Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids

Christian E. Zimmerman

Abstract: Analysis of otolith strontium (Sr) or strontium-to-calcium (Sr:Ca) ratios provides a powerful tool to reconstruct the chronology of migration among salinity environments for diadromous salmonids. Although use of this method has been validated by examination of known individuals and translocation experiments, it has never been validated under controlled experimental conditions. In this study, incorporation of otolith Sr was tested across a range of salinities and resulting levels of ambient Sr and Ca concentrations in juvenile chinook salmon (Oncorhynchus tshawytscha), coho salmon (Oncorhynchus kisutch), sockeye salmon (Oncorhynchus nerka), rainbow trout (Oncorhynchus mykiss), and Arctic char (Salvelinus alpinus). Experimental water was mixed, using stream water and seawater as end members, to create experimental salinities of 0.1, 6.3, 12.7, 18.6, 25.5, and 33.0 psu. Otolith Sr and Sr:Ca ratios were significantly related to salinity for all species ($r^2$ range: 0.80–0.91) but provide only enough predictive resolution to discriminate among fresh water, brackish water, and saltwater residency. These results validate the use of otolith Sr:Ca ratios to broadly discriminate salinity histories encountered by salmonids but highlight the need for further research concerning the influence of osmoregulation and physiological changes associated with smolting on otolith microchemistry.

Résumé : L’analyse des concentrations de strontium (Sr) et des rapports strontium:calcium (Sr:Ca) dans les otolithes est un outil puissant pour la reconstitution de la chronologie de la migration dans des environnements de salinité différente chez les samonidés diadromes. Bien que la méthode ait été validée par l’examen d’individus connus et des expériences de translocation, elle ne l’a jamais été dans des conditions expérimentales contrôlées. La présente étude vérifie l’incorporation du Sr dans les otolithes sur une gamme de salinités et mesure les concentrations ambiantes de Sr et de Ca en résultant chez de jeunes saumons quinnat (Oncorhynchus tshawytscha), saumons coho (Oncorhynchus kisutch), saumons rouges (Oncorhynchus nerka), truites arc-en-ciel (Oncorhynchus mykiss) et ombles chevaliers (Salvelinus alpinus). L’eau utilisée dans les expériences est un mélange d’eau de ruisseau et d’eau de mer de manière à obtenir des salinités de 0,1, 6,3, 12,7, 18,6, 25,5 et 33,0 ups. Chez toutes les espèces, il y a une relation significative entre la concentration de strontium et le rapport Sr:Ca des otolithes d’une part et la salinité d’autre part (étendue de $r^2$: 0,80–0,91); cependant, la relation n’est suffisamment prédictive que pour permettre de discriminer entre les séjours en eau douce, en eau saumâtre et en eau salée. Ces résultats permettent de valider l’utilisation des rapports Sr:Ca pour distinguer grossièrement les séjours passés des saumons dans des milieux de différentes salinités; ils démontrent cependant l’importance de recherches futures sur l’influence de l’osmoregulation et des changements physiologiques associés à la transformation en saumoneau sur la microchimie des otolithes.

Introduction

Otolith microchemistry is proving to be a powerful tool in reconstructing the chronology of migration by diadromous teleosts among fresh water, estuarine, and saltwater habitats (Campana 1999). Otolith Sr or Sr:Ca ratios have been used to describe migration in many species, ranging from American eel (Anguilla rostrata) (Casselman 1982) to striped bass (Morone saxatilis) (Secor 1992). Given the occurrence of anadromy within the Salmonidae, the method has been particularly useful in studying migration in this family. For example, otolith Sr or Sr:Ca ratios have been used to describe diadromous migrations in Arctic char (Salvelinus alpinus) (Radtke et al. 1996; Babaluk et al. 1997), brown trout (Salmo trutta) (Limburg et al. 2001), Atlantic salmon (Salmo salar) (Friedland et al. 1998), rainbow trout (Oncorhynchus mykiss) (Zimmerman and Reeves 2000), chinook salmon (Oncorhynchus tshawytscha) (Zimmerman et al. 2003), and inconnu (Stenodus leucichthys) (Howland et al. 2001). In addition to describing migration, increased Sr:Ca ratios in otolith primordia have been used to identify the progeny of anadromous salmonids (Kalish 1990; Volk et al. 2000; Zimmerman and Reeves 2002). Use of otolith microchemistry to reconstruct the chronology of migration among salinity environments, determine the presence of anadromous phenotypes, and identify maternal origin (i.e., anadro-
mous versus nonanadromous) offers significant opportunities in the study of life history variation and the evolution of diadromous migration in salmonids.

In spite of general acceptance of the use of otolith Sr:Ca ratios to describe migration in salmonids, the method has never been validated through controlled rearing studies under laboratory conditions. Radtké et al. (1996), Limburg et al. (2001), and Zimmerman et al. (2003) used transplantation studies to ensure that otolith Sr:Ca ratios associated with growth in various habitats were as expected (i.e., low in fresh water and higher in salt water). In each case, use of otolith Sr:Ca ratios was supported by the results of the transplantation experiment. In a more direct investigation of the relationship between ambient water chemistry and otolith composition in a salmonid, Wells et al. (2003) compared water chemistry and otolith composition in westslope cutthroat trout (Oncorhynchus clarkii lewisi) collected from throughout the Coeur d’Alene watershed in northern Idaho and found a linear relationship between water Sr:Ca and otolith Sr:Ca ratios. In that case, Sr:Ca ratios were not meant to be a correlate for salinity but, rather, represented varying ambient water chemistries resulting from variation in lithology and hydrology among watersheds.

In the absence of controlled rearing studies with salmonids, researchers have relied on results from other species. Several studies have examined the relationship of ambient Sr:Ca or salinity and otolith Sr:Ca ratios from fish reared in controlled laboratory conditions. Secor et al. (1995) reared juvenile striped bass at six different salinities ranging from 0 to 30 ppt (in this paper, salinity units are presented as reported in each study and ppt and psu are equivalent). Secor et al. (1995) found a positive linear relationship between salinity and otolith Sr:Ca ratios. Results from wild-caught adult striped bass indicated a logistic relationship between ambient salinity and otolith Sr:Ca ratios in subadult and adult striped bass (Secor et al. 1995). Tzeng (1996) captured elvers of the Japanese eel (Anguilla japonica) in an estuary and reared them in the laboratory for 7 months in salinities of 0, 10, 25, and 35 ppt. Mean Sr:Ca ratios in otolith regions associated with the laboratory rearing were highly correlated with salinity. Farrell and Campana (1996) reared Nile tilapia (Oreochromis niloticus) in fresh water and artificially enhanced Sr:Ca conditions to compare the incorporation of Sr and Ca from radioisotope-labeled food and water. Strontium and Ca in the otolith were primarily derived from water (through gill uptake), with 75% of Ca and 88% of Sr derived from water rather than from food. It was not clear, however, whether otolith Sr varied directly with Sr availability in the water or was a function of water Sr:Ca ratios (Farrell and Campana 1996). Bath et al. (2000) reared spot (Leiostomus xanthurus) in artificial seawater with Sr:Ca ratios corresponding to 1.2×, 1.4×, and 1.8× ambient seawater Sr and Ca levels and found that otolith Sr:Ca ratios were deposited in proportion to Sr:Ca ratios in the ambient water. Kraus and Secor (2003) tested the response of otolith Sr:Ca ratios to variation in salinity (0.5, 5, 10, 15, and 25 psu) and temperature in elvers of American eel. They concluded that broad-scale inference of fresh water, brackish, and ocean migrations was valid, but finer scale estuarine movements among intermediate salinities were not supported by their results. In contrast, Chesney et al. (1998) found no significant relationship between Sr or Sr:Ca ratios and salinity in juvenile gulf menhaden (Brevoortia patronus) reared in experimental salinities of 20, 26, and 33.4 psu. Elsdon and Gillanders (2002) tested the interaction of salinity and temperature on otolith Sr:Ca ratios in juvenile black bream (Acanthopagrus butcheri) in salinities ranging from 5.5 to 30 ppt and temperatures ranging from 12 to 28 °C. In single-factor experiments, temperature significantly affected otolith Sr:Ca ratios but there was no relationship between salinity and otolith Sr:Ca ratios (Elsdon and Gillanders 2002). In two-factor experiments, however, temperature and salinity interacted to significantly affect otolith Sr:Ca ratios. When considered together, the above studies indicate that use of otolith Sr and Sr:Ca ratios can be an appropriate tool for describing migration across significant salinity gradients.

Because all of the validation studies to date have been conducted on single species, it is difficult to assess the applicability of results for other species. As a result, interspecific variation in Sr incorporation can only be surmised based on comparisons between studies. Secor and Rooker (2000) conducted a metaanalysis of the relationship between otolith Sr:Ca ratios and ambient salinity for 27 species and found a positive relationship. Relatively few intermediate salinity (estuarine) examples were available and Secor and Rooker (2000) suggested that it was “remarkable” that a positive relationship emerged given the diversity of taxa included and range of methods contained in the data set. Ultimately, they suggested that “rigorous empirical testing” is needed to validate the use of otoliths as “faithful environmental chronometers”. In this study, I examined the response of otolith Sr:Ca ratios to salinity in five species of anadromous salmonids to calibrate otolith Sr:Ca ratios across the range of salinities typically encountered by juvenile salmonids and assess interspecific variation in otolith Sr and Ca incorporation.

**Materials and methods**

Juvenile chinook salmon ($n = 100$), coho salmon ($Oncorhynchus kisutch$) ($n = 125$), sockeye salmon ($Oncorhynchus nerka$) ($n = 125$), rainbow trout ($n = 125$), and Arctic char ($n = 125$) were obtained from local hatcheries (Alaska Department of Fish and Game, Fort Richardson Hatchery and Cook Inlet Aquaculture Corporation, Trail Lakes Hatchery) and transported to the Alaska Seafishic Center in Seward, Alaska. After collection and transportation, all fish were allowed to acclimate in fresh water for at least 7 days prior to initiation of the experiment. Fish were held in an array of six 200-L circular fiberglass tanks with one species in each tank. Fresh water and seawater collected from local sources were used as end members for mixing to produce experimental water representative of the range of salinities encountered in the environment. Fresh water and seawater were continuously supplied from a small stream and from two seawater intake structures located in Resurrection Bay (90 m offshore, 23 m deep), respectively. Fresh water and seawater were continuously mixed and aerated in a 300-L tank and gravity fed to the rearing tanks through a single distribution pipe. Inflow to each rearing tank was 6 L·min$^{-1}$ resulting in a turnover rate of 1.8 times·h$^{-1}$. Since water was only used once and turned over rapidly, it was assumed that Sr and Ca

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were continuously replenished and not diluted through uptake as seen in experiments using recirculated water.

After initiation of the experiment, salinity was increased by proportionally increasing the ratio of seawater to fresh water by 20% every 14 days (Fig. 1) resulting in 14-day experimental salinity periods of 0.1, 6.3, 12.7, 18.6, 25.5, and 33.0 psu. Fish were fed commercial salmon feed (1.2 mm and No. 1 Crumble; Moore-Clark, Vancouver, British Columbia) equal to 5% of fish weight and distributed throughout the day from belt feeders placed above each tank. Mortalities were removed and counted each day.

Water samples were collected from the distribution line at the beginning, middle, and end of each 14-day experimental salinity period. Strontium and Ca concentrations were determined using atomic absorption spectrometry (Laboratory for Oceanographic and Environmental Research, Texas A&M University, Galveston, Texas). Salinity in each tank was monitored on a weekly basis with an electronic salinity meter and temperature was continuously measured in each tank with temperature recording data loggers. To ensure that water samples collected at the distribution line were representative of each rearing tank, I also collected additional samples from all tanks on two occasions (at the midpoint of the 12.7- and 25.5-psu experimental periods). Coefficients of variation were calculated to determine the magnitude of differences in salinity, Ca, Sr, and Sr:Ca molar ratios among tanks.

To identify the beginning of the experiment, I marked otoliths by the immersion of fish in 50 ppm alizarin complexone for 4 h (Thomas et al. 1995; Beckman and Schulz 1996). Between experimental salinity periods, thermal or stress marks were induced on otoliths based on the methods of Lether and Tertiary (1998). Fish were held in 60-L tanks and allowed to warm at least 6 °C before being returned to the rearing tank at the next salinity level. The combination of temperature manipulation and stress associated with increased osmotic demands created a mark evident on the otolith (Fig. 2). Along with the alizarin complexone mark signifying the start of the experiment, the thermal marks were used to guide analysis of otolith microchemistry.

At the conclusion of the experiment, sagittal otoliths were removed from each fish, rinsed, and stored dry in plastic vials. Otoliths, for analysis, were collected from 22 coho salmon, 12 chinook salmon, 12 sockeye salmon, 22 rainbow trout, and 15 Arctic char. Otolith preparation and analyses followed the methods of Zimmerman and Reeves (2000, 2002) and Zimmerman and Nielsen (2003). One sagittal otolith from each fish was mounted sulcus side down with Crystal Bond 509 on a microscope cover slip attached on one edge to a standard microscope slide. The otolith was then ground with 1200-grit sandpaper in the sagittal plane to the level of the nucleus. The mounting medium was heated and the otolith turned sulcus side up. The otolith was then ground with 1200- and 2000-grit sandpaper in the sagittal plane to the level of the primordia and polished with a slurry of 0.05-µm alumina paste. The cover slip was then cut with a scribe so that several prepared otoliths could be mounted on a petrographic slide for microprobe analysis. The slide containing several otoliths was rinsed with deionized water, air dried, and coated with a 400-Å (40-nm) carbon layer.

Elemental analysis was conducted with a Cameca SX-50 wavelength dispersive electron microprobe (Cameca, Courbevoie, France). A 15-kV, 50-nA, 7-µm-diameter beam was used for all analyses. Strontianite and calcite were used as standards for Sr and Ca, respectively. Each element was analyzed simultaneously and a counting time of 40 s was used to maximize precision (Toole and Nielsen 1992). Using the alizarin complexone and thermal marks to guide sampling, Sr and Ca were measured within each experimental growth region (Fig. 2). One point was measured in each experimental region for each fish.

Differences in mean otolith Sr:Ca ratios (atomic ratio), otolith Sr (weight percent), and otolith Ca (weight percent) among treatments were tested by one-way ANOVA and Tukey–Kramer multiple comparisons (α = 0.05). With the exception of the rainbow trout treatment, all comparisons were tested using a balanced ANOVA design. Because only four of the 22 rainbow trout analyzed survived to the 33.0-psu salinity treatment, the analysis of this species was unbalanced. The relationship of otolith Sr:Ca ratios to salinity was tested using least-squares linear regression for each species. To examine if Sr and Ca incorporation rates differed among species, I tested homogeneity of the regression lines (slopes) using ANCOVA, and one-way ANOVA was used to test for significant differences in otolith Sr:Ca ratio among species within each salinity treatment. To assess the predictive power of otolith Sr:Ca ratio of salinity, I applied the methods of Prairie (1996) to determine the categorical resolution of otolith Sr:Ca ratio on salinity. This method essentially defines the predictive power of a regression model as the number of separate classes into which the dependent variable can be divided. In addition, the 95% confidence interval of individual measurements was determined to gauge the resolution in terms of salinity units. To compare incorporation rates of otolith Sr among treatments and species, I calculated the distribution coefficient of Sr (Dsr) according to the equation presented by Campagna (1999).

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D_{Sr} = \frac{[Sr:Ca]_{otolith}}{[Sr:Ca]_{water}}
\]
This relationship considers the otolith as a calcium carbonate system with dissolved and solid phases and a proportional relationship is expected between the otolith and dissolved Sr in the water (Morse and Bender 1990). The $D_{\text{Sr}}$ provides an estimate of rates of strontium incorporation and was calculated to compare with previously published values. A distribution coefficient of 0 would indicate that an element available in the ambient water is not incorporated into the otolith and a value of 1 would indicate that an element is incorporated directly into the otolith with no discrimination (Campana 1999).

The relationship of fish size to otolith Sr:Ca ratio was tested using least-squares linear regression. Fork length at the conclusion of the experiment was tested against otolith Sr:Ca ratio at each salinity level across all species and within each species. Fork length at the conclusion of the experiment was used as a measure of size across all treatments assuming that growth was constant for all fish throughout the experiment.

**Results**

Strontium and Ca were positively correlated with salinity in the experimental mixtures of seawater and fresh water (Ca: $P < 0.0001$; Sr: $P < 0.0001$) (Fig. 3). Ambient Sr ranged from 0.21 to 7.02 ppm and Ca from 35.4 to 386.8 ppm. Although the mixing curves for Sr and Ca were conservative, the Sr:Ca mixing curve was not conservative (Fig. 3). The relationship of Sr concentration and salinity was similar to that presented by Ingram and Sloan (1992) for San Francisco Bay (Sr = 0.051 + 0.23(salinity)) (Fig. 3). Strontium concentrations were highly correlated with those of Ca ($r = 0.99$, $n = 18$, $P < 0.0001$). Similarly, Sr:Ca ratio in water samples was correlated with the concentrations of Ca ($r = 0.83$, $n = 18$, $P < 0.0001$) and Sr ($r = 0.83$, $n = 18$, $P < 0.0001$).

Water temperature in the rearing tanks ranged from 4.86 to 10.74 °C and was positively correlated with the proportion of seawater and hence salinity ($r = 0.85$) (Table 1). On the two occasions when synoptic samples were collected from all five rearing tanks and the inflow, there was little to no variation in salinity, Ca, Sr, and Sr:Ca molar ratios as indicated by low coefficients of variation for each parameter (Table 2). As a result, water samples collected from the inflow appear to represent conditions in each rearing tank. Diel temperature variation was low in the fresh water treatment and increased as the proportion of salt water increased.

Survival to completion of the experiment was 100% in chinook salmon, coho salmon, and sockeye salmon. Survival of rainbow trout was 100% until the 18.8-psu salinity treatment and declined to 2% at the conclusion of the experiment. Similarly, survival of Arctic char was 100% through the 25.5-psu salinity treatment and declined to 65% at the conclusion of the experiment.

Within each species, otolith Sr:Ca ratios increased significantly with ambient salinity (Fig. 4). Mean otolith Sr:Ca ratios were significantly different among salinity treatments within each species (Table 3). As indicated by Tukey–Kramer multiple range tests, there were significant pairwise differences ($P < 0.05$) in otolith Sr:Ca ratios between all treatments in rainbow trout (Fig. 4d) and Arctic char (Fig. 4e). In chinook salmon (Fig. 4a), coho salmon (Fig. 4b), and sockeye salmon (Fig. 4c), however, mean otolith Sr:Ca ratios were not significantly different among all treatments. Mean otolith Sr:Ca ratios between the otolith growth regions asso-
mean otolith Sr:Ca ratios were not significantly different in chinook salmon (Fig. 4a) and sockeye salmon (Fig. 4c). Similarly, mean otolith Sr:Ca ratios between the otolith growth regions associated with the 18.6- and 25.5-psu growth regions were not significantly different in coho salmon (Fig. 4b) and sockeye salmon (Fig. 4c). Mean otolith Sr (weight percent) also varied significantly among salinity treatments (Table 3). Tukey–Kramer pairwise comparisons of otolith Sr were identical to those reported for otolith Sr:Ca ratios. Mean otolith Ca (weight percent) did not vary greatly among salinity treatments (Table 3). Only one pairwise comparison of otolith Ca was significant and that was between the fresh water treatment and the 33.0-psu treatment in sockeye salmon.

Otolith Sr:Ca ratios were positively correlated with salinity in each species: chinook salmon ($r^2 = 0.84$, $n = 77$, $P < 0.0001$), coho salmon ($r^2 = 0.81$, $n = 126$, $P < 0.0001$), sockeye salmon ($r^2 = 0.86$, $n = 73$, $P < 0.0001$), rainbow trout ($r^2 = 0.80$, $n = 109$, $P < 0.0001$), and Arctic char ($r^2 = 0.91$, $n = 90$, $P < 0.0001$). The slopes of these regressions were not homogeneous (ANVOA test for homogeneity of slopes, $F = 56.10$, $P < 0.001$), indicating that the rate of Sr incorporation varied among species. The predictive resolution (Prairie 1996) of salinity based on otolith Sr:Ca ratios ranged from 2.35 to 2.88 categories, suggesting that, at best, two or three salinity environments can be predicted based on this relationship. Similarly, the 95% confidence intervals for individual prediction ranged from 14 to 18 psu.

Differences among species were suggested by the lack of homogeneity of the slopes of the otolith Sr:Ca ratio to salinity relationship reported above. There were significant differences in otolith Sr:Ca ratios among species in the fresh water treatment (salinity = 0.1; $F_{[4,78]} = 4.45$, $P = 0.005$) and differences in otolith Sr:Ca ratios among species increased as salinity increased: 6.3 salinity ($F_{[4,78]} = 6.01$, $P < 0.001$), 12.7 salinity ($F_{[4,78]} = 18.63$, $P < 0.0001$), 18.6 salinity ($F_{[4,78]} = 36.19$, $P < 0.0001$), 25.5 salinity ($F_{[4,73]} = 50.55$, $P < 0.0001$), and 33.0 salinity ($F_{[4,55]} = 33.92$, $P < 0.0001$).

Estimates of $D_{Sr}$ ranged from 0.16 to 0.60 and varied among salinity treatments and among species (Fig. 5). Generally, $D_{Sr}$ was greatest in the fresh water treatment, declined to a minimum in the 6.3-ppt salinity treatment, and gradually increased, thereafter, with increasing salinity. For all species, estimates of $D_{Sr}$ during the fresh water treatment were similar to values reported by Wells et al. (2003) for westslope cutthroat trout in fresh water. For chinook salmon and coho salmon, estimates of $D_{Sr}$ during the salinity treatments were similar to those reported for marine species (Campana 1999; Bath et al. 2000). In the sockeye salmon, Arctic char, and especially rainbow trout treatments, $D_{Sr}$ continually increased with increasing salinity.

Mean fork length of fish varied among species at both the beginning and the end of the experiment (Table 4). Because the species were of different ages, they varied in size at the start of the experiment. The chinook salmon were over 1 year old and the other species were under 1 year old. When all species were pooled, otolith Sr:Ca ratios within
each salinity treatment (>0.1 psu) were negatively correlated with fork length at the conclusion of the experiment and this relationship became stronger as salinity increased: 0.1 salinity \((r = -0.07, n = 74, P = 0.55)\), 6.3 salinity \((r = -0.32, n = 76, P < 0.01)\), 12.7 salinity \((r = -0.67, n = 74, P < 0.0001)\), 18.6 salinity \((r = -0.77, n = 75, P < 0.0001)\), 25.5 salinity \((r = -0.82, n = 73, P < 0.0001)\), 33.0 salinity \((r = -0.83, n = 58, P < 0.0001)\).

**Discussion**

Use of otolith Sr or Sr:Ca ratios to describe migration among fresh water, brackish, and saltwater habitats by salmonids is supported by the results of this study. Otolith Sr and Sr:Ca ratios were linearly related to salinity and hence ambient Sr. Given the magnitude and variability of otolith Sr:Ca ratios observed, however, predictive capabilities provide only enough sensitivity to discriminate between fresh water, brackish water, and seawater. Kraus and Secor (2003) similarly concluded that inference from otolith Sr:Ca ratios was only sensitive enough to discriminate between periods of fresh water and saltwater occupation in elvers of the American eel. Because they reared elvers to approximately 22 ppt salinity, they suggested that extrapolation to 25 or 30 ppt might support discrimination between brackish and ocean occupation. In spite of this lack of sensitivity, the method will remain a powerful tool in the study of diadromous fishes as long as this sensitivity is considered. Depending on the question, this resolution may be adequate to describe migration and aid in the study of sympatric life history types. For example, Zimmerman et al. (2003) were able to confirm marine migration in precocious chinook salmon based on increases in otolith Sr:Ca ratios. Although it would have been beneficial to identify the extent of that migration in terms of salinity, identifying that these fish migrated to salt water at all was significant. More detailed analysis of the range of salinity encountered by these fish would require methods with a greater sensitivity and hence predictive capabilities.

Because this study did not include replicates for each species, it is inappropriate to draw too much from the comparisons among species. It does suggest, however, that there may be differences among species. Although the linear relationship of increasing otolith Sr:Ca ratios with salinity was true for all species, incorporation of Sr varied among even closely related species in the same genus. As a result, it may not be adequate to use the results from other species as reference.
points when determining migrations among salinity environments based on otolith Sr. Howland et al. (2001), for example, suggested that otolith Sr levels observed across otolith transects of inconnu were similar to those measured in anadromous Arctic char held in salt water, thus indicating that the inconnu had migrated to salt water. They warned, however, that rather than suggesting residence in salt water, these results could mean that Sr is incorporated at different rates by these two species. The variation among species observed in this study indicates that this warning is well founded and results from other species should be used as benchmarks with caution.

Although the results from this study followed the expected trend of increasing otolith Sr:Ca ratios with increasing salinity, results from the rainbow trout treatment lead to further questions. For example, the mean otolith Sr:Ca ratios observed in the 12.7-, 18.6-, 25.5-, and 33.0-ppt salinity treatments were all higher than values typically observed in the ocean growth region of wild steelhead (assumed salinity >32 ppt). Mean otolith Sr:Ca ratio in the saltwater growth region of steelhead (C.E. Zimmerman, unpublished data) from the Deschutes River, Oregon, was 0.0021 (SD = 0.0003, n = 31), from the Babine River, British Columbia, was 0.0021 (SD = 0.0004, n = 20), and from the Saichek River, Kamchatka, was 0.0024 (SD = 0.0003, n = 8), whereas in the rainbow trout in this study, mean otolith Sr:Ca ratio in the 25.5-ppt salinity treatment was 0.0031 (SD = 0.002, n = 18) and in the 33.0-ppt salinity treatment was 0.0038 (SD = 0.0004, n = 4). The reason for this is unclear but may be related to population-specific differences in Sr incorporation at higher salinity or to ontogenetic-related changes in osmotic regulatory capabilities. The hatchery population of rainbow trout used in this study was originally founded from a resident population and therefore may exhibit different osmotic capabilities and associated otolith incorporation processes than populations characterized by anadromous phenotypes (steelhead). Boula et al. (2002) showed that even sympatric resident and anadromous brook trout (char) (Salvelinus fontinalis) were characterized by physiological differences related to saltwater adaptability. Resident char did not show the same Na/K ATPase activity observed in anadromous char and anadromous char were characterized by higher plasma osmolality and Cl and Na concentrations. In this study, rainbow trout experienced increasing mortality as salinity increased in this experiment, suggesting that they were not capable of dealing with the saltwater transition. If the rainbow trout are not capable of dealing with increasing osmotic regulatory demands associated with transfer to salt water, changes in blood chemistry and ion balance may result in changes in otolith incorporation rates. In addition, steelhead juveniles typically do not migrate to sea until at least age 1. In this experiment, the rainbow trout were transferred into seawater at least a year earlier, and at a much smaller size than that observed in wild steelhead populations. Since osmo-

Table 3. Summary of ANOVA results for otolith Ca, otolith Sr, and otolith Sr:Ca ratios measured in otolith growth regions associated with experimental salinity treatments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ca (weight %)</th>
<th>Sr (weight %)</th>
<th>Sr:Ca ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F ratio df P</td>
<td>F ratio df P</td>
<td>F ratio df P</td>
</tr>
<tr>
<td>Chinook salmon (Oncorhynchus tshawytscha)</td>
<td>0.80 5,71 0.55</td>
<td>80.12 5,71 &lt;0.0001</td>
<td>79.91 5,71 &lt;0.0001</td>
</tr>
<tr>
<td>Coho salmon (Oncorhynchus kisutch)</td>
<td>2.25 5,120 0.05</td>
<td>218.06 5,120 &lt;0.0001</td>
<td>214.35 5,120 &lt;0.0001</td>
</tr>
<tr>
<td>Sockeye salmon (Oncorhynchus nerka)</td>
<td>2.33 5,67 0.05</td>
<td>94.33 5,67 &lt;0.0001</td>
<td>93.64 5,67 &lt;0.0001</td>
</tr>
<tr>
<td>Rainbow trout (Oncorhynchus mykiss)</td>
<td>0.76 5,103 0.26</td>
<td>79.53 5,103 &lt;0.0001</td>
<td>79.27 5,103 &lt;0.0001</td>
</tr>
<tr>
<td>Arctic char (Salvelinus alpinus)</td>
<td>0.87 5,84 0.50</td>
<td>158.60 5,84 &lt;0.0001</td>
<td>168.09 5,84 &lt;0.0001</td>
</tr>
</tbody>
</table>

Fig. 5. Mean (±SE) distribution coefficients of Sr (D) versus salinity for (a) chinook salmon (Oncorhynchus tshawytscha), (b) coho salmon (Oncorhynchus kisutch), (c) sockeye salmon (Oncorhynchus nerka), (d) rainbow trout (Oncorhynchus mykiss), and (e) Arctic char (Salvelinus alpinus).
regulation related to smolting is seasonal and related to fish size (Clarke and Hirano 1995), these fish may have been transferred to salt water at an age or size where smolting is not feasible. Further work is needed to elucidate the impacts of smolting and associated changes in osmoregulation and blood chemistry on otolith growth and incorporation of Ca and Sr. For example, calcitonin, a hormone that appears to regulate Ca exchange in gills and bones, increases following the transfer from fresh water to salt water in rainbow trout, during the upstream migration of adult sockeye salmon, and in relation to maturation (Watts et al. 1975; Fouchereau-Peron et al. 1986; Clarke and Hirano 1995). Kalish (1989) argued that an increase in Ca-binding proteins, such as albumin or vitellogenin, in the blood would result in reduced Ca in the endolymph and hence in the otolith. Given the potential role of calcitonin in regulating Ca and its fluctuation during the fresh water to marine transition and maturation of salmonids, its relationship to otolith formation should be investigated.

Along with the findings of Wells et al. (2003), this study suggests that further work is needed to examine the kinetics of Sr incorporation in fresh water and diadromous species. The $D_{Sr}$ values for all species in the fresh water treatment of this study were similarly high and comparable with that reported by Wells et al. (2003) for cutthroat trout in fresh water. The $D_{Sr}$ values associated with the salinity treatments, however, were similar to values reported for marine fishes (Kalish 1989, 1991; Bath et al. 2000). However, $D_{Sr}$ values were consistently high in rainbow trout, Arctic char, and sockeye salmon across the range of salinity treatments. The $D_{Sr}$ values were similar among species at the 6.3-ppt salinity treatment but diverged as salinity increased. The $D_{Sr}$ in chinook salmon was similar to values reported for marine fishes across the range of salinity treatments. The $D_{Sr}$ in rainbow trout, however, steadily increased as salinity increased. Similarly, Arctic char and sockeye salmon exhibited higher than expected $D_{Sr}$ as salinity increased. Perhaps increased $D_{Sr}$ in these cases reflects ontogenetic variation in seawater adaptation or population-level differences in seawater adaptability as discussed above. Wells et al. (2003) suggested that the elevated value observed in fresh water cutthroat trout reflected the difference between Sr and Ca uptake in fresh water versus marine environments. They suggested that this difference was the result of the different pathway of elemental uptake between marine and fresh water environments (through intestinal walls for marine fishes and through chlo-

ride cells in the gills for fresh water fishes) (Wells et al. 2003).

Geographic and seasonal variation of ambient Sr in fresh waters should be considered in all studies examining migration among salinity environments. Ambient concentrations of Sr and Ca should be determined among the habitats to be studied (Rieman et al. 1994; Wells et al. 2003; Zimmerman et al. 2003). Given the potential variability of ambient Sr resulting from differing lithology and hydrology, it is critical that analysis of ambient water chemistry is a part of all studies using otolith Sr:Ca ratios to reconstruct movements among salinity environments to ensure that fresh waters conform to the assumption of low Sr. Several questions remain concerning the application of otolith Sr and Sr:Ca ratios in the study of fresh water and marine migrations of salmonids. These questions include uncertainty concerning the role of smolting and maturation on incorporation of Ca and Sr in otoliths. This is especially critical, since these are processes occurring as fish make the transition between salinity environments (the very thing that we are seeking to identify using otolith Sr:Ca ratios). In addition, the roles of temperature (Elsdon and Gillanders 2002) and dietary Sr are still unresolved.

In spite of limited resolution in terms of predicting salinity, otolith Sr concentrations or Sr:Ca ratios provide an important tool in the study of migration in diadromous salmonids. Continued utility of the method, however, will require further refinement, validation, and research concerning the interactive effects of ambient water chemistry, temperature, and physiology.

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References


Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., and Jones, C.M. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d’Alene River, © 2005 NRC Canada


