RAPID COMMUNICATION / COMMUNICATION RAPIDE

Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history

Peter K. Weber, Ian D. Hutcheon, Kevin D. McKeegan, and B. Lynn Ingram

Abstract: We report a new ion microprobe method to reconstruct aspects of fish life history based on sulfur isotopes ($^{34}S/^{32}S$, expressed as $\delta^{34}S$). Selected hatchery raised and naturally spawned juvenile chinook salmon (*Oncorhynchus tshawytscha*) are shown to have a 12.96 ± 0.27‰ (mean ± 2 standard errors) difference in muscle $\delta^{34}S$ values, corresponding to $\delta^{34}S$ differences between the hatchery and freshwater diets. Isotopic microanalyses of otoliths demonstrate that this 13‰ difference is preserved in the otoliths. We interpret the otolith $\delta^{34}S$ record to be a chronology of dietary $\delta^{34}S$, with approximately one-week temporal resolution, preserved in these banded calcium carbonate structures. Potential applications of this method include identifying hatchery raised fish and reconstructing nutrition sources, migration, and other aspects of fish life history.

Résumé : Une nouvelle méthode qui utilise une microsonde ionique permet de reconstituer certains aspects du cycle biologique d'un poisson à l'étude des isotopes de soufre ($^{34}S/^{32}S$, soit $\delta^{34}S$). De jeunes saumons quinnat (*Oncorhynchus tshawytscha*) sélectionnés, certains élevés en pisciculture et d'autres nés en nature, présentent des différences de 12,96 ± 0,27‰ (moyenne ± 2 fois l'erreur type) dans les $\delta^{34}S$ de leurs muscles, ce qui correspond aux différences de $\delta^{34}S$ entre les régimes alimentaires en pisciculture et en milieu d'eau douce. Des microanalyses des isotopes des otolithes révèlent que cette différence de 13‰ se maintient dans les otolithes. Nous interprétons cet enregistrement du $\delta^{34}S$ dans les couches de carbonate de calcium des otolithes comme le reflet de l'évolution dans le temps du $\delta^{34}S$ alimentaire avec une précision de l'ordre de 1 semaine. L'identification des poissons élevés en pisciculture, ainsi que la reconstitution des sources alimentaires, de la migration et d'autres aspects du cycle biologique des poissons sont parmi les applications potentielles de cette nouvelle méthodologie.

[Traduit par la Rédaction]

Introduction

Sulfur isotopes are useful for the study of nutrient flows and migratory patterns in large part because marine and continental food webs typically have substantially different sulfur isotopic compositions (Peterson et al. 1986; Nriagu et al. 1991). Bacterial sulfate (SO_4^{2-}) reduction in the oceans enriches marine SO_4^{2-} in ^{34}S relative to sulfur in the earth's crust ($\delta^{34}S$ of +21‰ versus a mean of ~0‰) (Thode 1991). The $\delta^{34}S$ of river water SO_4^{2-} varies regionally between –5‰ and +15‰ according to bedrock lithology, anthropogenic inputs, and atmospheric deposition from natural sources, with a global average value of approximately +7‰ (Nriagu et al. 1991). Photosynthetic plants use inorganic sulfur, like SO_4^{2-}, to synthesize S-bearing compounds with a small amount of isotopic fractionation (0 to –3‰ for marine, and –5‰, on average, for freshwater plants) (Nriagu et al. 1991). The $\delta^{34}S$ signal established by primary producers is maintained in the food web because the essential sulfur-bearing compounds are incorporated into the tissue of consumers without significant fractionation (Peterson et al. 1986; Nriagu et al. 1991), generating a substantial difference in $\delta^{34}S$ between marine and most continental food webs. Smaller but still useful differences can be found within freshwater systems (Nriagu et al. 1991). In fish, $\delta^{34}S$ can readily be determined in muscle and organ samples by standard isotope ratio mass spectrometry techniques, providing time-averaged dietary $\delta^{34}S$ information (Hesslein et al. 1993).

We hypothesize that the otolith organic matrix contains a permanent record of a fish's dietary sulfur history. Otoliths are calcium carbonate concretions in the inner ear of bony fish (salmon, in this study) that accrete with daily and sea-

Received 4 January 2002. Accepted 10 April 2002. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 23 April 2002. J16683

P.K. Weber.[1] Department of Geography, University of California, Berkeley, CA 94720, U.S.A.
I.D. Hutcheon. Analytical & Nuclear Chemistry Division, Lawrence Livermore National Laboratory, Livermore, CA 94551, U.S.A.
K.D. McKeegan. Department of Earth and Space Sciences, University of California, Los Angeles, CA 90095, U.S.A.
B.L. Ingram. Department of Earth and Planetary Science, University of California, Berkeley, CA 94720, U.S.A.

[1]Corresponding author (email: pweber@socrates.berkeley.edu)

 DOI: 10.1139/F02-038

sonal growth increments, preserved by successive growth layers. Sulfur (100–600 $\mu g \cdot g^{-1}$) is associated with the otolith organic matrix (Kalish 1989). Otolith chemistry has been extensively analyzed (Campana 1999), but no measurements of otolith $\delta^{34}S$ have ever been reported. We expect the organic matrix in the centre of each salmon otolith to reflect the marine sulfur isotopic composition of the mother, transmitted via the egg to the fish. Once the juvenile salmon hatches out and begins to feed, the $\delta^{34}S$ of each otolith growth increment should reflect the diet of the fish at that particular time (i.e., the otolith layers preserve a temporal record of fish diet).

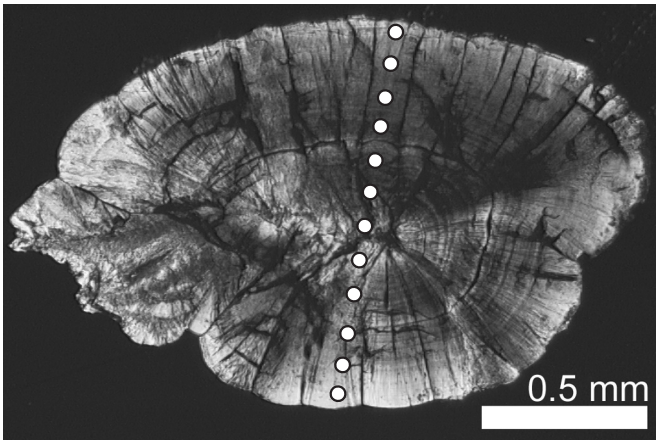
We test this hypothesis by making in situ measurements of otolith $\delta^{34}S$ for hatchery raised and naturally spawned juvenile chinook salmon (*Oncorhynchus tshawytscha*). Hatchery raised and naturally spawned salmon should have distinct juvenile dietary $\delta^{34}S$ histories in most river systems. In the hatchery, juvenile salmon are raised on commercial feeds typically consisting of 80–90% protein from marine sources, whereas in the wild, juvenile salmon have a freshwater diet. We analyze tissue and food samples to show that hatchery raised and naturally spawned juvenile chinook salmon have distinct dietary sulfur histories. We compare hatchery and source river water dissolved sulfur content to exclude significant additions of exogenous sulfur from hatchery operations (e.g., feeding). Finally, we analyze otolith $\delta^{34}S$ by ion microprobe, generating a spatially resolved $\delta^{34}S$ record.

Methods

Five sets of juvenile chinook salmon from the Sacramento – San Joaquin river system (~38°N, 122°W) were used in this study. One set of fish came from a river with no hatchery stocking (Butte Creek), another set was captured in August in a river that last received hatchery fish four months before (Sacramento River at Red Bluff), the third set was taken directly from a hatchery (Feather River), the fourth set were naturally spawned chinook salmon fry recently emerged from the gravel (34- to 38-mm fork length, FL), and the fifth set were tagged hatchery raised salmon (Merced River) released in the Tuolumne River and recaptured in the Sacramento – San Joaquin Delta 25 days later. With the exception of the fry, the fish caught in the wild were all larger than 60-mm FL, and the fish taken directly from the hatchery were larger than 45-mm FL.

The Butte Creek, Sacramento River, and Feather River Hatchery fish were used to characterize dietary and resultant bulk muscle tissue $\delta^{34}S$ for the naturally spawned and hatchery raised juvenile salmon because these fish were expected to be in equilibrium with their diets. Whole prey items were extracted from the stomachs of salmon caught in the wild, three commercial chinook salmon feeds were obtained from the Feather River and Merced River hatcheries, and stomach contents were extracted from three of the hatchery salmon. To characterize prefeeding salmon $\delta^{34}S$, two sets of chinook salmon eggs and the four naturally spawned chinook salmon fry were analyzed. All muscle, stomach content, feed, and egg samples were dried at 60°C and ground to a fine powder. Lipids were extracted using methylene chloride and methanol (2:1, v/v). The lipid-extracted samples were analyzed for ^{34}S:^{32}S by an Isochrom Continuous Flow Stable Isotope Mass Spectrometer (Micromass, Manchester, U.K.) coupled to a Carlo Erba Elemental Analyzer (ThermoFinnigan Italia, Milan, Italy) at the Environmental Isotope Laboratory, University of Waterloo, Ont., Canada.

Fig. 1. Transmitted light micrograph of a polished otolith from a juvenile chinook salmon (*Oncorhynchus tshawytscha*) showing concentric growth increments and ion microprobe traverse used to determine $\delta^{34}S$. In situ ion microprobe analyses reveal variations in $\delta^{34}S$, correlated with radial position (salmon age), that track changes in nutrient-source and (or) diet $\delta^{34}S$, which can in turn be related to aspects of salmon life history (Fig. 3).

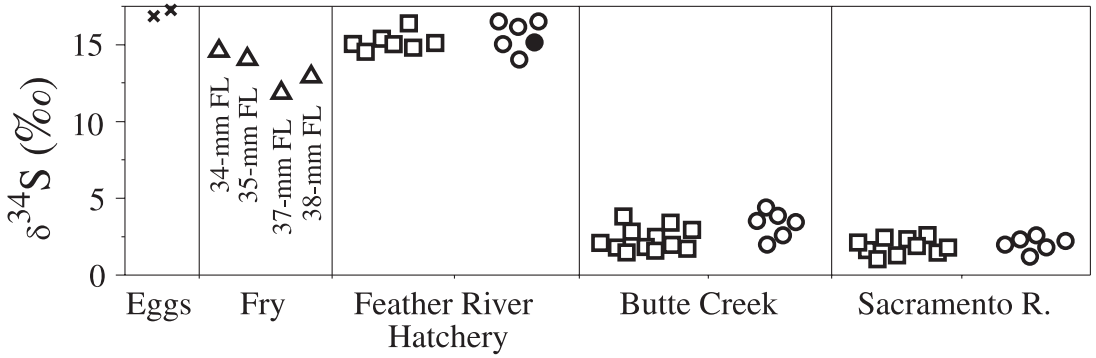


To test for sulfur additions in the hatchery (Merced River), matched pairs of filtered water samples (0.4 μm) were collected from the hatchery raceways and the adjacent river when salmon were present (February 1998, March 1998, and January 1999). Total sulfur was determined by inductively coupled plasma atomic emission spectrometer (Thermo Jarrell Ash Iris HR, Thermo Elemental, Franklin, Mass., U.S.A.) at the University of California, Berkeley. Replicate analyses of matched pairs were made using concentrated and spiked aliquots.

We selected otoliths from the Butte Creek juvenile salmon for analysis to characterize otolith $\delta^{34}S$ in the wild because these fish were the most likely to be naturally spawned. Otoliths from the recaptured Merced River Hatchery salmon were selected to characterize otolith $\delta^{34}S$ in the hatchery and after release. Otoliths were removed, cleaned in deionized water, dried at 60°C, mounted in Araldite epoxy, and polished to expose banding (Fig. 1).

The spatial distribution of sulfur isotopes in otoliths was determined using ion microprobes (Cameca, Courbevoie, France) at the University of California at Los Angeles (Cameca IMS 1270, National Ion Microprobe Facility) and the Lawrence Livermore National Laboratory (Cameca IMS 3f, Livermore, Calif.). Techniques for the IMS 1270 were similar to those described previously for oxygen-isotope analyses (McKeegan et al. 1998). A 20-keV, ~4-nA Cs^+ primary ion beam, defocused to produce a uniformly illuminated spot, was used to sputter gold-coated sections of otoliths. Low-energy (<30 eV) negative secondary ions were accelerated to 10 keV and mass analyzed at high resolving power ($m/\triangle m = 4000$). A normal incidence electron flood gun was used to provide charge compensation. Secondary ions were detected by static multicollection: ^{32}S (~4×10^6 ions$\cdot s^{-1}$) on a Faraday cup and ^{34}S by ion counting on an

Fig. 2. Sulfur isotope composition of muscle tissue (□) from hatchery raised (>45 mm fork length, FL) and naturally spawned juvenile (>60-mm FL) chinook salmon (*Oncorhynchus tshawytscha*), with comparison to $\delta^{34}S$ of diet (○; ● for Merced River Hatchery feed), eggs (×), and recently hatched, naturally spawned fry (△). The egg (n = 2) and fry (n = 4) data demonstrate that the maternal marine $\delta^{34}S$ signal is transmitted to the prefeeding juvenile fish. The data for the larger juvenile fish (n = 7, 12, and 10 from left to right) show an unambiguous difference in $\delta^{34}S$ (12.96 ± 0.27‰ (2 SE)) between hatchery raised and naturally spawned fish. The larger fish are in approximate equilibrium with their diets (n = 6, 6, and 6), as expected based on known history and size. Butte Creek salmon are expected to be naturally spawned in the wild because the river is not stocked with hatchery salmon. The Sacramento River samples were collected at the Red Bluff diversion dam four months after the last releases from the upstream hatchery; therefore, any hatchery fish still in the river would reflect a freshwater diet in the wild. Data point size represents two standard errors.



electron multiplier. Techniques for the IMS 3f were generally similar, with the major differences being a reduction in the Cs^+ beam energy to 14.5 keV, reduction in secondary ion energy to 4.5 keV, and the measurement of both S isotopes by pulse counting and magnetic peak switching. The ^{34}S–^{32}S ratios were corrected for instrumental mass-dependent fractionation by comparison to analyses of pressed standards composed of 95% calcium carbonate and 5% dried, lipid-extracted fish muscle with known sulfur isotopic compositions. All ^{34}S–^{32}S ratios are expressed as $\delta^{34}S$ values, the deviation in per mil relative to the Canyon Diablo Troilite (CDT) standard

$$\delta^{34}S = \left[\frac{(^{34}S/^{32}S)_{sample}}{(^{34}S/^{32}S)_{CDT}} - 1\right] \times 1000$$

Measurement uncertainty is presented as two standard errors (2 SE). The sulfur concentration in these otoliths (100–500 ppm) is sufficient to measure $\delta^{34}S$ at a single spot to 1–2‰ (external precision) with the Cameca IMS 1270, and to 3–5‰ with the Cameca IMS 3f. The temporal resolution of the analyses is limited by the ~20-μm diameter of the primary beam. Juvenile salmon otoliths have 2- to 10-μm daily growth increments and each single spot analysis averages four to eight daily bands in these otoliths. The $\delta^{34}S$ data thus provide a chronology with roughly one-week resolution.

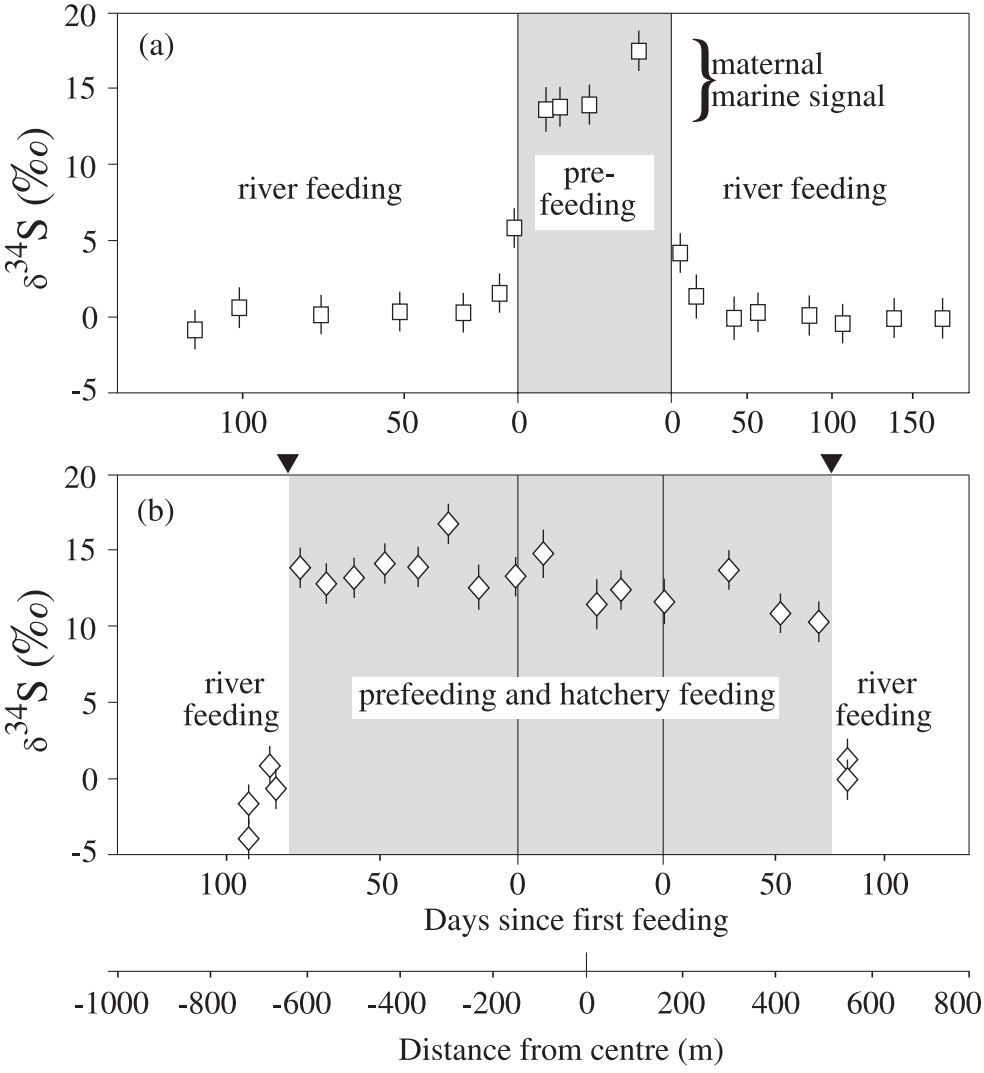
Results and discussion

Our $\delta^{34}S$ data for eggs and fry (Fig. 2) demonstrate that the adult salmon $\delta^{34}S$ signal (+17‰ to +18‰; Krouse et al. 1991) is transmitted to the juvenile fish via the egg. With time and growth (>45-mm FL), this signal in the tissue is replaced by the dietary $\delta^{34}S$ value (Fig. 2), as expected (Hesslein et al. 1993). Our results show a substantial difference in muscle tissue $\delta^{34}S$ (12.96 ± 0.27‰) between juvenile salmon feeding in the hatchery and those feeding in the rivers. This difference is large relative to both sample variability (2 standard deviations, SD, of 1.0–1.8‰) and measurement precision (2 SE of 0.6‰). Wild salmon $\delta^{34}S$ values for muscle (+1.0‰ to +3.8‰) and stomach content (+1.3‰ to +4.5‰) are in the range expected for freshwater sulfur sources. In contrast, the $\delta^{34}S$ values in the muscle tissue of the hatchery raised fish (+14.6‰ to +16.4‰) are indicative of sulfur derived from the hatchery feeds (+14.1‰ to +16.6‰), reflecting a marine signature modified by the admixture of sulfur from continental sources.

Filtered water samples from the Merced River Hatchery and adjacent river contain S at 0.7–1.5 $\mu g \cdot g^{-1}$. Matched pairs have the same sulfur content within error ($mean_{river-hatchery}$ = 0.03 ± 0.25 $\mu g \cdot g^{-1}$), suggesting that hatchery operation does not add significant amounts of exogenous dissolved sulfur to river water diverted to the hatchery raceways. This observation is consistent with strontium isotope and major and minor elemental data for this hatchery and others in the Sacramento – San Joaquin river system, which also suggest that hatchery operation does not alter source water chemistry (Weber 2002). If little exogenous sulfur is added to hatchery water, then the hatchery water sulfate $\delta^{34}S$ cannot be significantly elevated relative to river water sulfate $\delta^{34}S$.

We present the in situ analyses of $\delta^{34}S$ in two otoliths, from a naturally spawned juvenile salmon and a recaptured juvenile hatchery raised salmon (Fig. 3). The data show large differences in sulfur isotope composition correlated with nutritional history. For the naturally spawned fish, the $\delta^{34}S$ of the otolith core (14.4 ± 2.2‰, 2 SE, external precision) reflects the marine signal from the egg. The $\delta^{34}S$ values abruptly decrease to near zero (–0.4 ± 1.3‰) ~150 μm from the centre, reflecting growth of the salmon in fresh water. In contrast, the bulk of the otolith from the hatchery raised salmon (Merced River Hatchery) reflects the marine and hatchery-feed $\delta^{34}S$ signal (+12.9 ± 1.5‰). Only the outermost 25 growth bands (~100 μm wide) of the otolith, formed during the 25 days between release of the salmon and its subsequent capture downstream, have the freshwater $\delta^{34}S$ value (–0.6 ± 2.0‰). Pooling the data for the two oto-

Fig. 3. Spatially resolved otolith $\delta^{34}S$ values for (*a*) a juvenile naturally spawned chinook salmon (*Oncorhynchus tshawytscha*) and (*b*) a recaptured juvenile hatchery raised chinook salmon. The data reveal an unambiguous difference in the distribution of $\delta^{34}S$ values across individual otoliths, correlated with salmon diet and life history. Elevated $\delta^{34}S$ values in the core of the wild salmon otolith, reflecting the marine S signal transmitted to the fish from the mother via the egg, decrease to values near zero once the hatchling begins to feed in the river. The hatchery raised salmon otolith exhibits uniformly high $\delta^{34}S$ values until release to freshwater (indicated by ▼; shaded area represents the period when the nutrition source is marine), 25 days before capture. The difference in $\delta^{34}S$ (13.33 ± 0.97‰ (2 SE)) between hatchery-feeding and river-feeding zones reflects dietary and muscle tissue $\delta^{34}S$. Distance from the centre of the otolith is related to days since first feeding based on visible daily banding. These data were collected on the Cameca IMS 1270 ion microprobe and replicated on the Cameca IMS 3f ion microprobe (see Methods). Error bars represent two standard errors.



liths, the sulfur isotope composition of the hatchery-feeding and river-feeding zones differs by 13.33 ± 0.97‰ (2 SE, internal precision).

At our current levels of accuracy and precision, we are unable to distinguish between the marine $\delta^{34}S$ signal in the otolith core and the (largely) marine signal derived from the hatchery feed. We are also unable to determine whether the offset in otolith $\delta^{34}S$ relative to muscle $\delta^{34}S$ is an artifact of the mass-fractionation correction or whether it is an actual difference between otolith and muscle $\delta^{34}S$.

Based on the observations that (*i*) the spatial variation of otolith sulfur content is consistent with the spatial variation in otolith protein content and (*ii*) the sulfur content of an otolith is consistent with the total amount of sulfur in the proteins of the organic matrix, Kalish (1989) inferred that S-bearing amino acids in the organic matrix are the primary source of sulfur in otoliths. Otoliths are 0.2–10% protein, containing 1–3% cysteine and methionine (Degans et al. 1969), which are 26.5% and 21.5% S by weight, respectively (Kalish 1989). However, sulphated acid mucopolysaccharides have also been detected in otoliths (Asano and Mugiya 1993), and their abundance and provenance have not been studied. Mugiya and Iketsu (1987) demonstrated that inorganic sulfate in ambient water is incorporated into an otolith under experimental conditions, most likely in the organic matrix as sulphated acid mucopolysaccharides. Our sulfur concentration results for matched pairs of water samples exclude hatchery water sulfate as a significant source of

elevated $\delta^{34}S$ for the otolith hatchery-feeding zone, supporting the thesis that diet is the primary source of otolith sulfur under river and hatchery conditions.

Applications

Hatchery managers can use a number of physical, chemical, and thermal methods to tag hatchery fish, but not all hatchery fish are marked. In California's Sacramento – San Joaquin river system, only 10 to 20 percent of hatchery chinook salmon are tagged, primarily because of concerns about physiological stress to the fish and cost (compiled from personal communications with: Rich Bryant, Mokelumne River Hatchery, Clemments, Calif.; Mike Cozart, Merced River Hatchery, Snelling, Calif.; Anna Kastern, Feather River Hatchery, Oroville, Calif.; John Scott, Coleman Hatchery, Anderson, Calif.; Terry West, Nimbus Hatchery, Rancho Cordova, Calif.). In this river system, otolith $\delta^{34}S$ will be useful for identifying hatchery salmon. This method will also work in other systems where (*i*) fish are released from the hatchery after significant feeding and growth occur, (*ii*) the hatchery feed is marine-based, and (*iii*) exceptionally high $\delta^{34}S$ lithologies do not dominate the freshwater $\delta^{34}S$ signal (Nriagu et al. 1991; Thode 1991). Where hatchery raised fish can be identified, the timing of the release of fish to the river can also be determined.

More generally, in situ analyses of $\delta^{34}S$ can be used to reconstruct aspects of fish life history, given sufficient differences in $\delta^{34}S$ between geographic regions and food sources of interest. The presence of the marine $\delta^{34}S$ value in the centre of the otolith can be used to distinguish anadromous from resident populations. Regional differences in river $\delta^{34}S$ (Nriagu et al. 1991) should be useful for determining fish origin and reconstructing migration. For estuary or migratory fish, bulk tissue $\delta^{34}S$ provides time-averaged information on nutrition sources (Hesslein et al. 1993). By contrast, spatially resolved otolith $\delta^{34}S$ analyses provide a time-resolved record of dietary $\delta^{34}S$.

Acknowledgements

We thank the California Department of Fish and Game, the California Department of Water Resources, and the U.S. Fish and Wildlife Service for samples; C. Coath, M. Grove, and D. Phinney for ion probe technical assistance; R. Stewart for early discussions; C. Beeman, A. Kapuscinski, J. Kirchner, J. Stella, E. Volk, and an anonymous reviewer for comments on the manuscript; and T. Nguyen and E. Pickett for sample preparation. This work was supported financially by the California Department of Water Resources and the Institute for Geophysics and Planetary Physics, and performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48. The UCLA ion probe facility is partially supported by a grant from the National Science Foundation Instrument Fund.

References

Asano, M., and Mugiya, Y. 1993. Biochemical and calcium-binding properties of water-soluble proteins isolated from otoliths of the tilapia, *Orechromis niloticus*. Comp. Biochem. Physiol. B, **104**: 201–205.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. **188**: 263–297.

Degans, E.T., Deuser, W.G., and Haedrich, R.L. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. **2**: 105–113.

Hesslein, R.H., Hallard, K.A., and Ramlal, P. 1993. Replacement of sulfur, carbon and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can. J. Fish. Aquat. Sci. **50**: 2071–2076.

Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. **132**: 151–178.

Krouse, H.R., Stewart, J.W.B., and Grinenko, V.A. 1991. Pedosphere and biosphere. *In* Stable isotopes: natural and anthropogenic sulphur in the environment. *Edited by* H.R. Krouse and V.A. Grinenko. John Wiley & Sons, New York. SCOPE, **43**: 267–306.

McKeegan, K.D., Leshin, L.A., Russell, S.S., and MacPherson, G.J. 1998. Oxygen isotopic abundances in calcium-aluminum-rich inclusions from ordinary chondrites: implications for nebular heterogeneity. Science (Washington, D.C.), **280**: 414–418.

Mugiya, Y., and Iketsu, H. 1987. Uptake of aspartic acid and sulfate by calcified tissues in goldfish and tilapia. Bull. Fac. Fish. Hokkaido Univ. **38**: 185–190.

Nriagu, J.O., Rees, C.E., Mekhtiyeva, V.L., Lein, A.Y., Fritz, P., Drimmie, R.J., Pankina, R.G., Robinson, B.W., and Krouse, H.R. 1991. Hydrosphere. *In* Stable isotopes: natural and anthropogenic sulphur in the environment. *Edited by* H.R. Krouse and V.A. Grinenko. John Wiley & Sons, New York. SCOPE, **43**: 177–265.

Peterson, B.J., Howarth, R.W., and Garritt, R.H. 1986. Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow. Ecology, **67**: 865–874.

Thode, H.G. 1991. Sulfur isotopes in nature and the environment: an overview. *In* Stable isotopes: natural and anthropogenic sulphur in the environment. *Edited by* H.R. Krouse and V.A. Grinenko. John Wiley & Sons, New York. SCOPE, **43**: 1–26.

Weber, P.K. 2002. Geochemical markers in the otoliths of chinook salmon in the Sacramento-San Joaquin river system, California. Ph.D. thesis, University of California, Berkeley.