

Evaluating otolith Sr/Ca as a tool for reconstructing estuarine habitat use

Corey C. Phillis, David J. Ostrach, B. Lynn Ingram, and Peter K. Weber

Abstract: There is no standard method to determine the applicability of otolith Sr/Ca ratio to reconstructing estuary use. We have developed a novel method to determine the response of otolith Sr/Ca to changes in water Sr/Ca and salinity in San Francisco Estuary (California, USA). We perform correlated, spatially resolved Sr/Ca and Sr isotope measurements using otoliths from adult striped bass (*Morone saxatilis*) in the San Francisco Estuary to estimate the otolith–water Sr/Ca partition coefficient ($D_{Sr} = 0.305 \pm 0.009$). D_{Sr} did not vary significantly with salinity, and therefore the salinity–otolith Sr/Ca model was constructed by substituting the partition coefficient into the nonlinear salinity–water Sr/Ca mixing model for the system. The model demonstrates that the primary factor controlling the response of Sr/Ca to salinity is the Ca concentration in the freshwater source flowing into the estuary. A concentration of 60 ppm Ca is an approximate threshold below which estuary Sr/Ca increases rapidly to near the marine Sr/Ca at low salinities (5‰–15‰), thereby providing sharp delineation of estuary entrance, but little to no discrimination among higher salinity habitats. Our approach provides a general framework for assessing the potential utility of Sr/Ca in estuarine systems and specifically for the San Francisco Estuary.

Résumé : Il n'y a pas de méthode standard pour déterminer l'applicabilité du rapport Sr/Ca dans les otolithes pour la reconstitution de l'utilisation d'un estuaire. Nous avons mis au point une nouvelle méthode pour déterminer la réponse de Sr/Ca des otolithes aux changements de Sr/Ca et de salinité de l'eau dans l'estuaire de San Francisco (Californie, É.-U.). Nous avons obtenu des mesures corrélées et à résolution spatiale de Sr/Ca et de l'isotope Sr à partir d'otolithes du bar d'Amérique (*Moxone saxatilis*) dans l'estuaire de San Francisco afin d'estimer le coefficient de partitionnement otolithe–eau de Sr/Ca ($D_{Sr} = 0,305 \pm 0,009$). D_{Sr} ne varie pas de façon significative en fonction de la salinité; nous avons donc construit le modèle salinité–Sr/Ca de l'otolithe en substituant le coefficient de partitionnement dans le modèle de mélange non linéaire de salinité–eau Sr/Ca du système. Le modèle démontre que le facteur principal de contrôle de la réponse de Sr/Ca à la salinité est la concentration de Ca dans la source d'eau douce qui se déverse dans l'estuaire. La concentration de 60 ppm de Ca représente le seuil approximatif sous lequel le Sr/Ca de l'estuaire augmente rapidement pour s'approcher du Sr/Ca de la mer à salinités basses (5–15 ‰), ce qui produit une nette délimitation de l'entrée de l'estuaire, mais peu ou pas de discrimination entre les habitats de plus forte salinité. Notre méthodologie fournit un cadre général pour l'évaluation de l'utilité potentielle de Sr/Ca dans les systèmes estuariens et en particulier dans l'estuaire de San Francisco.

[Traduit par la Rédaction]

Introduction

Estuaries are increasingly degraded habitats. Species that use estuaries for part or all of their life cycle often suffer from threats ranging from contaminants to habitat loss. Populations of striped bass (*Morone saxatilis*) in the San Francisco Estuary (California, USA) have steadily declined since the mid-1960s. The cause of this decline is attributable to several potential factors, including water diversions, food

limitation, introduced species, and climate change (Bennett and Moyle 1996; Sommer et al. 2007; Stevens et al. 1985), as well as contaminant effects on early life stages, particularly from maternal transfer of xenobiotics (Bailey et al. 1994; Bennett et al. 1995; Ostrach et al. 2008). Relating habitat use to fish health and the bioaccumulation of contaminants may provide new insight into the problems with striped bass and other pelagic fish in the San Francisco Estuary (Feyrer et al. 2007b; Sommer et al. 2007). To suc-

Received 18 September 2009. Accepted 5 August 2010. Published on the NRC Research Press Web site at cjfas.nrc.ca on 29 January 2011.
J21422

Paper handled by Associate Editor Steven Campana.

C.C. Phillis^{1,2} and **B.L. Ingram**. Department of Earth and Planetary Sciences, University of California, Berkeley, CA 94720, USA.
D.J. Ostrach. Pathobiology, Conservation & Population Biology Laboratory, John Muir Institute of the Environment, Center for Watershed Sciences, University of California – Davis, One Shields Avenue, Davis, CA 95616, USA.
P.K. Weber.³ Chemical Sciences Division, Lawrence Livermore National Laboratory, L-231, P.O. Box 808, Livermore, CA 94551, USA.

¹Corresponding author (e-mail: phillis@biology.ucsc.edu).

²Present address: Department of Ecology and Evolutionary Biology, University of California – Santa Cruz, 100 Shaffer Road, Santa Cruz, CA 95060, USA.

³Corresponding author (e-mail: weber21@llnl.gov).

cessfully protect fish populations from these threats common in estuaries, managers need a thorough understanding of the populations' temporal and spatial use of the habitat.

Otolith microanalysis has become a popular method to reconstruct habitat use (Campana 1999). Otoliths are calcium carbonate concretions in the inner ear of bony fish that accrete incrementally (Campana and Neilson 1985). The chemistry of these increments can be directly related to the chemistry of the water the fish were living in and (or) the food they were eating at the time that the increments were deposited (Campana 1999). In many ways, otolith microchemistry provides advantages over traditional tagging techniques. Because otoliths will act as passive recorders of the environment in all bony fish, there is a near 100% tag rate in the case of natural markers (see Almany et al. 2007 for an example of artificial otolith markers). These properties allow a chronology of movement for any fish in a population to be developed.

Otolith Sr/Ca ratio is a commonly used marker for hindcasting habitat utilization and migration histories of euryhaline fish (Secor and Rooker 2000). It can readily identify habitat shifts of diadromous fish between fresh and saline waters in most systems (e.g., American shad (*Alosa sapidissima*; Limburg 1995), steelhead trout (*Oncorhynchus mykiss*; Kalish 1990), and European eel (*Anguilla anguilla*; Tzeng et al. 1997)). Inferring movements of fish within estuarine habitat, however, requires a model that accounts for the local water chemistry, as well as the response of individual species to that water chemistry (Kraus and Secor 2004).

The two methods that have been used to determine the otolith response to water Sr/Ca and salinity are laboratory rearing experiments and field caging experiments. These approaches are labor intensive and have not always resulted in data that is applicable to the natural system. A number of laboratory rearing experiments have encountered problems reproducing the natural system Sr/Ca or salinity curves of interest (Bath et al. 2000; Secor et al. 1995). In many cases, the problem appears to be the use of artificial seawater with Sr/Ca levels that are ~33% greater than normal seawater. In the case of Bath et al. (2000), the use of artificial seawater for rearing experiments with marine fish resulted in estimates of the Sr/Ca partition coefficient, D_{Sr} , ~33%–150% greater than commonly observed in marine systems. Caging experiments are problematic in estuaries because of biofouling and other unnatural stressors that can result in inconsistent otolith growth and samples lost to mortality (Kraus and Secor 2004).

Here, we report a new approach to determining the otolith response to water Sr/Ca and salinity using correlated Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratio measurements on otoliths from the study population of adult striped bass. Strontium isotopes, unlike elemental ratios, are not substantially biologically modulated (Sugerman et al. 1997) and therefore can be used to back-calculate water salinity directly. We use pre-existing Sr isotope and Sr concentration gradient data for the San Francisco Estuary (Ingram and Sloan 1992) and our correlated Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ ratio measurements on otoliths to infer striped bass otolith Sr/Ca response to changes in salinity and water Sr/Ca ratio. As part of this work, we develop a generalized mathematical approach for relating otolith Sr/Ca to water Sr/Ca and salinity. We also assess the usefulness of

otolith Sr/Ca given freshwater Sr and Ca concentrations in this estuary.

Materials and methods

Adult female and male striped bass were collected from the Sacramento River between Knights Landing and Colusa, California, on a weekly basis during the spawning seasons in 1999 and 2001 (Fig. 1). Standard electrofishing methods were used. Fish were transported live to Professional Aquaculture Service's Hatchery Facility in Chico, California. Twenty-four striped bass females were selected for otolith microchemistry analysis, ranging from 58 to 101 cm in standard length, 2.3 to 15.9 kg in mass, and between 5 and 10 years in age (Table 1).

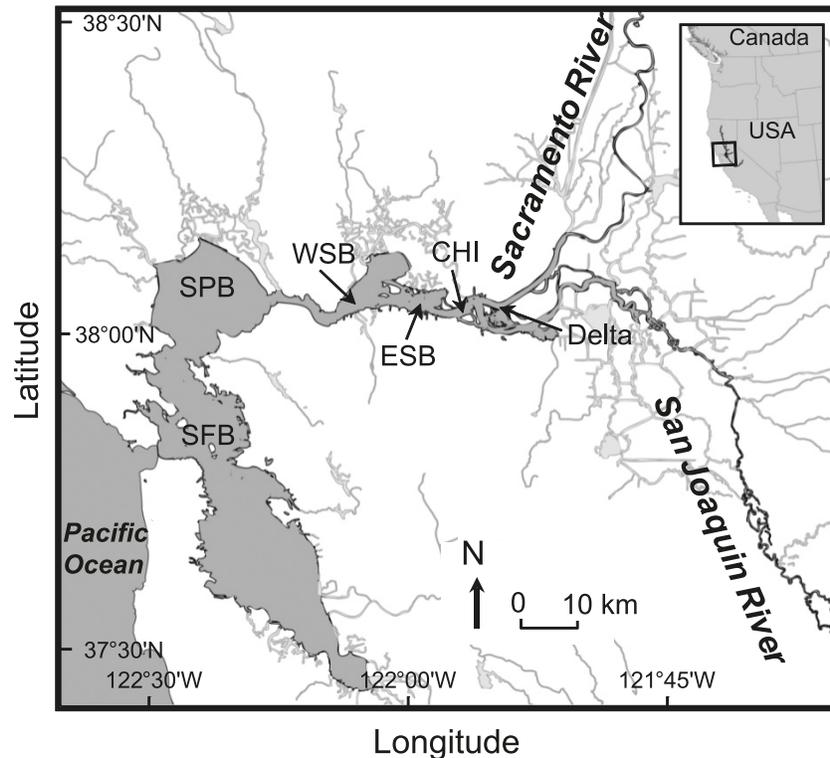
Females were euthanized using a solution of 250 mg·L⁻¹ tricaine methane sulfonate (MS222; Argent Chemical Laboratories, Redmond, Washington, USA). Heads were removed posterior to the gill arches and frozen at -20 °C until processing. Hatchery-reared F₂ generation striped bass from a San Francisco Estuary lineage were used as the controls in this study and treated in the same manner to obtain their otoliths. Otoliths were removed and stored in coded scintillation vials at room temperature until analysis.

For the purpose of ageing the fish, otoliths were baked at 400 °C for approximately 2 min to enhance the opaqueness of the annuli. Baking does not substantially affect the Sr/Ca ratio of aragonites (corals, echinoid, red algae, and foraminifera heated to 200 °C for 24 h; Gaffey et al. 1991; euhedral crystal powdered and heated to 400 °C for 2 h; Love and Woronow 1991), presumably because Sr substitutes for Ca in the aragonite matrix (Bath et al. 2000). Likewise, baking is not an issue for Sr isotope analyses and is sometimes used to oxidize the organic matrix of teeth and bone prior to Sr isotope analysis (Koch et al. 1992). Therefore, we would not expect the baking of the otoliths to have an effect on the chemical and isotopic properties of the otoliths investigated here.

Ageing and geochemical analysis required the otoliths to be sectioned and polished. After baking, otoliths were embedded in epoxy (Eponate 12) and sectioned in a transverse plane to expose the otoliths' cores. Sectioned otoliths were mounted on 1 inch (1 inch = 2.5 cm) glass rounds with epoxy and polished with a series of sandpapers to reach a 0.25–0.75 mm thickness suitable for viewing the otoliths' annuli under a transmitted light microscope. A final polish using 3 μm alumina eliminated surface imperfections that can cause artifacts in microprobe analysis (Kalish 1989).

Geochemical analyses of otoliths were performed on three instruments. An electron microprobe was used to measure Sr/Ca ratios in the otoliths of the 24 wild-caught and two hatchery-reared striped bass. Nine of these otoliths were also analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to obtain Sr/Ca ratios. The Sr isotopic composition ($^{87}\text{Sr}/^{86}\text{Sr}$) was measured in 13 otoliths by multicollector LA-ICP-MS (Table 1). Mean lifetime Sr/Ca of a hatchery otolith analyzed on both the electron microprobe and LA-ICP-MS were not significantly different (two-sample *t* test, $t = 0.814$, 106 df, $p = 0.417$); therefore, we correlated Sr isotope analyses to the LA-ICP-MS Sr/Ca analyses when possible. This enabled a

Fig. 1. Map of San Francisco Estuary (California, USA), including locations where surface water was sampled in Ingram and Sloan (1992) and used in this study to develop Sr/Ca and $\Delta^{87}\text{Sr}$ mixing curves. Striped bass for this study were collected between Knights Landing and Colusa, approximately 145–230 river kilometres upstream of the Delta site on the Sacramento River. SFB, San Francisco Bay; SPB, San Pablo Bay; WSB, West Suisun Bay; ESB, East Suisun Bay; CHI, Chippis Island.



direct correlation between Sr isotope and Sr/Ca analyses. The estimated partition coefficient from correlated Sr isotope and Sr/Ca (see Results) was not significantly different when instrument type was evaluated as a covariate (analysis of covariance, ANCOVA test for homogeneity of slopes, $F_{[1,108]} = 1.301$, $p = 0.256$), and hereafter the instrument used to acquire Sr/Ca is not considered.

Prior to electron microprobe analysis, the otoliths were carbon-coated in a high vacuum chamber to prevent the sample from charging during electron beam analysis. The Sr/Ca ratios were quantified using wavelength dispersive analysis with a JEOL JXA 8200 electron probe microanalyzer (EPMA) at the Institute for Geophysics and Planetary Physics, Lawrence Livermore National Laboratories, Livermore, California. Microprobe analyses (spot size 10 μm , 15 kV accelerating voltage, 20 nA probe current) were arranged in transects along the dorsal margin of the sulcal groove where annuli are most defined. Microprobe analyses were placed closer together in areas of narrower annuli to achieve approximately 13 analyses per year (age 0–2 years, step size 24 μm ; middle years, step size 12 μm ; and last 1–2 years of growth, step size ~6–8 μm). These analyses left minor damage marks on the otolith, which were used for chronological reconstruction and correlation with other analyses. The EPMA was calibrated using in-house standards of calcite and strontium titanate and checked against calcite with known Sr/Ca ratios (Weber et al. 2005).

Based on the electron microprobe results, seven otoliths representing the full range of observed Sr/Ca life-history

profiles were selected for correlated Sr/Ca and Sr isotope analysis at the University of California – Davis Interdisciplinary Center for Plasma Mass Spectrometry (<http://icpms.ucdavis.edu>). The Sr isotope data for these otoliths were skewed towards freshwater values, and therefore another six otoliths with regions of high Sr/Ca were analyzed for Sr isotopes in an effort to obtain a data set that represented the full range of possible habitat salinities. For this second set of otoliths, Sr isotopes were correlated to the original electron microprobe Sr/Ca results.

The seven otoliths representing the full range of observed Sr/Ca life-history profiles were additionally analyzed using an LA-ICP-MS with an Agilent 7500a ICP-MS. Prior to analysis, the carbon coating was removed using an ultrasound bath and a mild detergent, and then the otoliths were rinsed thoroughly in deionized water (Milli-Q). The otoliths were microsampled with a New Wave Research UP-213 laser ablation system equipped with an Nd:YAG deep UV (213 nm) laser (New Wave Research, Inc., Fremont, California). Single spots adjacent to the electron microprobe otolith analyses were assayed using a laser beam size of 20 μm , 100% laser power, and 15 Hz repetition rate. Helium was used as the carrier gas at a flow rate of 0.85 L·min⁻¹ and mixed with Ar at a flow rate of ~1.0 L·min⁻¹ prior to introduction to the mass spectrometer. Data were collected in 90 s increments in time-resolved mode, which includes background signal (30 s), the sample signal (30 s), and washout (30 s). A line of spots was placed on each otolith from core to rim with 40 μm steps. Measurements of the standard were taken before and after each line of spots.

Table 1. Age, length, and mass of fish sampled.

Otolith ID ^a	Age (years)	Length (cm)	Mass (kg)	EPMA (Sr/Ca)	LA-ICP-MS (Sr/Ca)	MC-LA-ICP-MS (Sr isotopes)
9R99	7	80	8.2	×		
10R99	—	88	15.9	×		
11R99	7	85	11.4	×		×
13R99	7	74	6.8	×	×	×
14R99	7	77	8.2	×	×	
15R99	6	73	7.3	×		
16R99	7	83	9.1	×	×	×
17R99	6	71	5.5	×		×
18R99	9	94	13.6	×	×	×
19R99	9	91	13.6	×		×
20R99	9	98	15.9	×	×	×
23R99	—	64	4.5	×		
25R99	10	101	13.6	×		
27R99	—	87	9.1	×		
28R99	5	60	3.2	×	×	×
1R01	7	74	6.8	×	×	×
3R01	7	79	9.1	×		×
4R01	7	76	6.8	×		×
5R01	10	100	15.9	×		
7R01	9	94	13.6	×		
11R01	7	79	5.5	×		
13R01	10	93	11.4	×		
14R01	6	58	2.3	×	×	×
15R01	10	94	15.9	×		×

Note: 13 of the 24 otoliths analyzed for Sr/Ca were subsampled for correlated Sr isotope analysis by multicollector laser ablation inductively coupled plasma mass spectrometry (MC-LA-ICP-MS). EPMA, electron probe microanalysis.

^aLast two digits of otolith ID correspond to year the fish was collected.

For this study, Sr was the analyte of interest. Counts per second of ⁸⁸Sr were background-subtracted, ratioed to ⁴³Ca (used as the internal standard), then standardized to NIST-612 glass standard (Pearce et al. 1997). Concentrations of total Sr were ratioed to Ca (expressed as mmol·mol⁻¹).

The Sr isotopic composition was measured in single spots adjacent the Sr/Ca measurements using a multicollector ICP mass spectrometer (Nu Plasma HR manufactured by Nu Instruments Ltd., UK). The same laser ablation system described above was used with 40–80 μm beam size with 80–160 μm steps, 80%–100% power, and 10 Hz. Typical ⁸⁸Sr signals of 2–6 V were obtained during the analyses. The laser was typically turned on for 30–60 s. Background signals, measured for 30 s prior to sample analysis, were subtracted from the measured signals automatically. After the sample change (i.e., exposing sample cell to the air), measurements were not made until ⁸⁴Kr and ⁸⁶Kr stabilized. A value of ⁸⁶Sr/⁸⁸Sr = 0.1194 was used to correct for instrumental fractionation in accordance with the exponential law. The peak intensities for ⁸⁸Sr, ⁸⁷Sr, ⁸⁶Sr, ⁸⁵Rb, and ⁸⁴Sr are measured simultaneously. The ⁸⁵Rb peak is monitored to correct for the ⁸⁷Rb interference on ⁸⁷Sr. The accuracy of the measured ⁸⁷Sr/⁸⁶Sr ratios was verified by measuring NIST-612 glass standard and an in-house marine carbonate standard (0.709189) throughout the analytical session. All ⁸⁷Sr/⁸⁶Sr ratios are expressed as Δ⁸⁷Sr values to allow for comparisons between otoliths run during different sessions and to allow for interlaboratory comparisons between otolith and water data:

$$(1) \quad \Delta^{87}\text{Sr} = [({}^{87}\text{Sr}/{}^{87}\text{Sr})_{\text{sample}} - ({}^{87}\text{Sr}/{}^{87}\text{Sr})_{\text{std}}] \times 10^5$$

Sr/Ca and Sr isotope analyses were matched to provide temporally correlated data for this study. Because of the difference in spot size between the Sr/Ca (10 and 20 μm for EPMA and LA-ICP-MS, respectively) and Sr isotope (40–80 μm) analyses, Sr/Ca analyses adjacent to a single Sr isotope analysis were averaged to represent the mean Sr/Ca ratio in the otolith material from which the Sr isotope signal was measured. Distance between correlated Sr/Ca and Sr isotope analyses generally ranged from 100 μm to complete overlap for some electron microprobe spots, in which case locations were reconstructed with before and after Sr isotope analysis images. A visual assessment of each set of correlated analyses was performed to ensure that common otolith growth increments were sampled. We assume that the increments visible at the surface reflect the material sampled at depth because these striped bass otoliths are large and transverse sectioned, resulting in growth increments with negligible curvature that are perpendicular to the section surface. Therefore, we assume that we can reliably relate the Sr/Ca and Sr isotope measurements for the same growth increments at the surface despite the difference in sampling depth between the electron microprobe (<5 μm) and laser ablation systems (~20–30 μm). This assumption may not hold in regions of early otolith growth where the radius of growth increments would be smallest. Therefore, we omitted correlated analyses that used electron microprobe Sr/Ca prior to the third otolith annuli.

Mixing models

The Sr composition of the San Francisco Estuary and its two principal freshwater inputs, the Sacramento and San Joaquin Rivers, have been studied previously (Ingram and Sloan 1992; Paulsen and List 1997; Weber 2002). Ingram and Sloan (1992) demonstrated that Sr concentration and Sr isotopes mix conservatively in this estuary. Here, we derive

a general mixing model to predict water salinity based on otolith Sr isotopic composition using measured salinity, water Sr concentration, and water Sr isotopic composition. The essential equations are derived in detail in Faure (1986).

For a mixture of components *A* and *B* with different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for the mixture (*M*) can be calculated from eq. 9.9 from Faure (1986):

$$(2) \quad (^{87}\text{Sr}/^{86}\text{Sr})_M = (^{87}\text{Sr}/^{86}\text{Sr})_A \times \left(\frac{[\text{Sr}]_A \times f}{[\text{Sr}]_M} \right) + (^{87}\text{Sr}/^{86}\text{Sr})_B \times \left(\frac{[\text{Sr}]_B \times (1-f)}{[\text{Sr}]_M} \right)$$

where $[\text{Sr}]$ is the Sr concentration for the mixture *M* or component *A* or *B*, and *f* is the fraction of component *A* in the mixture, $f = \frac{A}{A+B}$. In this equation, the isotopic ratio in the mixture is weighted for the Sr contributed by the two sources. It can be demonstrated that $\Delta^{87}\text{Sr}$ can be used in the place of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. First, eq. 1 is solved for $(^{87}\text{Sr}/^{86}\text{Sr})_{\text{sample}}$ and substituted for $(^{87}\text{Sr}/^{86}\text{Sr})_M$, $(^{87}\text{Sr}/^{86}\text{Sr})_A$, and $(^{87}\text{Sr}/^{86}\text{Sr})_B$ in eq. 2:

$$(3) \quad \Delta^{87}\text{Sr}_M \times 10^{-5} + (^{87}\text{Sr}/^{86}\text{Sr})_{\text{std}} = \left(\Delta^{87}\text{Sr}_M \times 10^{-5} + (^{87}\text{Sr}/^{86}\text{Sr})_{\text{std}} \right) \times \left(\frac{[\text{Sr}]_A \times f}{[\text{Sr}]_M} \right) + \left(\Delta^{87}\text{Sr}_B \times 10^{-5} + (^{87}\text{Sr}/^{86}\text{Sr})_{\text{std}} \right) \times \left(\frac{[\text{Sr}]_B \times (1-f)}{[\text{Sr}]_M} \right)$$

Rearranging and gathering like terms yields

$$(4) \quad \Delta^{87}\text{Sr}_M \times 10^{-5} = \Delta^{87}\text{Sr}_A \times 10^{-5} \times \left(\frac{[\text{Sr}]_A \times f}{[\text{Sr}]_M} \right) + \Delta^{87}\text{Sr}_B \times 10^{-5} \times \left(\frac{[\text{Sr}]_B \times (1-f)}{[\text{Sr}]_M} \right) + (^{87}\text{Sr}/^{86}\text{Sr})_{\text{std}} \times \left(\frac{[\text{Sr}]_A \times f}{[\text{Sr}]_M} + \frac{[\text{Sr}]_B \times (1-f)}{[\text{Sr}]_M} - 1 \right)$$

The term $\left(\frac{[\text{Sr}]_A \times f}{[\text{Sr}]_M} + \frac{[\text{Sr}]_B \times (1-f)}{[\text{Sr}]_M} - 1 \right)$ can be found to equal zero by substituting $\frac{[\text{Sr}]_M - [\text{Sr}]_B}{[\text{Sr}]_A - [\text{Sr}]_B}$ for *f*, based on the two-component mixing eq. 9.3 from Faure (1986). Thus eq. 4 can be simplified to

$$(5) \quad \Delta^{87}\text{Sr}_M = \Delta^{87}\text{Sr}_A \times \left(\frac{[\text{Sr}]_A \times f}{[\text{Sr}]_M} \right) + \Delta^{87}\text{Sr}_B \times \left(\frac{[\text{Sr}]_B \times (1-f)}{[\text{Sr}]_M} \right)$$

which by inspection is eq. 2 with the $^{87}\text{Sr}/^{86}\text{Sr}$ terms replaced by the respective $\Delta^{87}\text{Sr}$ terms. To use this equation to estimate water salinity from otolith $\Delta^{87}\text{Sr}$, we must eliminate $[\text{Sr}]_M$ and *f*. To achieve this goal, first we substitute $f([\text{Sr}]_A - [\text{Sr}]_B) + [\text{Sr}]_B$ for $[\text{Sr}]_M$ (eq. 9.3, Faure 1986) in eq. 5 and solve for *f*:

$$(6) \quad f = \frac{\Delta^{87}\text{Sr}_M \times [\text{Sr}]_B - \Delta^{87}\text{Sr}_B \times [\text{Sr}]_B}{\Delta^{87}\text{Sr}_A \times [\text{Sr}]_A - \Delta^{87}\text{Sr}_B \times [\text{Sr}]_B - \Delta^{87}\text{Sr}_M([\text{Sr}]_A - [\text{Sr}]_B)}$$

Next, *f* can be eliminated and this equation can be related directly to salinity because for the same mixture:

$$(7) \quad f = \frac{(\text{Salinity}_M - \text{Salinity}_B)}{(\text{Salinity}_A - \text{Salinity}_B)}$$

This equation is substituted in eq. 6 for *f*, subscripts are revised for our application, and the equation is solved for the salinity of the water where the otolith material was deposited:

$$(8) \quad \text{Salinity}_{\text{water}} = \text{Salinity}_{\text{Mar}} \times \frac{\Delta^{87}\text{Sr}_{\text{otolith}} \times [\text{Sr}]_{\text{FW}} - \Delta^{87}\text{Sr}_{\text{FW}} \times [\text{Sr}]_{\text{FW}}}{\Delta^{87}\text{Sr}_{\text{Mar}} \times [\text{Sr}]_{\text{Mar}} - \Delta^{87}\text{Sr}_{\text{FW}} \times [\text{Sr}]_{\text{FW}} - \Delta^{87}\text{Sr}_{\text{otolith}}([\text{Sr}]_{\text{Mar}} - [\text{Sr}]_{\text{FW}})}$$

Here, component *A* is defined as the marine (Mar) end member and component *B* is defined as the freshwater (FW) end member. The equation is simplified based on freshwater salinity being negligible (0.15 parts per thousand or ‰). Marine salinity (~35‰), $\Delta^{87}\text{Sr}_{\text{Mar}}$ (0, by definition), and $[\text{Sr}]_{\text{Mar}}$ (7.9 ppm by mass) are the global marine end members and $\Delta^{87}\text{Sr}_{\text{FW}}$ (-292) and $[\text{Sr}]_{\text{FW}}$ ($9.3 \pm 1.4 \times 10^{-2}$ ppm, median \pm

median absolute deviation; Weber 2002) are the locally measured freshwater end members (Table 2; Fig. 2).

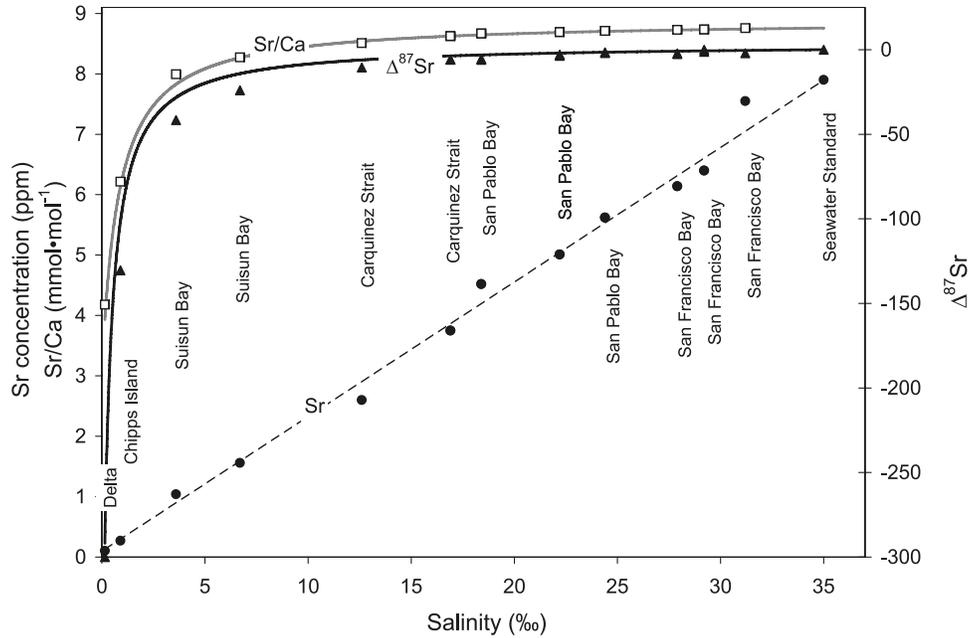
Salinity can also be predicted from water Sr/Ca using a mixing curve. As others have demonstrated (Ingram and Sloan 1992; Surge and Lohmann 2002), Sr and Ca concentrations in estuaries behave conservatively with the proportional mixing of fresh and marine waters:

Table 2. Freshwater and marine end members of the San Francisco Estuary.

End member	Salinity (‰)	Sr (ppm)	Ca (ppm)	Sr/Ca (mmol·mol ⁻¹)	⁸⁷ Sr/ ⁸⁶ Sr	Δ ⁸⁷ Sr
Freshwater	0.15	0.093	11	3.9	0.70627	-291.8
Marine	35	7.9	412	8.8	0.70919	0

Note: Measured freshwater values from Central Sacramento – San Joaquin River Delta (Weber 2002).

Fig. 2. Sr concentration (solid circles, left axis), Δ⁸⁷Sr (solid triangles, right axis), and Sr/Ca (open squares, left axis) at 12 sites within San Francisco Estuary. Calculated Δ⁸⁷Sr (solid black line) and Sr/Ca (solid gray line) mixing curves and the linear fit of Sr concentration (dashed black line) are plotted.



$$(9) \quad [Sr]_{\text{water}} = \text{Salinity}_{\text{water}} \times b_{\text{Sr}} + [Sr]_{\text{FW}}$$

$$(10) \quad [Ca]_{\text{water}} = \text{Salinity}_{\text{water}} \times b_{\text{Ca}} + [Ca]_{\text{FW}}$$

where *b* is the slope of the mixing line calculated by the marine and freshwater end members for salinity and concentration of the element of interest. The slope of the Sr mixing line (*b*_{Sr}), for example, would be

$$(11) \quad b = \frac{[Sr]_{\text{Mar}} - [Sr]_{\text{FW}}}{\text{Salinity}_{\text{Mar}} - \text{Salinity}_{\text{FW}}}$$

A general Sr/Ca mixing model as a function of salinity is the ratio of the mixing lines that describe the respective elemental concentrations:

$$(12) \quad Sr/Ca_{\text{water}} = \frac{\text{Salinity} \times b_{\text{Sr}} + [Sr]_{\text{FW}}}{\text{Salinity} \times b_{\text{Ca}} + [Ca]_{\text{FW}}}$$

By rearranging terms, we solve this equation for water salinity as a function of water Sr/Ca:

$$(13) \quad \text{Salinity}_{\text{water}} = \text{Salinity}_{\text{Mar}} \times \frac{Sr/Ca_{\text{water}} \times [Ca]_{\text{FW}} - [Sr]_{\text{FW}}}{[Sr]_{\text{Mar}} - [Sr]_{\text{FW}} - Sr/Ca_{\text{water}} \times ([Ca]_{\text{Mar}} - [Ca]_{\text{FW}})}$$

Again, the equation is simplified based on freshwater salinity being negligible. The marine end member for Ca is [Ca]_{Mar} = 412 ppm. For the San Francisco Estuary, the freshwater end members are [Sr]_{FW} = 9.3 ± 1.4 × 10⁻² ppm and [Ca]_{FW} = 11 ± 1.1 ppm (Weber 2002). Other constants are as reported above.

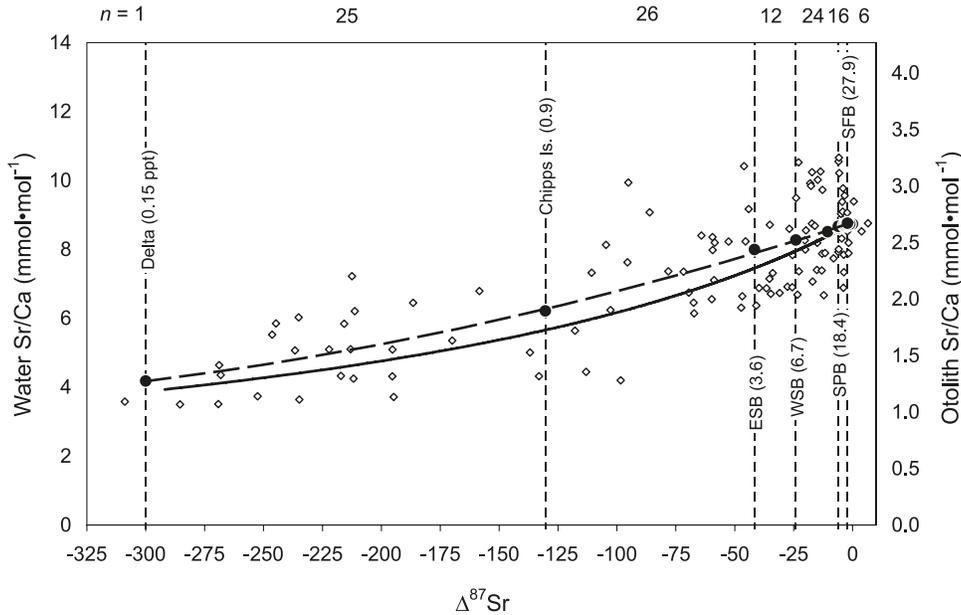
Water Sr/Ca ratio can be calculated from water Sr isotopic composition using eqs. 8 and 13, and the two equations can be combined to model the mixing relationship.

The empirical data of Ingram and Sloan (1992) can also be fit with a polynomial equation:

$$(14) \quad \text{Water Sr/Ca} = 2.29 \times 10^{-5} \Delta^{87}\text{Sr}^2 + 2.22 \times 10^{-2} \times \Delta^{87}\text{Sr} + 8.77$$

(Fig. 3; *r*² = 0.9991, *p* < 0.0001). The polynomial fit of the empirical data and a mixing model of the system's freshwater and marine end members are plotted (Fig. 3). Because

Fig. 3. Comparison of water Sr/Ca (solid circles, left axis) and otolith Sr/Ca (open diamonds, right axis) as functions of otolith $\Delta^{87}\text{Sr}$. The two axes are scaled to each other by the partition coefficient, D_{Sr} . Left axis: Measured water Sr/Ca versus $\Delta^{87}\text{Sr}$ is plotted for 12 sites within San Francisco Estuary (note overlap of eight sites at $\Delta^{87}\text{Sr} > -10$). A polynomial fit of the empirical data (dashed line; water Sr/Ca = $2.29 \times 10^{-5} \Delta^{87}\text{Sr}^2 + 2.22 \times 10^{-2} \Delta^{87}\text{Sr} + 8.77$) and a mixing model of the system's freshwater and marine end members (solid line) are plotted; the polynomial fit is used in this study. Right axis: Correlated otolith $\Delta^{87}\text{Sr}$ and Sr/Ca measurements (open diamonds) have been scaled on the right y axis using the partition coefficient, allowing the similarity in the water and otolith Sr/Ca with respect to $\Delta^{87}\text{Sr}$ to be observed. Six representative sites and their salinities (in parentheses) are displayed for reference (ESB = East Suisun Bay, WSB = West Suisun Bay, SPB = San Pablo Bay, SFB = San Francisco Bay); the number of otolith $\Delta^{87}\text{Sr}$ measurements within each region are listed along the top x axis.



the empirical data is available in this system, we have chosen to use the polynomial fit in this study. It is worth noting that because of the shapes of eqs. 8 and 13, the average otolith Sr/Ca or $^{87}\text{Sr}/^{86}\text{Sr}$ for an analysis spot may not equal the average salinity experienced by the fish over the period of time represented by that portion of the otolith if the fish moved, but the salinities estimated from Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ are not substantially different. Therefore, using otolith analyses to relate otolith Sr/Ca, $^{87}\text{Sr}/^{86}\text{Sr}$, and salinity is accurate.

Salinity is related to location using 1980–2002 salinity data courtesy of the Interagency Program for the San Francisco Estuary and California Department of Fish and Game's San Francisco Bay Study (K. Hieb, California Department of Fish and Game, Bay-Delta Region, 4001 North Wilson Way, Stockton, CA 95205, USA, unpublished data). Inter- and intra-annual variability is a function of proximity to the Pacific Ocean and seasonality, with greatest variability in San Francisco Bay (mean annual range of 19.7‰–29.7‰; March mean salinity of $19.7\text{‰} \pm 8.4\text{‰}$ (mean ± 1 standard deviation, or 1SD) and least variability in the Delta Sacramento River Channel (mean annual range of 0.2‰–2.1‰; February mean salinity of $0.8\text{‰} \pm 2.0\text{‰}$). Markers are placed in figures based on Ingram and Sloan (1992) values, which are representative of spring conditions (regulated flows).

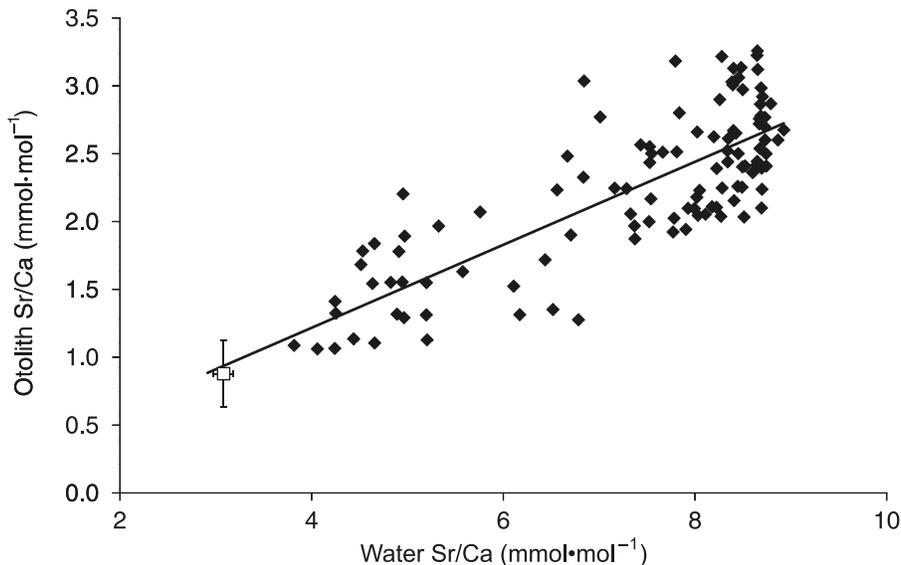
Results

In total 24 otoliths were analyzed by electron microprobe. The distribution of the Sr/Ca data (mean = $2.12 \text{ mmol}\cdot\text{mol}^{-1}$, 1SD = 0.63, $n = 5080$) suggests primarily freshwater and

low salinity estuary residence relative to the range reported for marine ratios in striped bass otoliths (Secor et al. 1995). A total of 13 of the 24 otoliths were selected for correlated Sr/Ca ratio and Sr isotope measurements, comprising seven otoliths representing the full range of observed Sr/Ca life-history profiles and six otoliths with regions of high Sr/Ca. Sr/Ca analyses adjacent to and within the same growth band as an Sr isotope analysis were averaged to represent the Sr/Ca of the material from which the adjacent Sr isotope measurement was taken. The range of Sr/Ca for these correlated measurements ($n = 110$) was $1.07\text{--}3.25 \text{ mmol}\cdot\text{mol}^{-1}$ with a mean of $2.24 \text{ mmol}\cdot\text{mol}^{-1}$ and was only marginally significantly greater than the mean Sr/Ca of all 24 otoliths analyzed on the electron microprobe (one-sample z test, $z = 1.951$, $p = 0.051$).

A total of 110 Sr isotope measurements in 13 otoliths were made (mean $\Delta^{87}\text{Sr} = -77$, range -309 to $+7$). As expected, the mean $\Delta^{87}\text{Sr}$ value of the six otoliths with extended regions of high Sr/Ca ($n = 44$, mean $\Delta^{87}\text{Sr} = -23$) was significantly higher than that of the seven otoliths selected to represent the full range of Sr/Ca life histories ($n = 66$, mean $\Delta^{87}\text{Sr} = -113$; two-sample t test, $t = 6.14$, 108 df, $p < 0.0001$). The mean $\Delta^{87}\text{Sr}$ value of all measurements ($\Delta^{87}\text{Sr} = -77$) and mean of otoliths selected to represent the full range of Sr/Ca life histories ($\Delta^{87}\text{Sr} = -113$) were in the range of the water isotopic composition in the region between east Suisun Bay and Chipps Island; the mean $\Delta^{87}\text{Sr}$ value for otoliths with extended regions of high Sr/Ca ($\Delta^{87}\text{Sr} = -23$) was similar to the water $\Delta^{87}\text{Sr}$ value for the western region of Suisun Bay (Fig. 3). Otolith $\Delta^{87}\text{Sr}$ was

Fig. 4. The otolith Sr/Ca incorporation rate as a function of water Sr/Ca. Water Sr/Ca is calculated from otolith $\Delta^{87}\text{Sr}$ using eq. 14. Data plotted are correlated analyses from 13 otoliths. The partition coefficient is the slope of the linear regression (solid line; $r^2 = 0.62$, $p < 0.0001$) and includes all 110 correlated otolith Sr/Ca and $\Delta^{87}\text{Sr}$ analyses. The combined lifetime mean (± 2 standard deviations, SD) otolith Sr/Ca for two control fish (open square) reared in a hatchery (well water Sr/Ca: 2.98 to 3.19 $\text{mmol}\cdot\text{mol}^{-1}$) are plotted for reference.



used to calculate the salinity at the time of otolith deposition using eq. 8. For all otoliths, salinity values skew toward the freshwater ($<5\text{‰}$) and estuarine (5‰ – 30‰) range; only three in 110 total analyses account for a salinity greater than 30‰ .

Otolith $\Delta^{87}\text{Sr}$ was also used to estimate D_{Sr} , the partition coefficient for Sr between water and otolith aragonite, which is defined as the slope in this relationship:

$$(15) \quad \text{Sr/Ca}_{\text{otolith}} = D_{\text{Sr}} \times \text{Sr/Ca}_{\text{water}}$$

Water Sr/Ca for each otolith Sr/Ca ratio was calculated from the correlated Sr isotope measurement using the curvilinear relationship between measured water Sr/Ca and $\Delta^{87}\text{Sr}$ in the San Francisco Estuary (eq. 14). The otolith $\Delta^{87}\text{Sr}$ -generated water Sr/Ca was plotted against correlated otolith Sr/Ca ($n = 110$; Fig. 4). The y intercept is not statistically different from zero ($p = 0.73$). Therefore, the linear regression was forced through zero to estimate the slope ($D_{\text{Sr}} = 0.305 \pm 0.009$; mean ± 2 standard errors; least-squares regression, $p < 0.0001$, $r^2 = 0.62$). The combined lifetime mean otolith Sr/Ca for the two control fish reared at Professional Aquaculture Service's Hatchery Facility (well water Sr/Ca: 2.98–

3.19 $\text{mmol}\cdot\text{mol}^{-1}$) was $0.88 \text{ mmol}\cdot\text{mol}^{-1}$ ($2\text{SD} = 0.25$) and falls on the regression but was not used to generate the regression. We do not see evidence for changes in D_{Sr} with salinity. At low salinities ($<5\text{‰}$ and $<1\text{‰}$) there is a large range in D_{Sr} for individually calculated correlated analyses; however, the mean value ($D_{\text{Sr}} = 0.304 \pm 0.013$ and 0.310 ± 0.021 for $<5\text{‰}$ and $<1\text{‰}$, respectively) is within error of the overall D_{Sr} (two-tailed z test, $z = 0.70$, $z = 0.43$, respectively). For visual assessment, this partition coefficient is used to scale water Sr/Ca relative to otolith Sr/Ca (Fig. 3), allowing the similarity in the water and otolith Sr/Ca with respect to $\Delta^{87}\text{Sr}$ to be observed.

The results for the relationship between otolith Sr/Ca and water Sr/Ca enable us to construct a model to estimate the salinity of the water that the fish was living in at the time of otolith deposition. Because we observe no change in the otolith–water Sr/Ca relationship, salinity is calculated from measured otolith Sr/Ca by first estimating habitat water Sr/Ca using the partition coefficient in the inverse equation $\text{Sr/Ca}_{\text{water}} = \text{Sr/Ca}_{\text{otolith}} \times D_{\text{Sr}}^{-1}$ and then inserting the estimated water Sr/Ca ratio into the Sr/Ca–salinity mixing model (eq. 13) described in the Materials and methods section:

$$(16) \quad \text{Salinity}_{\text{water}} = \text{Salinity}_{\text{Mar}} \times \frac{\text{Sr/Ca}_{\text{otolith}} \times D_{\text{Sr}}^{-1} \times [\text{Ca}]_{\text{FW}} - [\text{Sr}]_{\text{FW}}}{[\text{Sr}]_{\text{Mar}} - [\text{Sr}]_{\text{FW}} - \text{Sr/Ca}_{\text{water}} \times D_{\text{Sr}}^{-1} \times ([\text{Ca}]_{\text{Mar}} - [\text{Ca}]_{\text{FW}})}$$

Otolith Sr/Ca is positively and nonlinearly related to estimated habitat salinity (Fig. 5). These data are compared with the predicted range of otolith Sr/Ca versus habitat salinity using the otolith–water Sr/Ca partition coefficient substituted into the water Sr/Ca–salinity model. The otolith Sr/Ca data show significant variation for any given salinity

range (Table 3). This variability is consistent across all salinity groups, (Brown–Forsythe test of equal variance, $F_{[11,98]} = 1.32$, $p = 0.22$; $2\text{SD}_{\text{otoSr}, f(\text{sal})} = 0.70 \text{ mmol}\cdot\text{mol}^{-1}$). The 95% confidence interval (CI) on the model ($95\% \text{ CI}_{\text{fit}} = 0.29$ – 0.31) is calculated based on the partition coefficient. A similar number of analyses of otolith Sr/Ca and $\Delta^{87}\text{Sr}$ pre-

Fig. 5. Correlated measurements (open diamonds) of otolith Sr/Ca and salinity as calculated from otolith $\Delta^{87}\text{Sr}$ using eq. 8. The model-predicted salinity is plotted using the 95% confidence intervals of the partition coefficient (long dashed lines) and 1 standard deviation (SD) of the individual calculated partition coefficients from all correlated otolith Sr/Ca – water Sr/Ca measurements (short dashed lines). Four representative data points illustrate the nonlinear transformation of $\Delta^{87}\text{Sr}$ measurement error to calculated salinity (diamond with cross, y error bars represent typical 2 standard errors (SE) of an individual Sr isotope measurement).

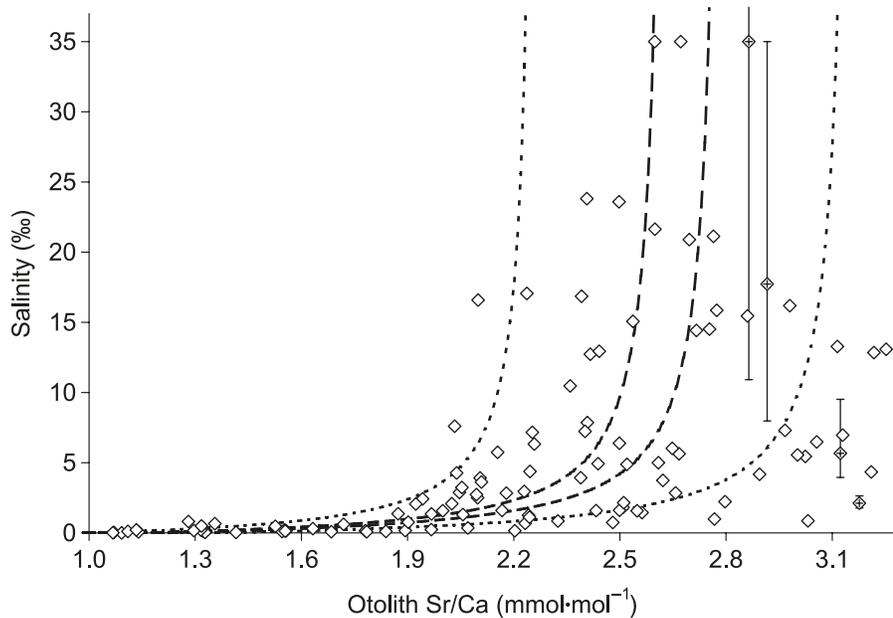


Table 3. Number of analyses (n), mean, and two standard deviations (2SD) of otolith Sr/Ca, grouped by correlated Sr isotope estimated salinity ranges.

Salinity range (‰)	n	Mean	2SD	Predicted Sr/Ca range
Hatchery	279	0.88	0.25	<1.70
<0.50	26	1.51	0.65	<1.70
0.50–0.99	9	2.12	1.21	1.71–1.96
1–1.99	12	2.26	0.50	1.97–2.21
2–2.99	12	2.31	0.79	2.22–2.34
3–3.99	5	2.26	0.49	2.35–2.41
4–4.99	7	2.57	0.78	2.42–2.46
5–5.99	5	2.79	0.79	2.47–2.50
6–6.99	5	2.72	0.74	2.51–2.52
7–7.99	5	2.41	0.69	2.53–2.54
8–12.99	4	2.61	0.81	2.55–2.60
13–17.99	12	2.72	0.70	2.61–2.63
18+	8	2.64	0.29	2.64–2.75

Note: Predicted Sr/Ca range is based on modeled otolith Sr/Ca – salinity relationship (eq. 16).

dict a freshwater growth (<5‰, 62% vs. 65%). However, otolith Sr/Ca predicts more instances of high salinity than $\Delta^{87}\text{Sr}$ (>30‰, 24% vs. 3%).

Discussion

Sr/Ca is an attractive marker in otoliths because Sr is chemically similar to Ca, it is not directly regulated by the fish, and, for a trace element, it occurs at relatively high concentrations in aragonite otoliths, making it relatively easy to measure. Furthermore, it is attractive for work in es-

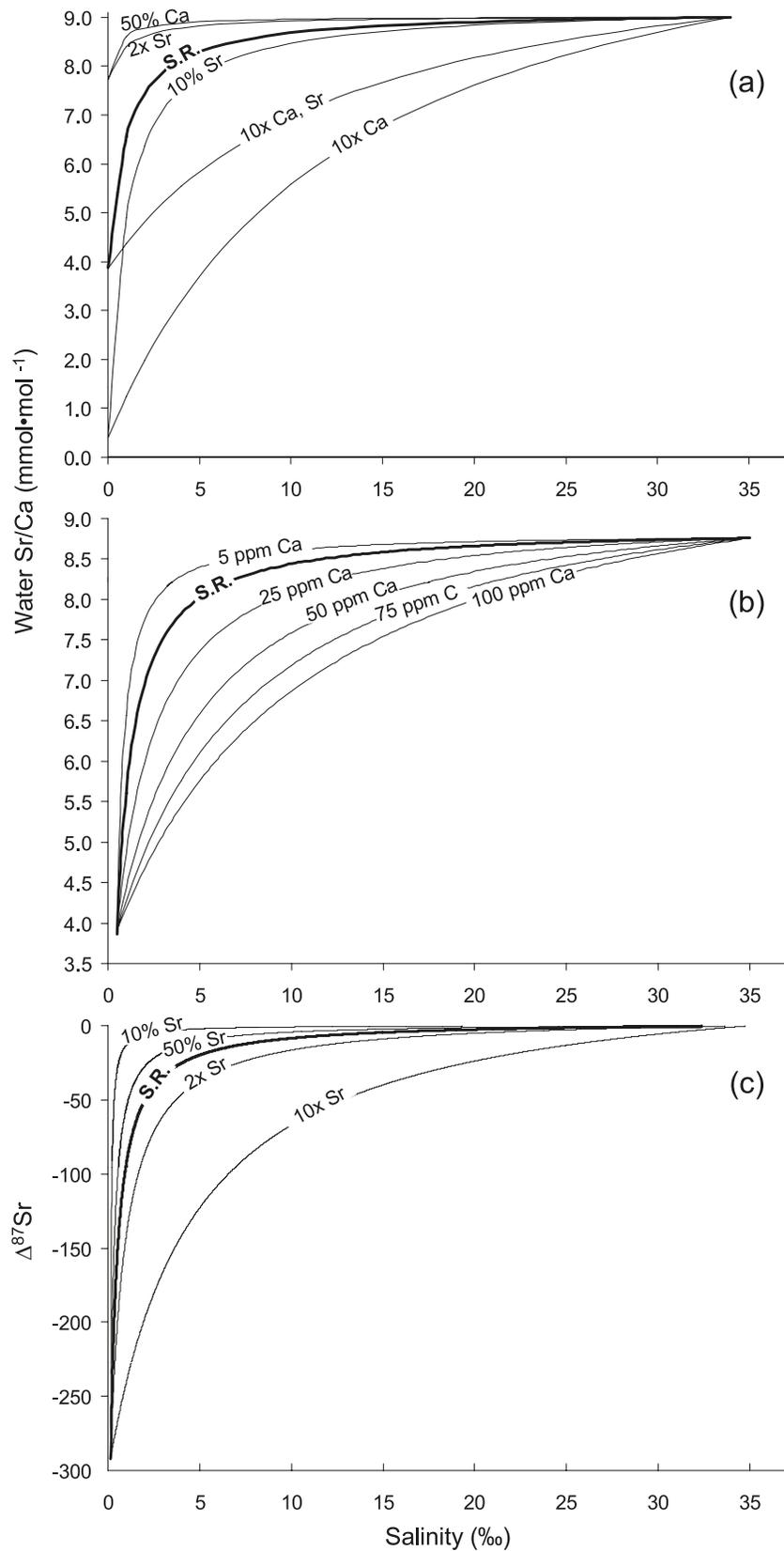
tuaries because the Sr concentration in seawater is significantly higher than that in inflowing river waters. Unfortunately, otolith Sr/Ca is not simply a function of the Sr concentration of the water, but also water's Ca concentration. This fact has made it substantially more difficult to predict the applicability of Sr/Ca in a given estuary setting. The goal of this study is to provide a streamlined framework for making this evaluation. Here, we discuss the methods, a general model for assessing the utility of Sr/Ca in other estuaries, and the utility of using Sr/Ca in the San Francisco Estuary. In addition, we address the utility of Sr isotopes and other potential markers for delineating estuarine habitat use.

Otolith Sr/Ca model development

To date, studies that have used trace elements and Sr isotopes in concert have done so to improve statistical classification of spawning location (Feyrer et al. 2007a), estuarine incursions of marine fish (McCulloch et al. 2005), and duration of maternal freshwater residence (Miller and Kent 2009). Milton and Chenery (2005) took the additional step of using correlated otolith Sr isotope and otolith Sr/Ca measurements to estimate a threshold otolith Sr/Ca ratio for freshwater residency.

In this study, we took a novel approach to determining the response of otolith Sr/Ca to salinity. We used correlated, spatially resolved Sr/Ca and Sr isotope measurements on otoliths from the study population, adult striped bass (*M. saxatilis*) in the San Francisco Estuary, to determine this response. By combining a mixing model with an estimated Sr/Ca partition coefficient (D_{Sr}), we were able to estimate the mean trend in otolith Sr/Ca response to salinity for

Fig. 6. The water Sr/Ca – salinity and $\Delta^{87}\text{Sr}$ – salinity mixing models for various Sr and Ca end members are plotted with the Sacramento River (SR) for reference. (a) The Ca freshwater end member primarily controls the linearity of the Sr/Ca – salinity mixing model. (b) Hypothetical mixing models are shown using various Ca freshwater concentrations and assuming a constant SR freshwater Sr/Ca end member ($3.9 \text{ mmol}\cdot\text{mol}^{-1}$). (c) In the case of the $\Delta^{87}\text{Sr}$ – salinity mixing model, freshwater Sr concentration controls the linearity of the response.



the San Francisco Estuary. To our knowledge, this is also the first estimate of D_{Sr} using correlated otolith Sr/Ca and Sr isotope measurements. This correlated analysis approach eliminates the effort and range of variables that need to be considered when undertaking the more traditional caging or laboratory rearing experiments. This approach also does not require a separate sample to establish the otolith Sr/Ca response, but can be taken from a subsample of the study population. It also eliminates issues of water chemistry averaging and comparison of otolith sampling to water chemistry.

Our results suggest that D_{Sr} is approximately constant (0.305 ± 0.009) across the range of salinities and is in the middle of a fairly large range of Sr partition coefficients reported in investigations of other fish species (0.131–0.52; Dorval et al. 2007). There is no evidence of a salinity effect on D_{Sr} as reported in other studies (de Vries et al. 2005; Martin et al. 2004; Zimmerman 2005).

The range and shape of the relationship between salinity and water Sr/Ca for any estuary can be characterized with the freshwater end members. The marine Sr and Ca concentrations are effectively fixed globally; therefore, the shape of the water Sr/Ca versus salinity mixing model is determined by the system's freshwater end members. The fact that the difference in Sr concentration between fresh water and the ocean is typically larger than the corresponding difference in Ca concentration results in a lower Sr/Ca ratio in fresh water than in the ocean (e.g., Surge and Lohmann 2002). The relative change in Sr and Ca concentrations between freshwater and seawater determines the shape of the curve.

Manipulating Sr and Ca freshwater end members illustrates the critical parameters of the mixing model (Figs. 6a, 6b). Because the marine end member is constant, the Sr/Ca ratio in the freshwater system determines the dynamic range of the signal (i.e., the range of possible Sr/Ca ratios between fresh water and seawater), and the Ca concentration in the freshwater system determines the rate of change in the signal moving from fresh to more brackish water (Fig. 6a). In systems with relatively low freshwater Ca concentration, such as the Sacramento – San Joaquin river system, the estuary Sr/Ca ratio rapidly increases at low salinities and then asymptotically approaches the marine Sr/Ca ratio. In systems with relatively high freshwater Ca concentrations, the estuary Sr/Ca ratio increases more gradually and continually with salinity, and Sr/Ca can be used over a broader range of salinity to delineate habitat use (Figs. 6a, 6b). Calcium concentrations of the Sacramento and San Joaquin rivers, the two principle freshwater inputs of the San Francisco Estuary, are lower than many other major rivers in the United States (Briggs and Ficke 1977), despite the freshwater Sr/Ca ($3.9 \text{ mmol}\cdot\text{mol}^{-1}$) being relatively common (see Kraus and Secor 2004 for a review of surface water Sr/Ca of coastal draining rivers and creeks in US coastal states).

Utility of otolith Sr/Ca in San Francisco Estuary

The factors that control the utility of otolith Sr/Ca in estuaries are the range in expected otolith Sr/Ca; the variability in otolith Sr/Ca for a given salinity; the precision with which otolith Sr/Ca can be measured; and the shape of the response to salinity, which is introduced above. Here, we discuss these factors in the context of the San Francisco

Estuary and introduce a signal-to-noise (S/N) ratio for the purpose of simplifying the evaluation of otolith Sr/Ca in this system.

For the San Francisco Estuary, the range of predicted otolith Sr/Ca is $\sim 1.5 \text{ mmol}\cdot\text{mol}^{-1}$ ($1.2\text{--}2.7 \text{ mmol}\cdot\text{mol}^{-1}$). Although fully marine samples are not well represented in this study, with the rapid rise in otolith Sr/Ca at low salinity, the upper estimate of the predicted range should be reliable. If we had found otolith regions that were fully marine, we would have used these to test the predicted marine otolith Sr/Ca mean value. At the lower limit, the hatchery fish indicate that freshwater residence could add $\sim 0.2 \text{ mmol}\cdot\text{mol}^{-1}$ to this range, but because these fish experience a very different life history, we do not include them in this range.

We estimate the variability in otolith Sr/Ca for a given salinity from the regression of the correlated data ($2SD_{\text{otoSr}, f(\text{sal})} = 0.70 \text{ mmol}\cdot\text{mol}^{-1}$). This estimate is designed to exclude the effect of salinity on otolith Sr/Ca. As presented in the Results, the empirical data show that the variability in otolith Sr/Ca does not significantly change with salinity. Our estimate of $2SD_{\text{otoSr}, f(\text{sal})}$ is consistent with previous studies of otolith Sr/Ca ($2SD_{\text{otoSr}, f(\text{sal})} \sim 0.6\text{--}1.2 \text{ mmol}\cdot\text{mol}^{-1}$ from Kraus and Secor (2004); $\sim 0.5\text{--}0.8 \text{ mmol}\cdot\text{mol}^{-1}$ from Zimmerman (2005); $\sim 0.3\text{--}1.2 \text{ mmol}\cdot\text{mol}^{-1}$ from Feyrer et al. (2007a)). This level of agreement suggests that our correlated method did not introduce significant error into our estimate of $2SD_{\text{otoSr}, f(\text{sal})}$. We do not take the variability in the two hatchery fish otolith Sr/Ca ($2SD = 0.25 \text{ mmol}\cdot\text{mol}^{-1}$) to be representative of $2SD_{\text{otoSr}, f(\text{sal})}$ because of the non-estuarine conditions in the hatchery and the low sample number. Systematic differences in otolith Sr/Ca among fish have been shown to be larger than spot-to-spot variability within individual otoliths (Feyrer et al. 2007a). Our study has insufficient data density to test for systematic differences among fish.

Measurement precision varies substantially among techniques and methods and is not likely to be limiting relative to true variability. In this study, the measurement precision (2σ) was $0.38 \text{ mmol}\cdot\text{mol}^{-1}$ for EPMA and $0.18 \text{ mmol}\cdot\text{mol}^{-1}$ for LA-ICP-MS. The electron microprobe and LA-ICP-MS measurement precision could have been significantly higher (Zimmerman and Nielsen 2003; Feyrer et al. 2007a). Likewise, higher precision could be achieved using secondary ion mass spectrometry ($\sim 0.03 \text{ mmol}\cdot\text{mol}^{-1}$; C.C. Phillis, unpublished data).

To assess the potential discrimination power of Sr/Ca in the San Francisco Estuary, it is convenient to define an S/N ratio as the dynamic range of predicted otolith Sr/Ca relative to the variability in otolith Sr/Ca in response to water Sr/Ca as a function of salinity. An S/N ratio of 1 indicates that a binomial population can be classified with 65% confidence, and an S/N ratio of 2 indicates 95% classification confidence. Using the otolith Sr/Ca range and $2SD_{\text{otoSr}, f(\text{sal})}$ in this study ($1.5 \text{ mmol}\cdot\text{mol}^{-1}$ from the model and $0.70 \text{ mmol}\cdot\text{mol}^{-1}$ for wild-caught fish), the S/N ratio is ~ 2.1 . This value indicates that otolith Sr/Ca can be used to distinguish between freshwater and marine residence, as others have demonstrated. However, 2‰ water would result in otolith Sr/Ca ratios in excess of $2.5 \text{ mmol}\cdot\text{mol}^{-1}$ 16% of the time, which would be interpreted as $\geq 9\%$. Therefore, in the San Francisco Estuary, this method can provide useful

data on estuary use at the population level, but at the individual fish level, the data cannot be unambiguously interpreted. Furthermore, subdividing the San Francisco Estuary is not feasible. In the San Francisco Estuary, otolith Sr/Ca rises rapidly between fresh water and 5‰, sharply delineating this transition. The combined estuarine and marine regions (5‰–35‰) span an otolith Sr/Ca equivalent of only 2.40–2.75 mmol·mol⁻¹, yielding an S/N ratio of 0.5, which indicates this range is too small to be distinguished even in a binomial situation, let alone to resolve individual fish movements within this region relative to freshwater residence. For example, 9‰ water would result in Sr/Ca ratios less than 2.22 mmol·mol⁻¹ 16% of the time and in excess of 2.9 mmol·mol⁻¹ 16% of the time each, which would be interpreted as ≤2‰ and ≥35‰, respectively.

Based on these results, specifically for striped bass in the San Francisco Estuary, otolith Sr/Ca is most useful for distinguishing low-salinity habitat use from moderate- to high-salinity habitat use (Suisun Bay and upstream). Excursions into brackish water by other species that utilize the lower salinity range of an estuary could also be identified. Because of the shape of the model, it is less sensitive to error in otolith Sr/Ca at low Sr/Ca.

In general, otolith Sr/Ca will be the most useful for distinguishing the transition from fresh to brackish water. Salinities above the inflection point of the model are nearly indistinguishable by water Sr/Ca in all but the most favorable conditions (i.e., high freshwater Ca concentration, low freshwater Sr/Ca). It is worth noting that in no cases can this method distinguish between the outer estuary (20‰–30‰) and the ocean. Furthermore, biologically mediated variability in otolith Sr/Ca in response to water Sr/Ca is a major limitation on the precision of this method in general.

⁸⁷Sr/⁸⁶Sr as an estuary marker

Strontium isotopes are an alternative to otolith Sr/Ca for hind-casting the salinity of fish habitats. Otolith Sr isotope composition is increasingly applied in freshwater systems (Barnett-Johnson et al. 2008; Ingram and Weber 1999; Kennedy et al. 2000), but in estuaries, its use has been limited to distinguishing habitat use in low-salinity regions (Hobbs et al. 2005) and marking the transition between fresh and marine habitats (Milton and Chenery 2003, 2005). The greatest advantage of Sr isotopes over Sr/Ca is that Sr isotopes are not biologically modulated and therefore will reflect the water chemistry at time of otolith deposition. This eliminates the need to quantify a partition coefficient and the variability inherent in such a biologic process. However, despite recent advances in instrumentation (Barnett-Johnson et al. 2005; Woodhead et al. 2005), Sr isotope measurements are generally more expensive and time consuming than Sr/Ca measurements, and they require more material to obtain sufficient analytical precision for most applications, which limits spatial resolution relative to Sr/Ca.

More fundamentally, there is the issue of the Sr-isotope-response-to-salinity curve. In systems such as the San Francisco Estuary, the change in water Sr isotopic composition in response to salinity produces a curve similar to that of Sr/Ca (Ingram and Sloan 1992). At low salinities (0‰–5‰), Sr isotopes are sensitive to small increases in salinity and increase rapidly ($\Delta^{87}\text{Sr}$: -292 to -19; S/N =

27.3). At salinities above 5‰, Sr isotopes increase asymptotically toward the marine end member ($\Delta^{87}\text{Sr} = 0$); in fact the range of Sr isotope values reflecting salinities above 5‰ is roughly equivalent to twice the average measurement precision in this study (2SE = $\Delta^{87}\text{Sr} \pm 10$), with salinities above 10‰ indistinguishable at this measurement precision (S/N = 1.9). Similar to the Sr/Ca mixing model, a freshwater end member — in this case Sr concentration — controls the rate at which Sr isotopes increase toward the marine value (Fig. 6c).

Error in salinity estimates

Salinities calculated by Sr/Ca and $\Delta^{87}\text{Sr}$ can underestimate average salinity because of the shape of the Sr/Ca – salinity and $\Delta^{87}\text{Sr}$ – salinity mixing curves. This is more important in the case of $\Delta^{87}\text{Sr}$ because of the relatively low spatial resolution (i.e., 40–80 μm spot size) of the analytical technique. For instance, an analysis point that represents otolith growth in equal parts from habitats with two different isotopic signatures will produce an Sr isotope value that is roughly their average, but also produce a salinity value that favors the less saline habitat, particularly in cases where the two habitats are on either side of the model's inflection point. To take an extreme example, an analysis point that represents equal otolith growth in the Delta (0.15‰, $\Delta^{87}\text{Sr} = -292$) and the Pacific Ocean (35‰, $\Delta^{87}\text{Sr} = 0$) would produce a mean otolith $\Delta^{87}\text{Sr}$ value of -146 and a salinity that is not an average of the two habitats (~17‰), but actually less than 1‰. Furthermore, there can be no overestimation of salinity. That is, the marine value is the maximum Sr isotope value in the system; only analysis spots representing entirely ocean otolith growth will produce a fully marine salinity. For these reasons, we analyzed the most recent otolith growth in four ocean-caught individuals (mean $\Delta^{87}\text{Sr} = -16.5$; range -35.6 to -6.7), but even in these cases we were unable to detect full marine salinities, suggesting these individuals had either (i) recently migrated to the marine environment from the San Francisco Estuary or (ii) were utilizing estuarine habitat of smaller coastal watersheds.

Other markers can be combined with Sr/Ca or Sr isotopes to further delineate habitat use. The use of isotopic ratios as environmental history tracers is less common than the use of elemental ratios, but their application in recent years has grown with advances in methodology and instrumentation (Campana 2005). Light stable isotopes can detect shifts in habitat related to salinity ($\delta^{18}\text{O}$), temperature ($\delta^{18}\text{O}$), and diet ($\delta^{13}\text{C}$, $\delta^{34}\text{S}$) (Gao and Beamish 1999; Weidman and Millner 2000; Weber et al. 2002). The most promising direction in this work is the application of secondary ion mass spectrometry to otolith analyses for light stable isotopes (Weber et al. 2002; Weidel et al. 2007), which enables high-precision analysis at high spatial resolution.

Otolith Sr/Ca will continue to be a valuable tool in the reconstruction of fish movements and environmental histories; however, application of this marker in estuarine habitats requires knowledge of the local freshwater chemistry to determine its potential. In most systems, otolith Sr/Ca will be best suited for delineating habitat use between fresh and more saline waters, though systems with high freshwater Ca and a large Sr/Ca dynamic range may allow further habitat

delineation. The low fidelity of otolith Sr/Ca to water Sr/Ca will limit its application to statistical analyses. Sr isotopes, though more precise and applicable to individuals, are limited in application to determining habitat use in the low salinity range (0‰–5‰). Researchers interested in studying within-estuary movements of fish should first assess the water Sr/Ca mixing curve of their system of interest.

Acknowledgements

We thank J.W. Moore, S. Campana, and two anonymous reviewers for comments that lead to substantial improvements in the manuscript. We are grateful for analytical assistance provided by F.J. Ryerson at Lawrence Livermore National Laboratory (LLNL) and M. Gras of the University of California – Davis Interdisciplinary Center for Plasma Mass Spectrometry. We thank B. Osburn and the University of California – Davis School of Veterinary Medicine for providing partial funding of the project; IEP-POD management team for their support and partial funding under DWR Contract No. 4600004664; and CalFed for partial funding under Project No. SCI-05-C179. Work at LLNL was performed under the auspices of the US Department of Energy under Contract DE-AC52-07NA27344.

References

- Almany, G.R., Berumen, M.L., Thorrold, S.R., Planes, S., and Jones, G.P. 2007. Local replenishment of coral reef fish populations in a marine reserve. *Science* (Washington, D.C.), **316**(5825): 742–744. doi:10.1126/science.1140597. PMID: 17478720.
- Bailey, H.C., Alexander, C., Digiorgio, C., Miller, M., Doroshov, S.I., and Hinton, D.E. 1994. The effect of agricultural discharge on striped bass (*Morone saxatilis*) in California Sacramento–San Joaquin drainage. *Ecotoxicology*, **3**(2): 123–142. doi:10.1007/BF00143410.
- Barnett-Johnson, R., Ramos, F.C., Grimes, C.B., and MacFarlane, R.B. 2005. Validation of Sr isotopes in otoliths by laser ablation multicollector inductively coupled plasma mass spectrometry (LA-MC-ICPMS): opening avenues in fisheries science applications. *Can. J. Fish. Aquat. Sci.* **62**(11): 2425–2430. doi:10.1139/f05-194.
- Barnett-Johnson, R., Pearson, T.E., Ramos, F.C., Grimes, C.B., and MacFarlane, R.B. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. *Limnol. Oceanogr.* **53**(4): 1633–1642. doi:10.4319/lo.2008.53.4.1633.
- Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., and Lam, J.W.H. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta*, **64**(10): 1705–1714. doi:10.1016/S0016-7037(99)00419-6.
- Bennett, W.A., and Moyle, P.B. 1996. Where have all the fishes gone? Interactive factors producing fish declines in the Sacramento–San Joaquin Estuary. In *San Francisco Bay: the ecosystem: further investigations into the Natural History of San Francisco Bay and Delta with reference to the influence of man*. Edited by J.T. Hollibaugh. Pacific Division of AAAS, San Francisco, Calif. pp. 519–542.
- Bennett, W.A., Ostrach, D.J., and Hinton, D.E. 1995. Larval striped bass condition in a drought-stricken estuary: evaluating pelagic food-web limitation. *Ecol. Appl.* **5**(3): 680–692. doi:10.2307/1941977.
- Briggs, J.C., and Ficke, J.F. 1977. Quality of rivers of the United States, 1975 water year – based on the National Stream Quality Accounting Network (NASQAN). US Geol. Surv. Open File Rep. 78–200.
- Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser.* **188**: 263–297. doi:10.3354/meps188263.
- Campana, S.E. 2005. Otolith science entering the 21st century. *Mar. Freshw. Res.* **56**(5): 485–495. doi:10.1071/MF04147.
- Campana, S.E., and Neilson, J.D. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* **42**(5): 1014–1032. doi:10.1139/f85-127.
- de Vries, M.C., Gillanders, B.M., and Elsdon, T.S. 2005. Facilitation of barium uptake into fish otoliths: influence of strontium concentration and salinity. *Geochim. Cosmochim. Acta*, **69**(16): 4061–4072. doi:10.1016/j.gca.2005.03.052.
- Dorval, E., Jones, C.M., Hannigan, R., and van Montfrans, J. 2007. Relating otolith chemistry to surface water chemistry in a coastal plain estuary. *Can. J. Fish. Aquat. Sci.* **64**(3): 411–424. doi:10.1139/F07-015.
- Faure, G. 1986. Principles of isotope geology. John Wiley & Sons, New York.
- Feyrer, F., Hobbs, J., Baerwald, M., Sommer, T., Yin, Q.Z., Clark, K., May, B., and Bennett, W. 2007a. Otolith microchemistry provides information complementary to microsatellite DNA for a migratory fish. *Trans. Am. Fish. Soc.* **136**(2): 469–476. doi:10.1577/T06-044.1.
- Feyrer, F., Nobriga, M.L., and Sommer, T.R. 2007b. Multidecadal trends for three declining fish species: habitat patterns and mechanisms in the San Francisco Estuary, California, USA. *Can. J. Fish. Aquat. Sci.* **64**(4): 723–734. doi:10.1139/F07-048.
- Gaffey, S.J., Kolak, J.J., and Bronnimann, C.E. 1991. Effects of drying, heating, annealing, and roasting on carbonate skeletal material, with geochemical and diagenetic implications. *Geochim. Cosmochim. Acta*, **55**(6): 1627–1640. doi:10.1016/0016-7037(91)90134-Q.
- Gao, Y.W., and Beamish, R.J. 1999. Isotopic composition of otoliths as a chemical tracer in population identification of sockeye salmon (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci.* **56**(11): 2062–2068. doi:10.1139/cjfas-56-11-2062.
- Hobbs, J.A., Yin, Q.Z., Burton, J., and Bennett, A.A. 2005. Retrospective determination of natal habitats for an estuarine fish with otolith strontium isotope ratios. *Mar. Freshw. Res.* **56**(5): 655–660. doi:10.1071/MF04136.
- Ingram, B.L., and Sloan, D. 1992. Strontium isotopic composition of estuarine sediments as paleosalinity–paleoclimate indicator. *Science* (Washington, D.C.), **255**(5040): 68–72. doi:10.1126/science.255.5040.68. PMID: 17739916.
- Ingram, B.L., and Weber, P.K. 1999. Salmon origin in California's Sacramento–San Joaquin river system as determined by otolith strontium isotopic composition. *Geology*, **27**(9): 851–854. doi:10.1130/0091-7613(1999)027<0851:SOICSS>2.3.CO;2.
- Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *J. Exp. Mar. Biol. Ecol.* **132**(3): 151–178. doi:10.1016/0022-0981(89)90126-3.
- Kalish, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish. Bull.* **88**(4): 657–666.
- Kennedy, B.P., Blum, J.D., Folt, C.L., and Nislow, K.H. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. *Can. J. Fish. Aquat. Sci.* **57**(11): 2280–2292. doi:10.1139/cjfas-57-11-2280.
- Koch, P.L., Halliday, A.N., Walter, L.M., Stearley, R.F., Huston, T.J., and Smith, G.R. 1992. Sr isotopic composition of

- hydroxyapatite from recent and fossil salmon: the record of lifetime migration and diagenesis. *Earth Planet. Sci. Lett.* **108**(4): 277–287. doi:10.1016/0012-821X(92)90028-T.
- Kraus, R.T., and Secor, D.H. 2004. Incorporation of strontium into otoliths of an estuarine fish. *J. Exp. Mar. Biol. Ecol.* **302**(1): 85–106. doi:10.1016/j.jembe.2003.10.004.
- Limburg, K.E. 1995. Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. *Mar. Ecol. Prog. Ser.* **119**(1–3): 25–35. doi:10.3354/meps119025.
- Love, K.M., and Woronow, A. 1991. Chemical changes induced in aragonite using treatments for the destruction of organic material. *Chem. Geol.* **93**(3–4): 291–301. doi:10.1016/0009-2541(91)90119-C.
- Martin, G.B., Thorrold, S.R., and Jones, C.M. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). *Can. J. Fish. Aquat. Sci.* **61**(1): 34–42. doi:10.1139/f03-143.
- McCulloch, M., Cappel, M., Aumend, J., and Muller, W. 2005. Tracing the life history of individual barramundi using laser ablation MC-ICP-MS Sr-isotopic and Sr/Ba ratios in otoliths. *Mar. Freshw. Res.* **56**(5): 637–644. doi:10.1071/MF04184.
- Miller, J.A., and Kent, A.J.R. 2009. The determination of maternal run time in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) based on Sr/Ca and $^{87}\text{Sr}/^{86}\text{Sr}$ within otolith cores. *Fish. Res.* **95**(2–3): 373–378. doi:10.1016/j.fishres.2008.09.030.
- Milton, D.A., and Chenery, S.R. 2003. Movement patterns of the tropical shad hilsa (*Tenualosa ilisha*) inferred from transects of $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratios in their otoliths. *Can. J. Fish. Aquat. Sci.* **60**(11): 1376–1385. doi:10.1139/f03-133.
- Milton, D.A., and Chenery, S.R. 2005. Movement patterns of barramundi *Lates calcarifer*, inferred from $^{87}\text{Sr}/^{86}\text{Sr}$ and Sr/Ca ratios in otoliths, indicate non-participation in spawning. *Mar. Ecol. Prog. Ser.* **301**: 279–291. doi:10.3354/meps301279.
- Ostrach, D.J., Low-Marchelli, J.M., Eder, K.J., Whiteman, S.J., and Zinkl, J.G. 2008. Maternal transfer of xenobiotics and effects on larval striped bass in the San Francisco Estuary. *Proc. Natl. Acad. Sci. U.S.A.* **105**(49): 19354–19359. doi:10.1073/pnas.0802616105. PMID:19033204.
- Paulsen, S.C., and List, E.J. 1997. A study of transport and mixing in natural waters using ICP-M.S.: water-particle interactions. *Water Air Soil Pollut.* **99**(1–4): 149–156. doi:10.1007/BF02406854.
- Pearce, N.J.G., Perkins, W.T., Westgate, J.A., Gorton, M.P., Jackson, S.E., Neal, C.R., and Chenery, S.P. 1997. A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. *Geostand. Newsl.* **21**(1): 115–144. doi:10.1111/j.1751-908X.1997.tb00538.x.
- Secor, D.H., and Rooker, J.R. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fish. Res.* **46**(1–3): 359–371. doi:10.1016/S0165-7836(00)00159-4.
- Secor, D.H., Henderson-Arzapalo, A., and Piccoli, P.M. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes. *J. Exp. Mar. Biol. Ecol.* **192**(1): 15–33. doi:10.1016/0022-0981(95)00054-U.
- Sommer, T., Armor, C., Baxter, R., Breuer, R., Brown, L., Chotkowski, M., Culberson, S., Feyrer, F., Gingras, M., Herbold, B., Kimmerer, W., Mueller-Solger, A., Nobriga, M., and Souza, K. 2007. The collapse of pelagic fishes in the Upper San Francisco Estuary. *Fisheries*, **32**(6): 270–277. doi:10.1577/1548-8446(2007)32[270:TCOPFI]2.0.CO;2.
- Stevens, D.E., Kohlhorst, D.W., Miller, L.W., and Kelley, D.W. 1985. The decline of striped bass in the Sacramento San Joaquin Estuary, California. *Trans. Am. Fish. Soc.* **114**(1): 12–30. doi:10.1577/1548-8659(1985)114<12:TDOSBI>2.0.CO;2.
- Sugarman, P.J., McCartan, L., Miller, K.G., Feigenson, M.D., Pekar, S., Kistler, R.W., and Robinson, A.G. 1997. Correlation of Miocene sequences and hydrogeologic units, New Jersey Coastal Plain. In *Proceedings of the Ocean Drilling Program, Scientific Results. Edited by K.G. Miller and S.W. Snyder. Ocean Drilling Program, College Station, Tex.* pp. 147–159.
- Surge, D.M., and Lohmann, K.C. 2002. Temporal and spatial differences in salinity and water chemistry in SW Florida estuaries: effects of human-impacted watersheds. *Estuaries*, **25**(3): 393–408. doi:10.1007/BF02695982.
- Tzeng, W.N., Severin, K.P., and Wickstrom, H. 1997. Use of otolith microchemistry to investigate the environmental history of European eel *Anguilla anguilla*. *Mar. Ecol. Prog. Ser.* **149**(1–3): 73–81. doi:10.3354/meps149073.
- Weber, P.K. 2002. Geochemical markers in the otoliths of Chinook salmon in the Sacramento–San Joaquin River System, California. Ph.D. thesis, Department of Geography, University of California, Berkeley, Calif.
- Weber, P.K., Hutcheon, I.D., McKeegan, K.D., and Ingram, B.L. 2002. Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history. *Can. J. Fish. Aquat. Sci.* **59**(4): 587–591. doi:10.1139/f02-038.
- Weber, P.K., Bacon, C.R., Hutcheon, I.D., Ingram, B.L., and Wooden, J.L. 2005. Ion microprobe measurement of strontium isotopes in calcium carbonate with application to salmon otoliths. *Geochim. Cosmochim. Acta*, **69**(5): 1225–1239. doi:10.1016/j.gca.2004.05.051.
- Weidel, B.C., Ushikubo, T., Carpenter, S.R., Kita, N.T., Cole, J.J., Kitchell, J.F., Pace, M.L., and Valley, J.W. 2007. Diary of a bluegill (*Lepomis macrochirus*): daily $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ records in otoliths by ion microprobe. *Can. J. Fish. Aquat. Sci.* **64**(12): 1641–1645. doi:10.1139/F07-157.
- Weidman, C.R., and Millner, R. 2000. High-resolution stable isotope records from North Atlantic cod. *Fish. Res.* **46**(1–3): 327–342. doi:10.1016/S0165-7836(00)00157-0.
- Woodhead, J., Swearer, S., Hergt, J., and Maas, R. 2005. In situ Sr-isotope analysis of carbonates by LA-MC-ICP-M.S.: interference corrections, high spatial resolution and an example from otolith studies. *J. Anal. At. Spectrom.* **20**(1): 22–27. doi:10.1039/b412730g.
- Zimmerman, C.E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. *Can. J. Fish. Aquat. Sci.* **62**(1): 88–97. doi:10.1139/f04-182.
- Zimmerman, C.E., and Nielsen, R.L. 2003. Effect of analytical conditions in wavelength dispersive electron microprobe analysis on the measurement of strontium-to-calcium (Sr/Ca) ratios in otoliths of anadromous salmonids. *Fish. Bull.* **101**(3): 712–718.