15	15	13
Snake River fall Chinook salmon							
1993	25						
1994	10						
2007	18						
2008	8						

BVA Bear Valley Creek; *ELK* Elk Creek; *LAK* Lake Creek; *LBG* lower Big Creek; *MAR* Marsh Creek; *SFS* South Fork Salmon River; *VAL* Valley Creek

across years (Table 1). The spring/summer Chinook ranged from 34 to 93 mm, and the fall Chinook ranged from 53 to 168 mm. The two ESUs demonstrated clear differences in their fish length/otolith radius relationships (Fig. 2, Table 2). Using AIC values as an index of model performance, models that contained the ESU term (Table 2, models 3–5) performed better than those that did not (Table 2, models 1–2): inclusion of the ESU term conferred a decrease in AIC of over 200. The best model was one that contained an interaction between ESU and O (Table 2, model 4). The more complex model (Table 2, model 5), which also had an interaction between ESU and O^2, had a comparable AIC, but the interaction term was not significantly different from 0 ($P=0.160$).

Based on R^2, all models fit the data similarly well (within an ESU) when an intercept term was included (Fig. 3, Table 3, odd numbered models). However, when models were constrained to pass through the biological intercept, the logarithmic model (Table 3, models 6 and 8; Fig. 3c and d) fit the data poorly. In these cases, constraining the models produced such poor fits that the R^2 values were <0.0. Overall, the linear model (Table 3, models 2 and 4; Fig. 3a and b) did not perform as well as the quadratic or power models (Table 3, models 10, 12, 14, and 16). In fact, the fits of quadratic (Table 3, models 9–12; Fig. 4e and f) and power models (Table 3, models 13–16; Fig. 4g and h) were similar in all cases, and both of these models fit the biological intercept without much drop off in performance compared to the corresponding unconstrained models. Both the quadratic and power models had the best overall performance, and
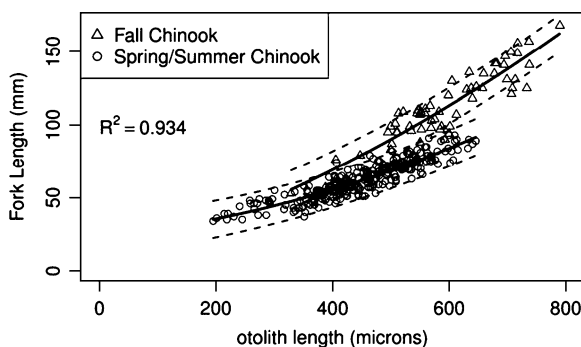
Table 2 Model results for regressions between fish length and otolith radius that also included the factor ESU, which refers to Evolutionarily Significant Unit. NP refers to the number of parameters in each model; L refers to fish length; O refers to otolith length; ESU refers to evolutionarily significant unit

Model	NP	R^2	AIC
1. $L=O$	2	0.766	2783.8
2. $L = O + O^2$	3	0.839	2651.7
3. $L = O + O^2 + ESU$	4	0.927	2372.1
4. $L = O*ESU + O^2$	5	0.934	2334.5
5. $L = (O + O^2)*ESU$	6	0.935	2334.5

despite the power model being more prevalent in the literature, we adopted the quadratic model because it is linear and thus easier to apply to the hypothesis testing exercises that follow.

The best fitting model (based on AIC) in the population analysis was the one that contained an unconstrained intercept and an interaction between population and otolith radius (Table 4, model 2; Fig. 4a). However, we do not recommend this model because the predicted relationships were inconsistent in the region of the biological intercept (Fig. 4a). When the model was constrained to pass through the biological intercept, the best model still contained an interaction between population and otolith radius (Table 4, model 4; Fig. 4b). However, this model contains several non-significant terms ($P>0.05$), and we therefore preferred the simplicity of the model that assumes no effect of population without suffering much of a loss in performance (Table 4, model 3; Fig. 4c). We selected the quadratic model constrained to pass through the biological intercept because the unconstrained model exhibited unrealistic curvature near the biological intercept (see Fig. 3f), and the linear model did not fit as well (see Fig. 3b) and exhibited biased residuals.

Neither otolith growth rate, which ranged from 1.64 to 3.92 microns per day, nor daily average temperature, which ranged from 10.97 to 16.15°C, improved the model fits for spring/summer Chinook (Table 5, models 1–5). In addition, the residuals from a model without growth rate were not related to otolith growth rate ($R^2=0.0125$, $P=0.302$). For fall Chinook, the addition of otolith growth rate, which ranged from 4.20 to 6.18 microns per day, conferred a slight advantage in model fit (AIC decreased slightly in models 8 and 9 compared to models 6 and 7).

Fig. 2 Relationship between fish length and otolith radius for Snake River fall Chinook (*triangles*) and Snake River spring/summer Chinook salmon (*circles*). The dashed lines are 95% prediction intervals

Fig. 3 Relationship between fish length and otolith radius for populations within the Snake River spring/summer Chinook ESU. In **a**, each population had a separate quadratic relationship, but the individual relationships were not constrained at the biological intercept. In **b**, each population had a separate relationship, but all relationships were constrained to pass through the biological intercept. In the **c**, all populations shared a common relationship that was constrained to pass through the biological intercept. In this plot, the dashed lines represent the 95% prediction interval. Abbreviations: *BVA* Bear Valley Creek; *ELK* Elk Creek; *LAK* Lake Creek; *LBC* Lower Big Creek; *MAR* Marsh Creek; *SF* South Fork Salmon River; *VAL* = Valley Creek
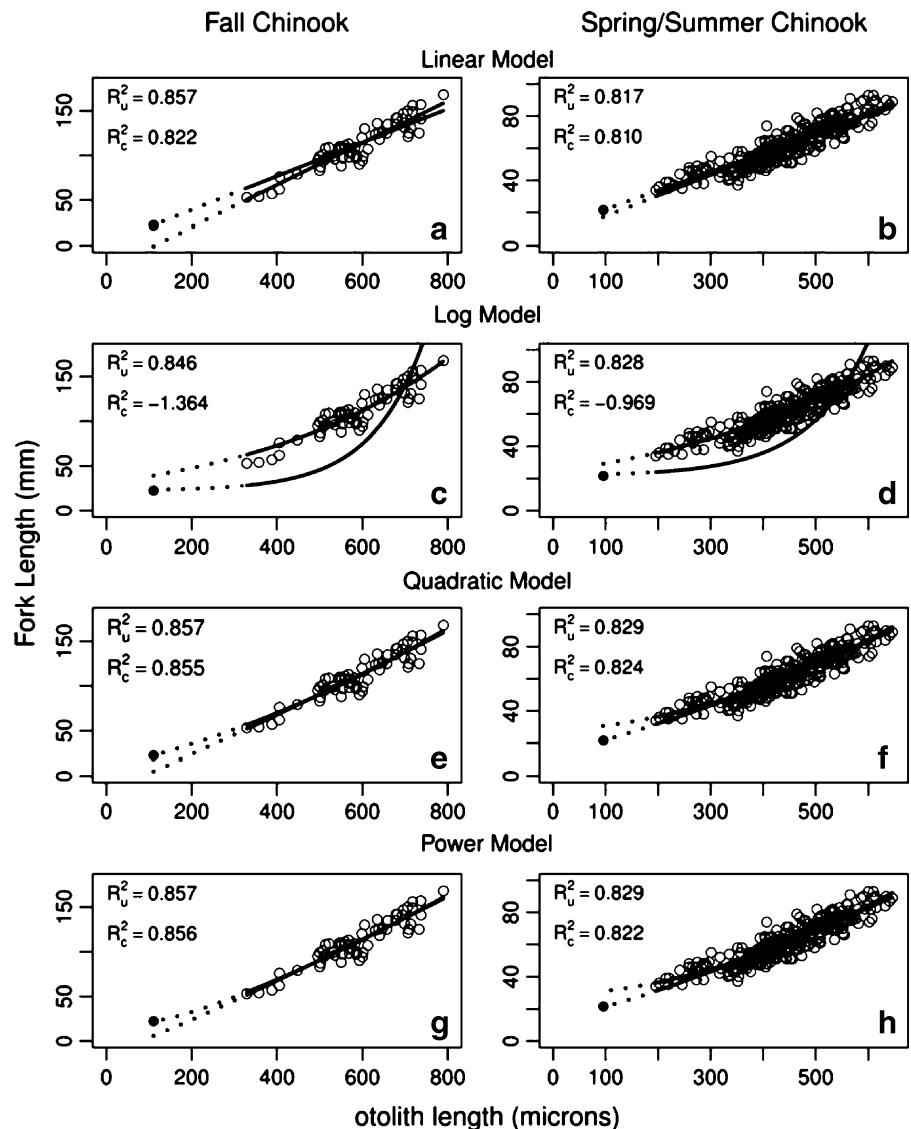
Similarly, the regression between the residuals and otolith growth rate was marginally significant ($R^2 = 0.103$, $P = 0.0495$). When we combined the two ESUs, models that contained growth rate (Table 5, models 12, 13, 16 and 17) fit the data substantially better than those that did not (Table 5, models 10, 11, 14, and 15). However, models that contained only ESU as a factor (Table 5, models 14 and 15) explained more variability than those without ESU but with otolith growth rate (Table 5, models 12 and 13). In addition, when added to models with ESU, the otolith growth rate term did not improve model fit (comparing model 16 to 14 and 17 to 15) and the associated coefficients were not significantly different from zero.

For the 17 fall Chinook included in the validation study, the mean number of days between tagging and recapture was 26.3 (s.d. = 9.7 days), and mean growth during this interval was 30.5 mm FL (s.d. = 11.6 mm). The linear models and the quadratic model using the biological intercept were reasonably unbiased when back-calculating fish length at PIT tagging, with mean deviations from observed lengths close to 1 mm FL or less (Fig. 5a, b, and d). However, the quadratic model with unconstrained intercept performed poorly (Fig. 5c), with a bias of nearly −6.5 mm FL. In addition, the unconstrained linear model (Fig. 5a) had a relatively large standard deviation in spite of having the smallest mean deviation. Thus, back-calculation

Table 3 Model results for regressions between fish length and otolith radius using four different equations. Int. refers to intercept, and U means the intercept was unconstrained at the biological intercept, and B means the equation was constrained to pass through the biological intercept. L refers to fish length, and L_B refers to fish length minus the biological intercept. O refers to otolith radius, and O_B refers to otolith radius minus the biological intercept

Model	Int.	Fall Chinook			Spring/Summer Chinook		
		AIC	R^2	Equation	AIC	R^2	Equation
Linear	U	454.5	0.857	1. $L = -26.54 + 0.23 \cdot L$	1851.7	0.817	3. $L = 5.06 + 0.13 \cdot L$
	B	465.8	0.822	2. $L_B = 0.19 \cdot O_B$	1860.9	0.810	4. $L_B = 0.12 \cdot O_B$
Log	U	458.9	0.846	5. $L = \exp(3.44 + 0.0021 \cdot O)$	1833.3	0.828	7. $L = \exp(3.17 + 0.0021 \cdot O)$
	B	623.5	−1.364	6. $L_B = \exp(0.0081 \cdot O_B)$	2555.6	−0.969	8. $L_B = \exp(0.0088 \cdot O_B)$
Quad	U	456.4	0.857	9. $L = -17.4 + 0.20 \cdot O + 0.000029 \cdot O^2$	1833.7	0.829	11. $L = 27.9 + 0.017 \cdot O + 0.00013 \cdot O^2$
	B	455.2	0.855	10. $L_B = 0.133 \cdot O_B + 0.00011 \cdot O_B{}^2$	1839.8	0.824	12. $L_B = 0.096 \cdot O_B + 0.000053 \cdot O_B{}^2$
Power	U	456.5	0.857	13. $L = -13.55 + 0.104 \cdot O^{1.11}$	1833.5	0.829	15. $L = 29.15 + 0.00044 \cdot O^{1.84}$
	B	454.8	0.856	14. $L_B = 0.0328 \cdot O_B{}^{1.28}$	1843.5	0.822	16. $L_B = 0.0458 \cdot O_B{}^{1.16}$

methods that constrained the growth trajectory to pass through the biological intercept performed best, with the quadratic model performing slightly better than the linear model due to its slightly lower standard deviation and its more symmetric distribution of deviations (Fig. 5c and d).

Discussion

In this study we investigated fish length/otolith radius relationships of Chinook salmon from the Columbia River Basin to determine whether this relationship varied between two Evolutionarily Significant Units

Fig. 4 Model fits for different forms of the fish length/otolith radius relationships. Data from Snake River fall Chinook are in the left column and data for Snake River spring/summer Chinook are in the right column
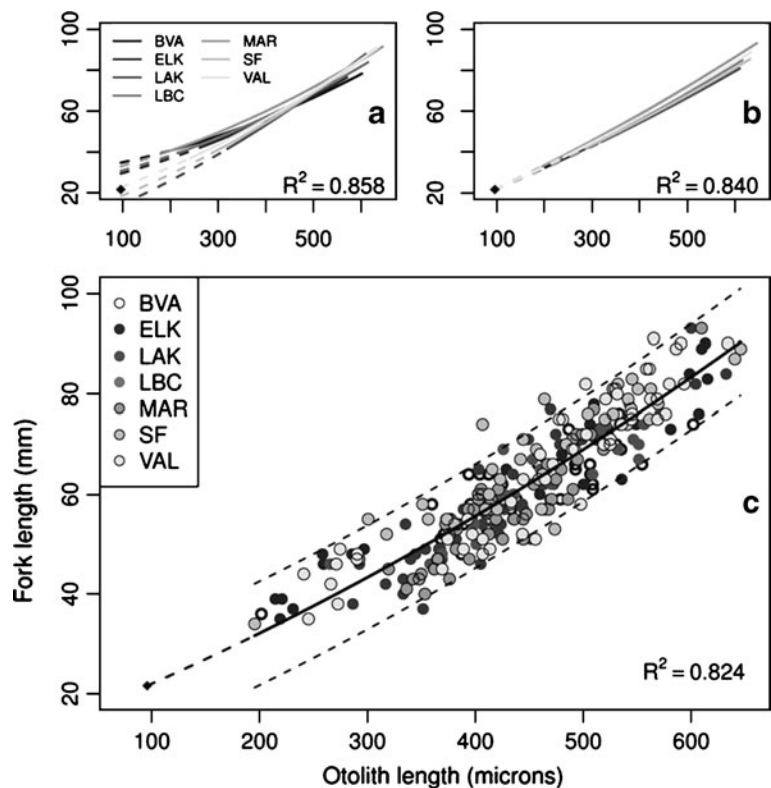
Table 4 Model results for regressions between fish length and otolith radius that also included the factor POP, which refers to Population. NP refers to the number of parameters in each model. L refers to fish length, and L_B refers to fish length minus the biological intercept. O refers to otolith radius, and O_B refers to otolith radius minus the biological intercept

Model	NP	R^2	AIC
1. $L = O + O^2$	3	0.829	1833.7
2. $L = O*POP + O^2$	15	0.858	1803.3
3. $L_B = O_B + O_B{}^2$	2	0.824	1839.8
4. $L_B = O_B : POP + O_B{}^2$	8	0.840	1824.4

(ESUs) and among populations within the spring/ summer Chinook ESU. Our analyses demonstrated clear differences in fish length/otolith radius relationships between ESUs of Chinook salmon, but much finer difference among populations that were more closely related to each other than were fish from the two ESUs. These results demonstrate the need to determine the

Table 5 Model results for regressions between fish length and otolith radius that also included mean otolith growth rate (G_O) and mean temperature (T, degrees C). NP refers to the number of parameters in each model; L refers to fish length; O refers to otolith length; ESU refers to evolutionarily significant unit

Model	NP	R^2	AIC
Spring/Summer Chinook			
1. $L=O$	2	0.634	565.7
2. $L = O + O^2$	3	0.637	567.1
3. $L = O + O : G_O$	3	0.635	567.4
4. $L = O + T$	3	0.636	567.3
5. $L = O*T$	4	0.643	567.6
Fall Chinook			
6. $L=O$	2	0.768	287.8
7. $L = O + O^2$	3	0.795	285.1
8. $L = O + O : G_O$	3	0.797	284.7
9. $L = O + O : G_O + O^2$	4	0.810	284.3
Combined			
10. $L=O$	2	0.672	1029.8
11. $L = O + O^2$	3	0.720	1012.1
12. $L = O + O : G_O$	3	0.856	928.6
13. $L = O + O : G_O + O^2$	4	0.862	925.2
14. $L = O + ESU$	3	0.891	894.1
15. $L = O + O^2 + ESU$	4	0.915	865.4
16. $L = O + O : G_O + ESU$	4	0.893	893.8
17. $L = O + O^2 + O : G_O + ESU$	5	0.915	866.4

proper taxonomic level at which to combine fish data when developing fish length/otolith radius relationships. Otherwise, as demonstrated by Strelcheck et al. (2003), studies using back-calculations derived from distinct populations lumped together can produce unrealistic conclusions regarding the variability in growth among populations. Below we conduct a sensitivity analysis that demonstrates the same potential for error with Chinook. Researchers will need to develop the ability to detect these differences in their sampling designs.

The differences in fish length/otolith radius relationships among ESUs were consistent with predictions from the growth rate hypothesis (Secor and Dean 1989) that fall Chinook has greater growth rate and is larger than spring/summer Chinook with the same otolith radius (Fig. 2). Although models that contained variability in otolith growth rate could explain some of the difference between ESUs, using only an ESU factor resulted in much stronger fits to the data. Further, we observed very little effect of otolith growth rate on the fish length/otolith radius relationship within ESUs, lending support to the conclusion that the differences we observed between ESUs were not solely the result of variability in growth rates. In addition, we do not believe the differences exhibited between were due to samples being collected in different years (spring Chinook: 2004–2006; fall Chinook 1993, 1994, 2007, and 2008). First the differences in fish growth rates we observed were consistent with the expectation that fall Chinook grow faster the spring/summer Chinook, which rear in higher elevation streams and require an extra year to reach the smolt stage. Further, when we included year effects in models within ESUs, it did not improve model fits, indicating no year-to-year variability in fish length/ otolith radius relationships within ESUs.

It is therefore unclear why these two ESUs demonstrated differences in the fish length/otolith radius relationship. Because these populations have been separated for thousands of years, and because the fish length/otolith size relationships have a genetic basis (Fey 2006), it is quite possible that the differences we observed in fish length/otolith radius relationships also have a genetic basis (as a result of selection or drift). Differences in these relationships may also be due to factors, environmental or otherwise, that were not measured in this study. One way to approach this issue would be to conduct investigations whereby fish from the two ESUs are raised in common environments.
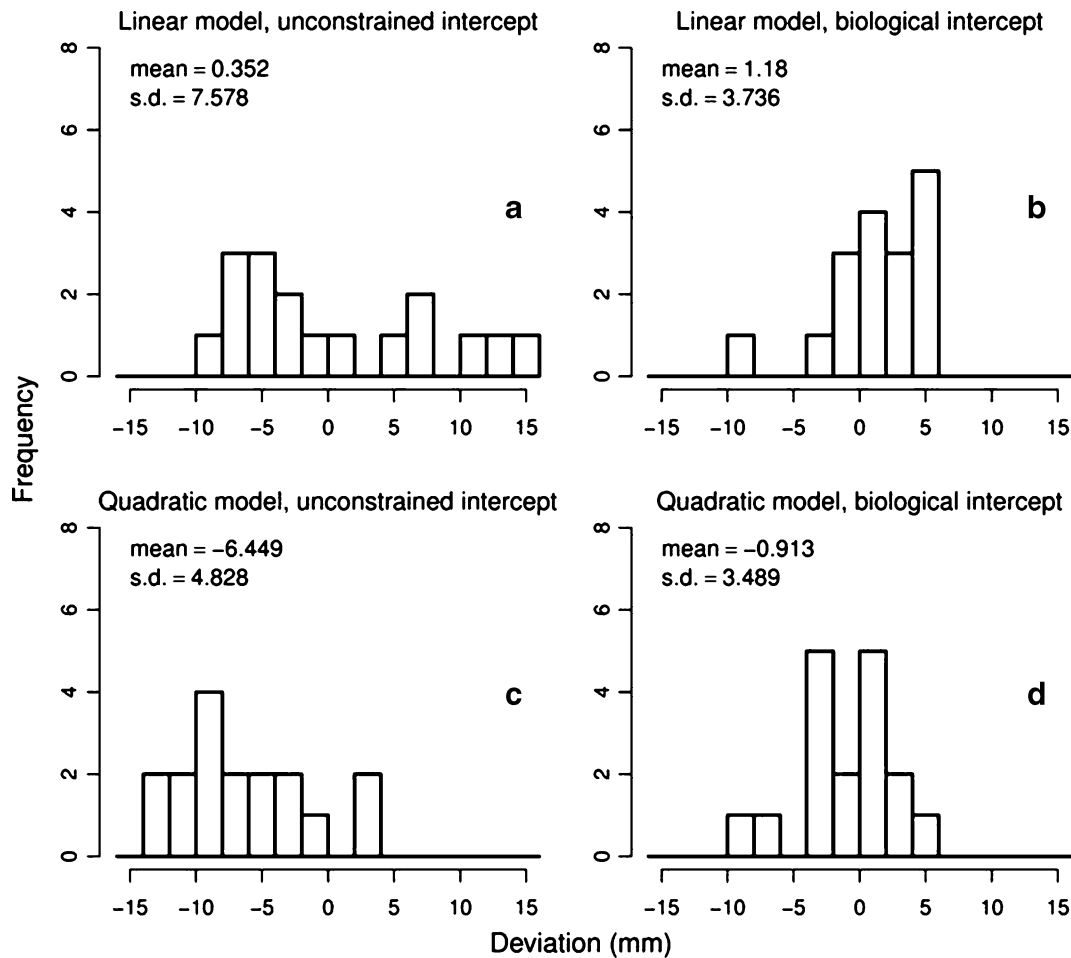
Fig. 5 Histograms of deviations between observed and predicted (from back-calculations) fish lengths for four methods of back-calculation

Our simple back-calculation methodology performed well when comparing our predictions of fish length to the observed lengths of fish tagged 26 days earlier (on average). Although our validation method was not ideal, given that we needed to estimate the otolith increment corresponding to the day of tagging by counting increments, the results were informative and encouraging. The validation exercise was clearly able to distinguish among alternative methods and confirmed the advantage of using methods that constrain back-calculated growth trajectories to pass through a meaningful biological intercept.

As a demonstration of how important it is to treat the salmon ESUs separately, we repeated the validation exercise, but instead of using model parameters specific to fall Chinook salmon, we used parameters obtained from fitting both ESUs together. When we then predicted size of fish at tagging using the constrained

quadratic model (with the biological intercept set to the mean of both ESUs), the bias increased from absolute value less than 1.0 mm FL (Fig. 5d) to almost 15 mm FL. Clearly this magnitude of bias would severely hinder the utility of these back-calculations.

Several other studies have examined fish length/ otolith size relationships in Chinook salmon (Neilson and Geen 1982; Zhang et al. 1995; Titus et al. 2004). Although each study uses slightly different approaches, our method is comparable to the Titus et al. (2004) study. Comparing our linear model to theirs, it appears that our slopes are approximately twice as large as theirs, further reinforcing the need to treat populations of Chinook separately when developing fish size/otolith size relationships.

We note that our study was conducted on fish reared in natural conditions, which created some logistical challenges, such as not having complete control over

sample sizes and distributions of fish sizes. Most studies of this type have been performed in controlled laboratory conditions that allow for clear resolution of the effects of various factors. However, the value of methods used in this study is to understand the population dynamics of natural populations, particularly those at risk, such as the Chinook salmon populations in this study. Thus, understanding the response of otolith growth to fish growth under a variety of natural conditions and across closely related taxa will be an important tool for the management of at-risk fish populations.

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