Abstract: For many species, understanding the processes underlying variation in life history strategies is limited by the difficulty of tracking individuals throughout their lives. Within the rapidly expanding field of otolith microchemistry, novel approaches are being combined with state-of-the-art analytical techniques to provide new and valuable information about the environmental history of fishes. However, no approach to date allows the reconstruction of fish movements at high temporal resolution (weeks to months) over relatively small spatial scales (1–10 km). We used micromilling techniques to extract strontium (Sr) isotopic signatures from the otoliths of four returning Atlantic salmon (Salmo salar) adults. Distinct Sr isotopic signatures were detectable from four life cycle stages, including prefeeding hatchery development, rearing stream growth, smolt out-migration, and ocean residence. High-resolution analyses of Sr isotope records establish that natal stream signatures are recoverable and show that both site fidelity within the freshwater stage and the timing of migration vary considerably among individuals. Results made possible with this approach provide insight into a long-standing debate on the mobility of salmon during their nonmigratory stage. The ability to resolve flexible behaviors of salmon increases our understanding of their population biology and conservation needs.

Résumé : Chez de nombreuses espèces, la difficulté de suivre les individus tout au cours de leur vie limite la compréhension des processus qui sous-tendent la variation des stratégies de leur cycle biologique. Des méthodes nouvelles dans le domaine en pleine expansion de la micro-chimie des otolithes, combinées à des techniques analytiques toutes récentes, fournissent des informations nouvelles et précieuses sur l’histoire environnementale des poissons. Cependant, aucune technique ne permet actuellement de retracer les déplacements des poissons sur une haute échelle temporelle (semaines à mois), sur une échelle spatiale relativement réduite (1–10 km). Nous avons utilisé des techniques de microfraisage pour extraire les signatures isotopiques de strontium (Sr) des otolithes de 4 saumons atlantiques (Salmo salar) adultes de retour de la mer. Des signatures distinctes du Sr isotopique dans les otolithes se reconnaissent à quatre stades du cycle, le développement en pisciculture antérieur à la naissance, la croissance dans le cours d’eau d’élevage, la migration des saumoneaux vers la mer et le séjour en eau saumâtre. L’analyse de haute précision des isotopes de Sr dans les otolithes révèle que la signature du cours d’eau d’origine peut être décelée et démontre que la fidélité au site durant la phase d’eau douce et le calendrier de la migration varient considérablement d’un individu à un autre. Cette méthodologie apporte des perspectives nouvelles dans le vieux débat sur la mobilité des saumons durant leur phase non migratrice. La possibilité de déterminer les comportements flexibles chez les saumons vient améliorer notre compréhension de la biologie des populations et de leurs exigences en vue d’une meilleure conservation.

Introduction

The anadromous life cycle of salmon is generally regarded as highly predictable. Spawning adults return to their natal streams, fry hatch and grow in single stream reaches, and migratory smolts travel to the ocean in a relatively synchronized fashion. The classic view of juvenile salmon during their freshwater phase has been that populations comprise territorial individuals that exhibit high site fidelity and rarely move beyond 50 m (Gerking 1959; Rodríguez 2002). These
generalizations have formed the conceptual basis for years of work on the population dynamics and life history of salmon (e.g., Hansen and Quinn 1998). They also provide the basis for predominately localized conservation approaches. Relatively little is known about the extent to which individual strategies vary at different life history stages for salmon, or most other fish species, because of difficulties in tracking individuals.

Recent studies suggest that the paradigms of predictability and restricted movement in salmon reflect inherent biases in conventional tagging techniques that focus either on the recaptured, nonmobile fraction of the population or on only fish large enough to receive tags (Gowan et al. 1994). For instance, radiotelemetry has provided compelling evidence that some salmon move more extensively than previously thought during their freshwater phase. However, telemetry cannot be used to study movements of small fish or entire populations (e.g., Gowan et al. 1994). Tracking individual fish of all sizes as they move through river networks would significantly improve our understanding of life history variability, habitat use, and energy and material transport by many fish species.

Our study shows that stable isotopes of strontium (Sr) can be used to discern movements over an individual’s life. This information may help to resolve debates over salmonid life history and has valuable management applications. Over 10 million Atlantic salmon (Salmo salar) are stocked annually into tributaries of the Connecticut River, U.S.A. Less than 500 return each year. Tracing the lifetime movements of the successful adults would be extremely helpful for guiding restoration and management policies.

Our approach builds upon recent studies using geochemical signatures in fish otoliths, or ear stones, to identify natal stream habitats and the movements of fish from stocked or marked locations (Kennedy et al. 2000; Thorrold et al. 2001). Otoliths are small mineral structures in which increments of calcium carbonate, in the form of aragonite, are accreted daily in thin concentric rings (approximately 1–10 μm). Because the aragonite rings remain unaltered after death, they can be referenced to specific ages, information recovered at different locations within the otolith can be used to reconstruct events throughout the lifetime of a fish. Changes in the elemental composition across the length of an otolith reflect either changes in the ambient water conditions or, in the case of some trace elements and isotopes, modifications resulting from changes in diet, stress, temperature, or other physiological factors (Kennedy et al. 2000). Third, because of the regulated deposition of aragonite in otoliths, the concentrations of some elements can be extremely low, which effectively constrains their analytical resolution in otolith subsamples. Yet, the high concentration of Sr in otoliths enables elemental analysis of tiny subsamples of otolith material and allows for resolution over comparatively short time intervals.

In this study, we isolated Sr isotopic signatures from otoliths of spawning adult salmon at a resolution that allows us to (i) recover natal stream signatures, (ii) reconstruct the freshwater movements of Atlantic salmon over relatively small spatial scales (1–10 km) and short time scales (weeks to months), and (iii) identify the timing of migration for salmon smolts. We combine micromilling techniques with Sr isotope analysis from otolith increments to address the long-standing hypotheses that juvenile salmon movements are rare and that their timing is predictable. In so doing, we reconstruct the environmental history of individuals and find evidence challenging key aspects of salmon life history paradigms.

Methods

We measured age-specific ${}^{87}$Sr/$^{86}$Sr ratios within the sagittal otoliths of four adult Atlantic salmon, which were captured as they returned to spawn in the Connecticut River. The fish were collected at Holyoke Dam on the Connecticut River in Massachusetts, U.S.A., which is located 140 km upstream of the river mouth and is the first dam encountered by returning salmon. The fish were randomly chosen from a cohort of 154 returning salmon in 1999.

Otoliths were cleaned, mounted, and sanded so that daily increments could be resolved when magnified to 1000x with a Leica compound scope (Fig. 1). Otolith images were digitized and projected onto a computer monitor so that otoliths could be systematically drilled using a computer-controlled micromill. Otolith subsamples came from drilled paths (40–50 μm) along parallel, concentric growth curves at known distances from the otolith center using a Merchantek xyz-stage micromill. Powdered aragonite (<25 μg) was carefully transferred to individually labeled, clean Teflon vessels and gently digested in quartz-distilled concentrated Seastar HNO₃. Digestion, elemental separation, and Sr isotope analysis were performed using established methods (Kennedy et al. 2000). The solution was dried to completeness and then redissolved in ultra-pure 3N HNO₃. Sr was separated from this solution using Sr-specific cation exchange resin. An aliquot of the Sr solution containing ~20 ng of Sr was evaporated to dryness, transferred onto single tungsten filaments with Ta₂O₅ activator, and loaded
into the mass spectrometer. All Sr isotopic compositions were measured using a Finnegan MAT 262 thermal ionization mass spectrometer. \( ^{87}\text{Sr}/^{86}\text{Sr} \) were normalized to \( ^{86}\text{Sr}/^{88}\text{Sr} = 0.1194 \). Twenty analyses of a National Institute of Standards and Technology standard reference material (SRM-987) were completed during the 5-month duration of this study and yielded a mean \( ^{87}\text{Sr}/^{86}\text{Sr} \) of 0.710250 ± 0.000005 (2 standard errors (SE)).

Changes in the Sr isotope ratios along the accretionary axis of an otolith imply a change in the environment experienced by a fish. To first quantify the precision of replicated Sr isotope measurements for a fish that lived within a single environment, we analyzed otolith increments from one 4-year-old hatchery salmon that had been reared to adulthood at the White River National Fish Hatchery (WRNFH) in Bethel, Vt., U.S.A. Sr isotope ratios in the otolith of this individual served as a control against which we compared the variability of Sr isotope ratios of our field-caught fish.

To identify natal stream signatures and to test for movement during the freshwater phase, we measured the isotopic signatures for these individuals at several points before smoltification. The otolith of a salmon at the time of stocking has a radius of approximately 400 \( \mu m \). To establish rearing stream signatures, otoliths were drilled sequentially at radial distances between 600 and 1000 \( \mu m \), representing at least four different time points throughout about 12 months of growth for each individual (first spring–summer to second spring–summer).

Most Atlantic salmon in the Connecticut River basin smolt during their third spring. However, a subset of the population leave their rearing tributaries after only their second spring. Using micromilled otolith increments, we compared the migratory behavior of the returning adult salmon as smolts. Because modern ocean water has a uniform global Sr isotopic signature (\( ^{87}\text{Sr}/^{86}\text{Sr} = 0.70918 \)), it can provide the most precise marker to date for identifying the incorporation of a marine-derived signature in fish, and hence, the timing and duration of ocean residence.

**Results and discussion**

Our micromilling technique was successful for characterizing distinct Sr isotopic signatures from the four major stages in the life cycle of adult Atlantic salmon: (1) prefeeding hatchery development (core), (2) rearing-stream growth, (3) smolt out-migration, and (4) ocean residence (Fig. 2). Micromilled otolith subsamples from our control fish, an adult salmon that lived exclusively at WRNFH, displayed very little variability around an average value of 0.71049 ± 0.00002 (SE) (Fig. 2). In contrast, Sr isotope ratios from micromilled otolith subsamples of the returning adult salmon varied significantly. Because all returning salmon were originally spawned in one of five hatcheries, the isotopic signature at the core of their otoliths must match that of prestocked fry from one those hatcheries. The Sr isotopic signatures from the otolith cores of three of the four returning adult salmon were similar to that of hatchery fry sampled at WRNFH (0.7123, 0.7127, 0.7129 vs. 0.7122; Fig. 2), making it their likely source. Since 1996, WRNFH has supplied more than 70% of stocked fry. The fourth fish was probably spawned in one of the two other hatcheries located in central and western Vermont. Those regions are underlain
Fig. 2. Age-specific Sr isotope ratios from adult Atlantic salmon (Salmo salar). Signatures are measured along a growth axis for four returning salmon (salmon 1 to salmon 4) and one hatchery fish. The $^{87}$Sr/$^{86}$Sr signature is expressed as a function of distance from the center of the otolith and records distinct environments as the otolith accretes increments. Sr isotope ratios of unfed, unstocked fry from the White River National Fish Hatchery (WRNFH, shaded triangle) correspond closely with the values for otolith cores from three of the four salmon. Precise natal stream signatures, identified by boxes, are quantifiable in three of the four individuals. Measurement error is expressed as the standard error of 20 analyses of a National Institute of Standards and Technology standard reference material (SRM-987) throughout the course of this experiment and is represented as the thin horizontal line within the symbol (marked with arrow).

![Sr isotope ratios graph](image)

Table 1. Natal stream $^{87}$Sr/$^{86}$Sr signatures recovered from freshwater portions of adult otoliths.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$^{87}$Sr/$^{86}$Sr</th>
<th>Radius of measurement (µm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.71268 (0.00012)</td>
<td>600–1000</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0.71676 (0.00006)</td>
<td>600–1000</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0.72443 (0.00017)</td>
<td>600–1000</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.71834 (0.00068)</td>
<td>600–1000</td>
<td>3</td>
</tr>
<tr>
<td>Hatchery fish</td>
<td>0.71049 (0.00002)</td>
<td>400–1100</td>
<td>5</td>
</tr>
<tr>
<td>Range and variability of stream water</td>
<td>0.7107–0.7235 (0.00015)</td>
<td>NA</td>
<td>6</td>
</tr>
<tr>
<td>Range and variability of age-0 resident fish</td>
<td>0.7123–0.7285 (0.00005)</td>
<td>NA</td>
<td>17</td>
</tr>
</tbody>
</table>

Note: Sample numbers correspond to fish designations in Fig. 2. Sr isotope ratios are the mean isotopic composition (± standard error (SE)) of micromilled otolith increments taken exclusively from the freshwater portion of each otolith. Mean SE from replicate streamwater and resident fish samples is calculated from the average SE of samples for water (6 sites) and fish (17 sites) throughout Vermont (Kennedy et al. 2000) and expressed here for comparison to the variability from within a single otolith. NA, not applicable.

primarily by old, Cambrian, silicate-bearing rock that yields a high $^{87}$Sr/$^{86}$Sr ratio (0.72337) similar to the signature found in the otolith core of this individual (Kennedy et al. 2000).

In two of the four fish, the natal signatures were precise and repeatable throughout the freshwater phase. This is consistent with the hypothesis that Atlantic salmon juveniles undergo restricted movement (i.e., these fish remained in a single stream the entire time). The isotopic signatures of all fish were also statistically distinguishable from one another (Tukey’s Honestly Significant Difference, $p < 0.001$; Fig. 2). This indicates that these four salmon were reared in different streams and highlights the promise of this technique for distinguishing among natal sources for mixed fish stocks. The standard error of replicated drilled paths from the freshwater phase for individual fish ranged from 0.008% to 0.023% of the Sr ratio (or $±0.00006$ to $0.00017$) (Table 1). The within-stream variability of field-caught fish was slightly larger than the variability we measured for the control hatchery-reared adult. It was comparable to the average within-site variation for water samples collected throughout a growing season (Table 1). Hence, minor fluctuations in a stream water Sr isotopic signature appear to be reflected in the otolith signature.

Counter to the hypothesis of restricted juvenile movement, the other two fish moved significantly among habitats during their freshwater rearing (or natal) phase. The “natal” stream signature for the third fish (salmon 3 in Fig. 2) was interrupted by periodic movements to different habitats. Rapid shifts in the Sr isotope ratios of this fish suggest that it moved extensively at times during the freshwater phase. However, it appears to...
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have returned to the same rearing stream during the summer growing periods. The spacing and timing of the isotopic shift is consistent with an over-wintering movement from a headwater stream in the summer (where it was probably stocked) with a high $^{87}\text{Sr}/^{86}\text{Sr}$ ratio into a deeper downstream tributary with a lower $^{87}\text{Sr}/^{86}\text{Sr}$ value. The fourth fish underwent a different movement pattern. Unlike the other three fish, it had no distinguishable natal stream signature (Fig. 2). Instead, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in its otolith decreased with distance from the center of the otolith resulting in isotopic variability during the first year of growth that is four times greater than those of more sedentary individuals (SE = 0.00068 and coefficient of variation = 0.094%). This steady decline is consistent with a pattern of slow progressive movement downstream throughout its two-year freshwater residency.

Scientists know considerably more about variability in migration and breeding dates among salmon populations (Quinn et al. 2000) than in movement during their nonmigratory stage. Retrospective approaches based on otolith microchemistry can be used to examine this variability further by definitively linking individual salmon survival with specific smolting strategies (e.g., Limburg 2001). Sr isotopic values from the outermost otolith increments from all salmon demonstrated the same distinctive trend over time toward the ocean water value (Fig. 2). In two of the four fish, we analyzed multiple subsections of the outer otolith rings to confirm the constancy of this value and found that there was little variability around a distinct modern ocean signature (range = 0.70912–0.70952; Fig. 2). Significant differences in the point at which the different otoliths incorporated a marine signature indicated that individuals smolted at different ages. Based on Sr isotope ratios and confirmed by both age–length analysis and annual checks in the otoliths, salmon-1 migrated to the ocean during its second spring, 1 year earlier than the other three fish.

Despite the increasing research into geochemical signatures and their recognized potential for discerning among populations, many of the assumptions that underlie their successful application in aquatic and fisheries sciences have not been critically assessed (Campana 1999). This study provides additional evidence that Sr isotope ratios (see also Kennedy et al. 2000) are incorporated unchanged from their aqueous environment, and therefore, they circumvent the confounding physiological or structural factors that can complicate the use of alternative elemental or isotopic markers (e.g., Kalish 1989). As a result, we used Sr isotope ratios in the otoliths from four randomly selected adult Atlantic salmon to distinguish hatchery marks, natal stream signatures, interstream movements, and migratory behaviors. Our examination of individual fish revealed clearly that all four fish adopted different strategies over their lives. This high level of variability in salmon life history strategies could not have been described by conventional marking techniques. We suggest that geologically derived fingerprints, like Sr isotopes, yield critical information about the habitat use and conditional behaviors of individuals. When applied to globally imperiled species like Atlantic salmon, this approach may help to inform management practices, which are otherwise limited to static local measures of performance.

Acknowledgements

We would like to thank K. Gillette, M. Novak, J. Revita, and J. Rowan of the U.S. Fish and Wildlife Service for their assistance in acquiring the salmon specimens and K. Lohmann, A. Dutton, and M. Kelley for access to instruments and laboratory assistance. We appreciate the constructive comments of Simon Thorrold and one anonymous reviewer on an earlier draft of the manuscript. The study was supported by grant #NA86FL0538 from the National Oceanic and Atmospheric Administration.

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