Using natural strontium isotopic signatures as fish markers: methodology and application

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Abstract: To distinguish Atlantic salmon (*Salmo salar*) populations in tributaries of the Connecticut River, we studied the incorporation and stability of Sr isotopes in juvenile salmon. We established the geologic basis for unique isotopic signatures in 29 salmon sites. Stream-specific Sr isotopic ratios ($^{87}Sr/^{86}Sr$) were found in calcified tissues of salmon parr within 3 months of stocking. We found little seasonal variation in the Sr signatures of stream water or fish tissue. There were no significant differences among the Sr signatures of otoliths, scales, and vertebrae. For mature salmon raised under constant conditions, 70% of the Sr isotopic signature in calcified tissues was derived from food sources. We developed a criterion for identifying moving fish based upon the isotopic variability of genetically marked fish. Applying this criterion to our streams, 7% of the fish in our study had incorporated Sr from multiple streams. Strontium isotopes distinguished all 8 regions in the White River basin and 7 of the 10 regions in the West River basin. When watersheds are considered together, Sr isotopes differentiated 11 unique signatures from 18 regions. We conclude that Sr isotopes are an effective marking tool and discuss ways in which they can be combined with other marking techniques over larger spatial scales.

Résumé : L'incorporation et la stabilité des isotopes du strontium (Sr) chez les Saumons de l'Atlantique (*Salmo salar*) juvéniles ont permis de distinguer les populations de différents tributaires du fleuve Connecticut. Les signatures isotopiques particulières de 29 sites à saumons ont pu être reliées à des conditions géologiques. Des rapports isotopiques de strontium ($^{87}Sr/^{86}Sr$) spécifiques à chaque cours d'eau apparaissent dans les tissus calcifiés des tacons moins de 3 mois après l'empoissonnement. Il y a cependant peu de variation saisonnière de la signature de Sr dans l'eau ou dans les tissus des poissons. Aucune différence significative n'apparaît entre les signatures de Sr dans les otolithes, les écailles ou les vertèbres. Chez les saumons à maturité élevés en conditions constantes, 70% de la signature isotopique de Sr dans les tissus calcifiés provient de l'alimentation. En étudiant la variabilité isotopique chez des poissons marqués génétiquement, il est possible d'établir un critère d'identification des poissons qui se déplacent d'une rivière à une autre. Ce critère appliqué aux cours d'eau étudiés a révélé que 7% des poissons avaient incorporé du Sr provenant de plusieurs milieux. Les isotopes du Sr ont permis de distinguer les poissons des huit régions du bassin de la rivière White et de sept des dix régions du bassin de la rivière West. Si les deux bassins sont regroupés, les isotopes du Sr rendent possible la reconnaissance de 11 signatures particulières dans les 18 régions. Les isotopes du Sr sont donc un outil efficace de marquage. Nous examinons comment ils peuvent être combinés à d'autres techniques de marquage pour une utilisation à plus large échelle.

[Traduit par la Rédaction]

Introduction

Fisheries scientists are increasingly using natural geochemical signatures as a population marking technique (Campana and Gagne 1995; Thorrold et al. 1998; Ingram and Weber 1999). This approach is based upon the observation that tissues of fish comprise the elemental or isotopic abundances particular to the water in which they have grown. Thus, in some cases, these "geochemical signatures" can be used to determine the geographic derivation of individuals (Bagenal et al. 1973; Lapi and Mulligan 1981; Limburg 1995). Geochemical signatures arise from the incorporation of particular elements (e.g., Sr) in fish tissue (e.g., scales, backbones) via water and (or) food (Simkiss 1974; Farrell and Campana 1996). Fish from one region can be distinguished from those of another if the chemical signatures in the water and food between regions are sufficiently different and if the fish have remained in a site long enough to incorporate a clear chemical signature. Kennedy et al. (1997) first demonstrated the potential for using Sr isotope ratios ($^{87}Sr/^{86}Sr$) to discriminate among juvenile Atlantic salmon (*Salmo salar*) populations that were reared in 10 different tributaries in Vermont, U.S.A. They found that the ratio of Sr isotopes varied among tributaries and that there was a strong positive correlation between the Sr isotopic ratios in salmon tissues (i.e., backbones and otoliths) and in the water. Many previous studies have used elemental *concentrations* as environmental markers. However, employing Sr isotope *ratios* eliminates the in-

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fluence of factors such as temperature and salinity (Radtke and Shafer 1992; Townsend et al. 1992; Fowler et al. 1995), growth and maturation (Kalish 1989; Gauldie 1996; Friedland et al. 1998), or genetics (Behrens Yamada et al. 1987). These physical and biological factors have been shown to alter elemental concentrations but will not influence the relative abundance of Sr isotopes. As a result, isotope ratios allow increased precision and can be more useful than element concentrations as environmental tracers (Gunn et al. 1992; Fowler et al. 1995).

This emerging methodology has several valuable applications in fisheries research. For example, as a method for stock discrimination, stable isotopes have certain advantages over conventional marking techniques. Isotopic signatures are quickly incorporated into small growing fish and are derived from the water and food where the animal lives. An alternative to using Sr isotopes is to mark fish prior to stocking into rearing streams and to use these markers to identify individuals when they are recaptured later in life. However, this approach is problematic. Physical tags are impossible to apply to such small individuals, and recovered tags can be used to estimate contributions from only a limited number of stocked populations (Bergman et al. 1992; Niva 1995). Most importantly, some species, such as salmon, may move extensively before acquiring a territory (Elliott 1987; Gowan et al. 1994; Armstrong et al. 1997), so the release location (determined by tracing the tag) may not be the actual rearing habitat. Alternatively, natural Sr isotope signatures are incorporated into both exchangeable (e.g., vertebrae) and nonexchangeable (e.g., otoliths) calcified tissues. This makes geochemical signatures recoverable from discrete time periods and, therefore, a potentially powerful tag for both short-term dispersal studies and longer-term studies of population demography. Finally, in situ incorporation of geochemical markers makes them applicable to both naturally reproducing and stocked populations.

Strontium can serve as a geochemical marker because Sr substitutes for Ca in geological and biological materials and is therefore found at relatively high concentrations in both calcium carbonate rich rocks and calcified tissue of fish. ^{87}Sr is produced through the radioactive decay of ^{87}Rb (half-life = 49×10^9 years) over geological timescales, whereas the absolute amount of ^{86}Sr does not change over time. Therefore, older rocks and (or) rocks with higher Rb/Sr ratios develop higher $^{87}Sr/^{86}Sr$ ratios (Faure 1977; Åberg 1995). The stable isotope ratios of dissolved Sr in stream water reflect the age and the composition of underlying soils and rocks in the watershed. In food webs, Sr isotopes do not fractionate with trophic levels like some isotopes (Graustein 1989; Blum et al. 2000); therefore, fish are likely to incorporate the same isotopic signature from both the dissolved Sr in stream water and the Sr in diet items.

Herein, we seek to refine the methodology for using Sr signatures as tags for fish populations by (*i*) quantifying Sr isotopic variability within and among fish in situ and (*ii*) placing this variability in the context of isotopic turnover in fish, fish movements, and regional geology. Currently, studies of geochemical markers rely on extensive site-specific analysis to determine the spatial variability of chemical signatures among different geographic areas. By placing the Sr isotopic variability in the context of the bedrock geology, we can predict in advance (e.g., from geological maps) whether sites are likely to show sufficient geochemical variation for the technique to be useful. Harrington et al. (1998) demonstrated the utility of a similar approach based upon land use patterns in six Vermont streams where the ^{15}N value of stream water nitrate and juvenile salmon was positively correlated with the agricultural coverage of the watershed.

We test the feasibility of this method in a case study to differentiate populations of juvenile Atlantic salmon in tributaries of the Connecticut River. This research builds upon our earlier work with Sr isotopes in juvenile Atlantic salmon (Kennedy et al. 1997) and is part of ongoing efforts to restore Atlantic salmon to their native ranges in the northeastern United States (Nislow et al. 1999). Successful application of this technique to the salmon restoration program underway in the Connecticut River would allow us to distinguish the rearing origin (the tributary in which individuals have grown) among mixed stocks of smolts and returning adult salmon and ultimately to establish relationships between juvenile habitat quality, individual survival, and adult fitness.

Materials and methods

Study system

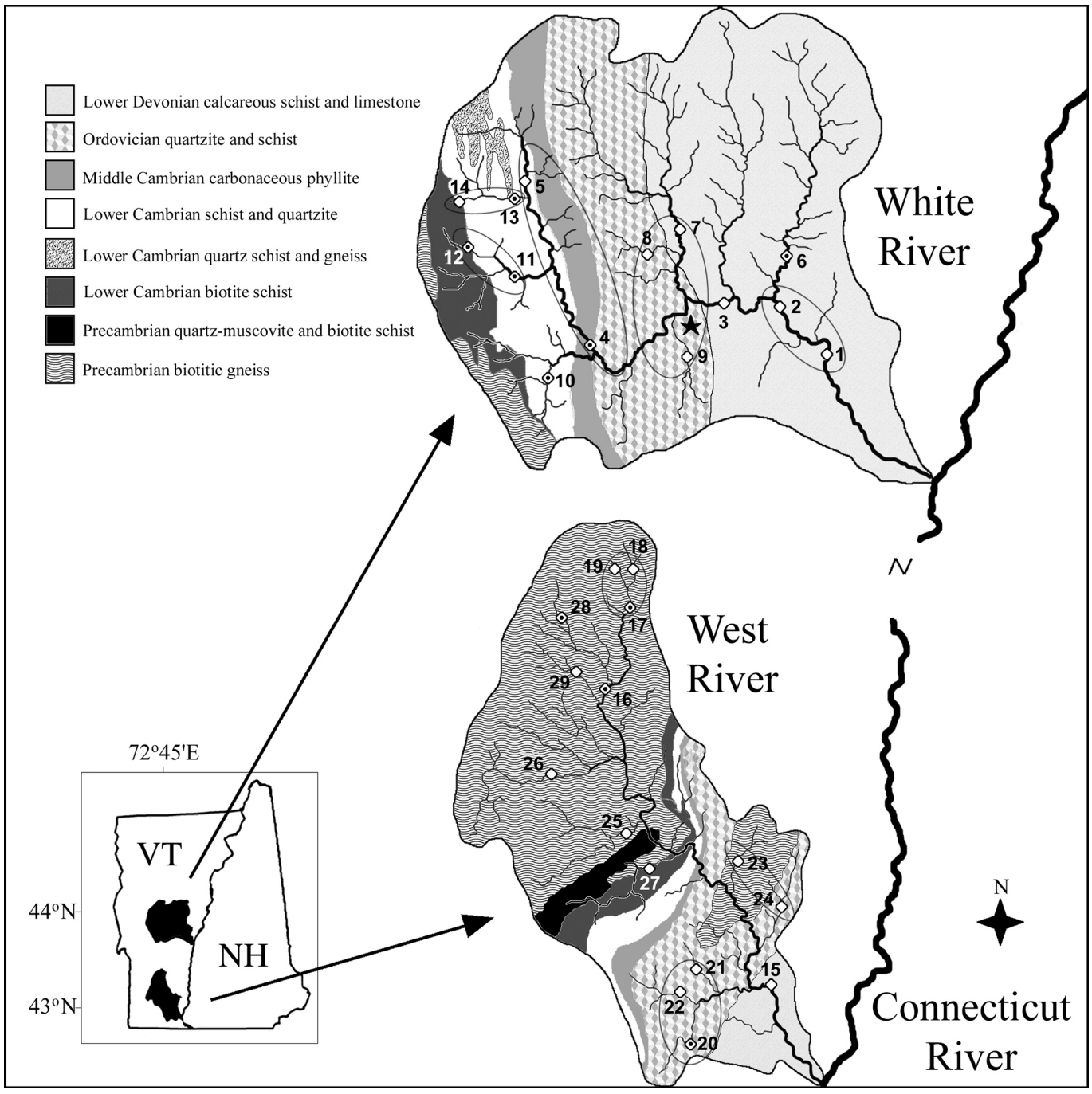
We studied Sr isotopic variability in the West and White rivers, two major watersheds of the Connecticut River in central and southern Vermont, U.S.A. (Fig. 1). Atlantic salmon were eliminated from the Connecticut River in the early nineteenth century when dams hindered passage to spawning grounds (Connecticut River Atlantic Salmon Commission 1998). For the past 30 years, juvenile salmon have been stocked into many watersheds in the Connecticut River basin in an effort to restore salmon to their historic range (McMenemy 1995). The West and White rivers receive approximately 15% of the more than 10 million hatchery fry that are stocked annually. After smolts leave their rearing tributaries and throughout their adult life, Atlantic salmon from many stocking regions along the Connecticut River are mixed and indistinguishable. Of the several hundred adult salmon that return to the Connecticut River each year, 90% are collected and used for brood stock, eliminating the possibility of simply following adults to their natal rearing habitat. Consequently, there is currently no way to determine which rearing stream produced a smolt or a returning adult salmon, although such information would be extremely useful to guide restoration and management strategies.

Our study addresses four separate aspects of Sr isotopic expression in stream habitats and salmon populations. First, we establish the Sr isotopic differences among 29 Vermont stream sites and examine these differences in light of the bedrock geology and the stability of the stream water signatures. Second, we address technical questions about the sources, turnover rates, and the variability of Sr isotopes both within individuals and among sympatric Atlantic salmon. Third, we discuss the potential complications introduced when fish incorporate isotopic signals from more than one region and we present a scenario for using isotopes to study the movements of fish among tributaries. Fourth, we apply Sr isotopic signatures to differentiate stream populations of juvenile Atlantic salmon.

Watershed geology and isotopic signatures in streams

To characterize the Sr isotopic variability of the region, we sampled juvenile Atlantic salmon vertebrae and sagittal otoliths and water from 29 study sites throughout the West (15 sample sites) and White (14 sample sites) rivers in 1995, 1996, 1997, and 1998. The 29 sampling locations (hereafter referred to as sites) are small

Fig. 1. Geologic map of the White and West rivers in Vermont, U.S.A. The star in the White River represents the White River National Fish Hatchery. Numbered symbols identify study sites and are referenced in Table 1. Site symbols with a dot in the center represent the 10 sites used to compare Atlantic salmon and water isotopic signatures. Sites aggregated by oval outlines denote geographic areas that were combined for regional analyses.



(approximately 50 m) reaches of second- to fourth-order streams from which both fish and water samples can be obtained. In most cases the designation "site" corresponds to an individual stream, with the exception of the mainstem West and White rivers, which had multiple sites. Sites were chosen to maximize the watershed area covered. In total, we analyzed Sr isotopes in approximately 150 fish tissue and water samples. In addition to the overall comparison among sites for which all of the data were used, we used subsets of the entire data set for other tests and comparisons. The sampling sizes were not balanced across all sites. In some sites, both stream water and fish were sampled at multiple times, in some sites, both fish and water were sampled only once, and in others, either fish or stream water was sampled.

To quantify the temporal variability of isotopic signatures in streams, seasonal and annual water samples were taken from four sites at baseflow during three ice-free months (May, August, October). The water from one of these four sites was sampled across two years. Juvenile salmon were sampled in late summer and early fall by electroshocking. Stream water was sampled in acid-washed polyethylene bottles and centrifuged to remove ≤0.45 µm particles. Samples were returned to the laboratory on ice and prepared for analysis in a clean laboratory facility as discussed below. Geologic

information is derived from the geologic maps of Vermont (Doll et al. 1961).

Incorporation of Sr signatures in Atlantic salmon

In this section, we address technical questions about the relationship between the Sr isotopic composition of a fish and its environment. As a part of this section, we also quantify two sources of isotopic variation: (*i*) the intra-individual variation of different calcified tissues and (*ii*) the intra-population variation by measuring the isotopic values of marked sympatric Atlantic salmon.

To compare the Sr isotopic ratio of stream fish with that of their stream water, we sampled both fish and water in a subset of the sites above (10 of 29) in summer and early fall between 1995 and 1998. Fish and water samples from multiple years were combined. These 10 sites spanned the range of isotopic values measured in the region. In 2 of the 10 sites, we sampled fish and water twice during the first growing season to measure the rate at which Sr isotopes become incorporated into fry tissue. At these sites (Bingo Brook and West Branch), stream water and age-0 salmon were sampled in August and October 1995 (3 and 5 months after stocking).

To quantify the relative contributions of Sr in food and water to salmon tissue, we sampled three mature domestic Atlantic salmon from the White River National Fish Hatchery in Bethel, Vermont. Hatchery adults were used for this experiment because they had lived for 4 years in a controlled environment in which water and food sources were constant. This allowed all calcified tissues to equilibrate with a single environment without introducing the potentially confounding effects of fish movements between streams. In the hatchery, water is continually delivered to flow-through salmon holding pens via two nearby groundwater wells. The Sr isotopic values of the two wells are similar (0.714545 and 0.714661) and were averaged to derive a mean isotopic contribution from water. Hatchery food is a special Fish and Wildlife trout chow blend produced by Purdue Feeds for Atlantic salmon (B. Jensen, White River National Fish Hatchery, Bethel, Vt., personal communication). Because the isotopic signatures of hatchery food and water were distinct from one another and because the isotopic values of salmon tissue fell between that of the food and water, we were able to apply a simple isotopic mixing model to establish the relative contribution of these two exclusive sources of Sr to the analyzed tissues. The equation for the model of Sr contributed by food is

$$(1)\qquad \%\ \mathrm{Sr}_{(\text{from food})} = \left(1 - \left(\left({}^{87}Sr/{}^{86}Sr_{\text{fish}} - {}^{87}Sr/{}^{86}Sr_{\text{food}}\right)/\left({}^{87}Sr/{}^{86}Sr_{\text{water}} - {}^{87}Sr/{}^{86}Sr_{\text{food}}\right)\right)\right) \times 100$$

and represents the fraction of the isotopic difference between food and water that is accounted for by the difference between the fish and food isotopic ratios. Similarly, the equation for water Sr contribution is

$$(2)\qquad \%\ \mathrm{Sr}_{(\text{from water})} = \left(1 - \left(\left({}^{87}Sr/{}^{86}Sr_{\text{water}} - {}^{87}Sr/{}^{86}Sr_{\text{fish}}\right)/\left({}^{87}Sr/{}^{86}Sr_{\text{water}} - {}^{87}Sr/{}^{86}Sr_{\text{food}}\right)\right)\right) \times 100$$

To compare the isotopic values of different calcified tissues, we extracted vertebrae, scales, and otoliths from three adult salmon. Both sagittal otoliths from each fish were combined and analyzed collectively. Scales (8 to 10) were removed from the front left side of the fish dorsal to the lateral line and combined for each sample. Two vertebral segments from the posterior end of the backbone were taken from each fish and combined into a single sample. All tissues were prepared for analysis as described below (see Analytical procedures). A one-way analysis of variance (ANOVA; JMP version 3.2.1, SAS Institute Inc., Cary, N.C.) was used to test for isotopic differences among tissue types.

To quantify intra-population isotopic variation, we conducted an experiment using marked fish in a single stream reach. Genetically marked salmon fry were created from controlled crosses of mature sea-run Atlantic salmon as part of a larger project on the genetics of Atlantic salmon in the Connecticut River (Letcher and King 1999). Fry were spawned and raised at the White River National Fish Hatchery. Approximately 200 fry with known genetic marks were stocked into a 100-m study reach of Flood Brook on May 12, 1998. The study reach was resampled on August 12, 1998. The $^{87}Sr/^{86}Sr$ values in vertebrae of four recaptured genetically marked fish were analyzed. We concluded that these four genetically marked individuals had spent 3 months in close proximity to where they were stocked and could therefore serve as a control for intra-population variability. The sample size is small due to a low recapture rate; however, it represents the average sample size of individuals in this study and a robust estimate for the isotopic signature and variance from a single site. We compared the standard deviations of $^{87}Sr/^{86}Sr$ values at this study site with those of salmon at all other sites. The variability expressed in the isotopic values of these fish serves as our benchmark for the natural variability of sympatric fish reared at the same site in the wild (within-stream variability). Below, we use this benchmark to identify fish that have incorporated a significant portion of their Sr signature outside the site in which they were caught.

Identifying "movers" based upon Sr isotopic signatures

Based upon our experiment with genetically marked individuals, we know that juvenile salmon raised under identical conditions develop similar signatures. However, some juvenile salmon move long distances, feed in different streams with dissimilar isotopic values, and therefore are likely to develop an isotopic signature that is a combination of multiple source sites (hereafter referred to as "movers"). If movers go unidentified, their $^{87}Sr/^{86}Sr$ values contribute to the overall isotopic variance within a site, and they can misrepresent the relationship between a stream water signature and the fish that have lived exclusively in that stream (hereafter referred to as "residents"). Based upon our measures of within-site isotopic variability (described above), we developed a criterion to distinguish straying from nonstraying fish. We classified movers as individuals whose $^{87}Sr/^{86}Sr$ value was beyond 2 SD from the mean value at a site. Hence, our metric for movers depends upon the confidence intervals for the isotope signature value within each site. On average, these confidence intervals are based upon the mean isotopic value of three to five samples.

The confidence intervals across all sites were four times greater than the standard deviation of the mean isotopic value from the genetically marked individuals in Flood Brook (±0.00035 versus ±0.00009). Although this is a subjective definition of movers, we believe that it provides a very conservative estimate for straying within these stream populations based upon the results from our genetic marking experiment. As such, our classification is much more likely to fail to identify movers (and therefore underestimate the occurrence of long-distance movements) than to incorrectly identify a resident fish as a mover. To estimate the frequency with which these movements occur, we analyzed 68 age-0 fish from 12 tributaries in the West River and 17 age-0 fish and 32 age-1 fish from 9 tributaries in the White River.

Distinguishing Atlantic salmon populations

To assess the feasibility of using isotopic signatures to differentiate fish, we tested for differences in isotopic signatures at two spatial scales: (*i*) among the 29 sites and (*ii*) among geographic regions, wherein close or connected sites are combined. For among-site comparisons, each of the 29 individual sampling sites was treated independently. For regional comparisons, we grouped values from geographically related sites based upon two criteria. First, we grouped sites if they were within 10 km of each other (e.g., Mill Brook and Grassy Brook in the West River). Second, we grouped sites if they shared a common tributary of origin (e.g.,

Bingo Brook and the West Branch in the White River). These regional groupings decreased the number of comparisons from 29 to 18 (eight in the White River and 10 in the West River). To compare Sr values across sites and regions, all fish and water samples were pooled to establish a mean signature. For site comparisons, sample sizes averaged between three and five. Fish that were identified as movers based upon our criterion for within-site variability were excluded from this analysis. All comparisons of $^{87}Sr/^{86}Sr$ values by site and region were made using one-way ANOVA (JMP version 3.2.1, SAS Institute Inc. Cary, N.C.) followed by Fisher's least significant difference multiple comparison ($\alpha = 0.05$) to test for significant differences in Sr isotopic signatures across sites.

Analytical procedures

All fish were frozen immediately upon sampling and were stored at –20°C. Water samples were refrigerated. Subsequent work was performed in clean laboratory facilities. We removed the entire vertebrae (approximately 50–100 mg) and the sagittal otoliths (<1 mg) using acid-washed Teflon® forceps or clean stainless steel tools when needed. Vertebrae and otoliths were rinsed three times with distilled and deionized water, placed into clean Teflon® beakers, ultrasonically cleaned in deionized water, dried at 60°C, and weighed. They were dissolved in double-distilled concentrated Seastar® HNO_3, evaporated to dryness, and taken into solution in 3 N HNO_3.

Strontium was separated using Sr-specific cation-exchange resin in quartz columns. Aliquots containing 50–350 ng of Sr were taken up in 1 μL of 0.3 M H_3PO_4 and loaded onto out-gassed tantalum or tungsten filaments with Ta_2O_5 powder. The Sr isotopic compositions were measured using either the VG-Sector or the Finnegan MAT 262 thermal ionization mass spectrometer at Dartmouth College. The VG-Sector is a single-collector instrument, whereas the Finnegan MAT 262 is a multicollector instrument with eight Faraday collectors. Reported uncertainties are ±2 SE calculated from the measurement of 100–300 ratios on each individual sample. Strontium isotope values are generally reported as ratios of ^{87}Sr to ^{86}Sr. $^{87}Sr/^{86}Sr$ ratios were normalized to $^{88}Sr/^{86}Sr = 0.1194$. Replicate analysis of National Institute of Standards and Technology SRM-987 completed during the course of this study yielded mean $^{87}Sr/^{86}Sr = 0.710215$ (SD = 0.000062, $n = 12$) and 0.710262 (SD = 0.000013, $n = 32$) for the VG-Sector and the Finnegan MAT 262, respectively. Data from the VG-Sector were corrected to the National Institute of Standards and Technology value of 0.710262.

Results

Watershed geology and isotopic signatures in streams

Water and geology of the West and White rivers

Patterns in the stream water Sr isotope values are generally predictable based upon the underlying stream basin geology. The most important geologic factor affecting the Sr isotopic values of stream water in the study watersheds is the presence or absence of calcium carbonate in the rocks because this mineral generally contains high concentrations of Sr with a low $^{87}Sr/^{86}Sr$ ratio and is easily dissolved by rainwater. In general, the bedrock of the White River is younger and contains more calcium carbonate rich rock, whereas the bedrock of the West River is older and almost exclusively silicate. The lowest $^{87}Sr/^{86}Sr$ ratios are in the First Branch of the eastern White River, which drains mostly carbonate-rich schist bedrock from the Devonian Gile Mountain and Waits River formations (Fig. 1). Streams in this region also have the highest dissolved Ca and Sr concentrations (200 ppb Sr compared with 10–25 ppb elsewhere in the White River). The combination of low $^{87}Sr/^{86}Sr$ and high overall Sr concentrations in the First and Second branches has a significant impact on the mainstem White River signal, which drops from 0.71829 to 0.71220 after collecting water from tributaries east of Bethel.

The western portion of the White River basin is roughly divided into five parallel distinct north–south geologic zones. In general, the bedrock in these five bands is a heterogeneous blend of four silicate rock types (phyllite, schist, gneiss, and amphibolite) that increase in age from east to west. The bedrock in this area contains significantly less calcium carbonate and has higher Rb/Sr ratios than that of the eastern White River. The first band is middle to lower Ordovician in age and influences the White River tributary chemistry between Bethel and the Tweed River confluence. This includes all of Locust Creek and the western tributaries of the Third Branch, such as Gilead Brook. These streams have $^{87}Sr/^{86}Sr$ values between 0.71936 and 0.72020. Next, the headwater tributaries of the White River are composed of phyllite of upper Cambrian age. $^{87}Sr/^{86}Sr$ values in this region of the White River range from 0.71757 to 0.71798.

The Cambrian age Pinney Hollow Formation runs the length of the upper White River and controls the chemistry of Hancock Branch, a tributary in the northern White River. This schist gives the Hancock Branch an $^{87}Sr/^{86}Sr$ value of 0.71876. The westernmost tributaries (e.g., Bingo Brook, West Branch, and Tweed Branch) have the oldest bedrock and highest stream water $^{87}Sr/^{86}Sr$ ratios of the White River (range = 0.72241 to 0.72358). Robbins Branch, which drains into Hancock, is influenced by contributions from both of these geologic terrains, with $^{87}Sr/^{86}Sr = 0.72023$.

In general, the West River contains very little of the Devonian calcium carbonate rich rocks that dominate the eastern White River. However, the easternmost portion of the West River contains bedrock formations similar to parts of the White River. Overlap in $^{87}Sr/^{86}Sr$ values is greatest here. For example, Grassy Brook and Mill Brook in the West River have $^{87}Sr/^{86}Sr$ values that are similar to those of Tweed River in the White River (0.72171 and 0.72188 versus 0.72258), but some of the stream Sr isotope patterns lack the predictability of those in the White River. For example, Marlboro Branch lies in a geologic terrain similar to that of Locust Creek in the White River but has a much higher $^{87}Sr/^{86}Sr$ ratio (0.72882 compared with 0.71963). Additionally, a major portion of the upper West River and its tributaries lies in the southern Green Mountains, which are underlain by homogenous Precambrian gneiss. In this region, we expected to find uniformly high $^{87}Sr/^{86}Sr$ ratio streams. Although we found some of the most radiogenic signals in this area (e.g., 0.73183), we also found a range of stream isotopic signals including some anomalously low $^{87}Sr/^{86}Sr$ values (e.g., Winhall Brook, $^{87}Sr/^{86}Sr = 0.71233$).

Stability of water signal through seasons and years

The Sr isotopic signal in stream water is very stable across seasons and years (Table 1). The largest seasonal difference among replicate samples is found in Bingo Brook where $^{87}Sr/^{86}Sr$ values changed from spring to summer by 0.00035 (0.72299–0.72334). Replicate samples collected during different seasons generally do not deviate from the mean isotopic value for that site by more than 0.02%.

Table 1. Strontium isotope ratios for juvenile Atlantic salmon from 29 sites in the West and White rivers, Vermont, U.S.A.

		$^{87}Sr/^{86}Sr$					
Map reference No. and site	Year	0+ vertebrae	1+ vertebrae	Water	Site average	Regional average	*n*
White River and tributaries							
1. White River	1995			0.712519±23*	0.712519±310 *a*	0.712360±159 *a*	2
2. White River	1998			0.712201±09	0.712201±310 *a*		
3. White River	1998			0.714524±08	0.714524±310 *b*	0.714524±310 *b*	1
4. White River	1996		0.718000±39*	0.718292±08	0.718067±57 *c*	0.717984±94 *c*	6
	1997		0.718050±08				
			0.721770±08				
			0.718010±09				
			0.717983±10				
5. White River	1997			0.717571±14	0.717571±310 *c*		
6. First Branch	1995		0.710582±36*	0.710770±28*	0.710680±54 *d*	0.710680±54 *d*	3
	1996			0.710687±38*			
7. Third Branch	1996	0.719523±12	0.719462±27*		0.719587±79 *e*	0.719705±73 *e*	19
		0.719544±13					
		0.719817±18					
8. Bethel Gilead	1996	0.719314±18	0.719428±09		0.719763±112 *e*		
	1998	0.719387±16	0.719870±09				
		0.719249±09	0.719784±06				
			0.720082±22				
			0.720115±09				
			0.719360±17				
			0.720129±144				
			0.720195±19				
			0.720238±11				
9. Locust Creek	1996	0.719637±13			0.719634±06 *e*		
		0.719642±13					
		0.719623±12					
10. Tweed Branch	1995	0.722070±27*	0.721528±30*	0.722793±27*	0.722036±206 *f*	0.722036±206 *f*	6
	1996		0.721505±07	0.722411±09			
	1997		0.721907±09				
			0.719702±12				
11. West Branch	1995	0.723529±32*	0.722587±06	0.723625±27*	0.723235±147 *g*	0.723216±80 *g*	13
	1996	0.723294±30*	(0.722332±19)	0.723549±39*			
	1997		0.723205±42*				
			0.722854±09				
			*0.720404±19**				
			0.719471±08				
12. Bingo Brook	1995	0.723235±30*	0.723051±29*	0.723037±36*	0.723193±55 *g*		
	1996	0.723265±29*	(0.722802±12)	0.723390±25*			
			*0.722310±14**	0.723181±30*			
13. Hancock Branch	1997		0.719250±10	0.718762±12	0.718346±237 *c*	0.718975±350 *e*	9
			0.717795±11				
			0.717769±12				
			0.718380±11				
			0.718117±14				
14. Robbins Branch	1996	0.720149±16			0.720233±56 *h*		
		0.720210±17					
		0.720339±16					
West River and tributaries							
15. West River	1995			0.719768±36*	0.719280±487 *e*	0.719280±487 *e*	2
				0.718793±27*			
16. West River	1996	*0.722429±11*		0.721246±12	0.721113±134 *i*	0.721113±134 *f*	2
	1998	0.720979±13					
		0.719261±14					

Table 1 (*concluded*).

Map reference No. and site	Year	$^{87}Sr/^{86}Sr$ 0+ vertebrae	1+ vertebrae	Water	Site average	Regional average	*n*
17. West River	1995	*0.731875±40*[1]		0.726818±417*	0.727463±172 *j*	0.727849±308 *h*	8
		(*0.731008±14*[1])					
		0.727665±22					
		0.727948±14					
		0.727639±09					
		0.727620±09					
		0.727085±09					
18. West River	1998			0.729652±13	0.729652±310 *k*		
19. Greendale Brook	1998			0.728363±09	0.728363±310 *l*		
20. Marlboro Branch	1995	0.728786±42*		0.728868±29*	0.728770±62 *l*	0.726651±622 *i*	8
		0.728656±103*					
21. Baker Brook	1995			0.725505±41*	0.725505±310 *m*		
22. Rock River	1996	0.725418±12			0.725348±89 *m*		
		0.725533±20					
		0.725110±09					
		0.725333±12					
23. Mill Brook	1996	0.721843±12			0.721887±27 *f*	0.721802±43 *f*	8
		0.721854±08					
		0.721886±11					
		0.721964±12					
24. Grassy Brook	1996	0.721850±10			0.721717±58 *f*		
		0.721596±11					
		0.721649±08					
		0.721772±10					
25. Ball Mountain Brook	1996	0.724596±09			0.724353±89 *n*	0.724353±89 *j*	4
		0.724169±10					
		0.724310±09					
		0.724339±12					
26. Winhall Brook	1996	0.712305±10			0.712334±17 *a*	0.712334±17 *a*	3
		0.712364±10					
		0.712332±09					
27. Wardsboro Branch	1996	0.725286±07			0.725340±39 *m*	0.725340±39 *k*	3
		0.725417±08					
		0.725318±11					
28. Utley Brook	1995	0.719483±36*[2]		0.719838±36*	0.720459±48 *h*	0.720459±48 *f*	32
	1998	(0.719247±17[2])					
		0.719599±33*					
		0.720543±08†					
29. Flood Brook	1998	0.714950±06			0.714870±44 *b*	0.714870±44 *b*	4
		0.714778±12					
		0.714813±12					
		0.714939±19					

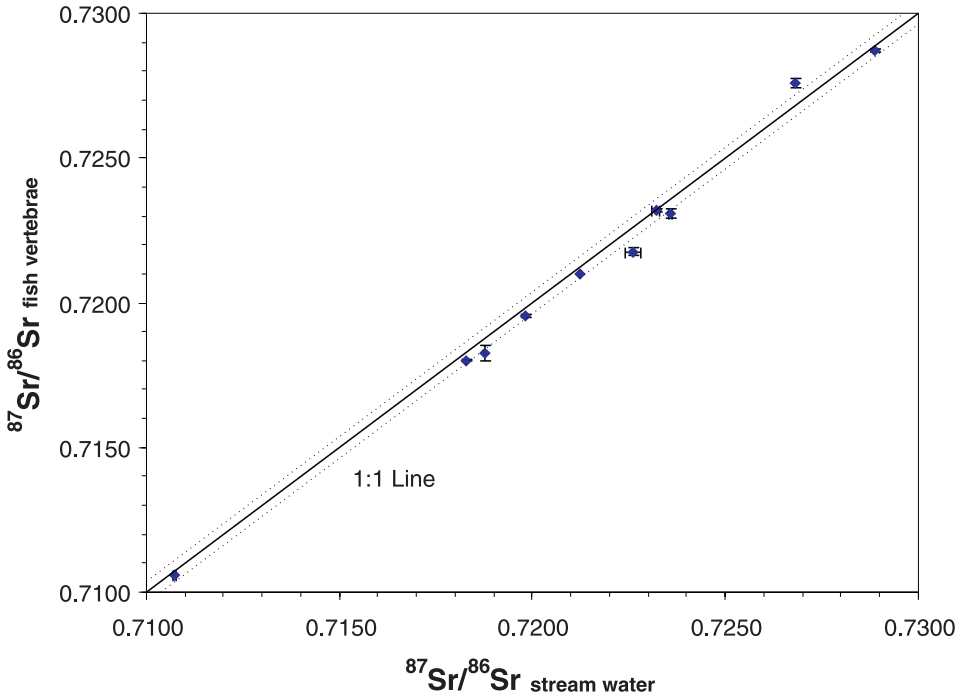
Note: All ratios in columns 3–5 represent individual samples except for the single value marked with a dagger at site 28, which is the average from 29 samples taken on a single sample date. Reported uncertainties are ±2 SE calculated from the measurement of 100–300 ratios of an individual sample run. Values marked with an asterisk denote samples that were analyzed on a VG-Sector (pre-1998) with appropriate correction (see Materials and methods). Other samples were analyzed on a Finnegan MAT 262. Matching superscript numbers represent coupled vertebrae and otolith (in parentheses) samples analyzed from the same age-0 individual. Italicized values represent straying individuals (see Materials and methods) and were excluded from multiple comparison tests. Ratios in columns 6 and 7 are the site and regional means and standard errors. Site averages are the means from all fish and water samples from an individually numbered site. Regional averages combine all samples from grouped sites, designated by horizontal broken lines (and groupings on Fig. 1). Sample sizes for regional comparisons given are in the last column. Results from an ANOVA of site and regional comparisons are summarized by letters in row averages. Sites and regions with different letters are significantly different (ANOVA, Fisher's least significant difference multiple comparison test, $p < 0.05$).

Incorporation of Sr signatures in Atlantic salmon

Strontium uptake in situ by stocked fry

Stream water Sr isotope ratios are incorporated quickly within the calcified tissues of growing fry. Three months after stocking, the vertebral signatures in salmon are indistinguishable from the Sr signature of the stream into which they were stocked (Table 1). The acquired signatures do not change between 3 and 5 months in age-0 fish. Moreover, the new signal is significantly different from the hatchery value

Fig. 2. Relationship between dissolved $^{87}Sr/^{86}Sr$ of 10 streams and the $^{87}Sr/^{86}Sr$ of the vertebrae of Atlantic salmon from those streams. Error bars on individual symbols are 1 SE of the mean when more than one sample was run per site (8 of 10 vertebrae samples and 4 of 10 water samples). The broken lines represent two times the average standard deviation of the mean across all sites (±0.00037).



for all 29 stream sites in this study. The hatchery signal in the backbone is overwhelmed by the accumulation of Sr from the stream environment. This accretion of Sr accompanies the explosive growth (the vertebrae grows from <1 mg to approximately 100 mg during this time) that salmon undergo during the first 3 months after stocking.

Relationship between stream water and Atlantic salmon tissue $^{87}Sr/^{86}Sr$

There is a strong correlation (r^2 = 0.995) between the Sr isotopic composition of the stream water and that of the resident juvenile salmon (Fig. 2). Most values lie within the analytical uncertainty of the 1:1 line.

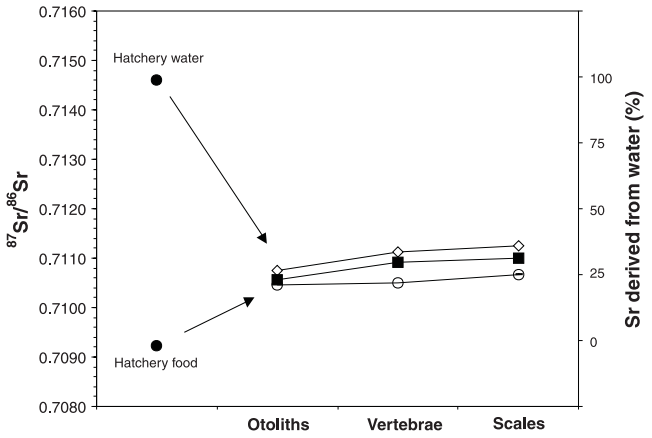
Hatchery adults and within-individual isotopic variation

An important finding is that all tissues studied (otoliths, scales, and backbones) have essentially the same isotopic signature (Fig. 3). Minor differences between Sr ratios of tissues (<0.03%) were consistent among fish but were not statistically significant ($F_{2,6}$ = 1.66, p = 0.27). Otoliths had the lowest $^{87}Sr/^{86}Sr$ ratios and scales had the highest $^{87}Sr/^{86}Sr$ ratios (Fig. 3).

Strontium sources (water versus food) at the hatchery

At the hatchery, dissolved $^{87}Sr/^{86}Sr$ ratios reflect the local groundwater (0.71460). The salmon diet has much lower $^{87}Sr/^{86}Sr$ ratios (0.70923), reflecting the marine origins of the major food ingredients (i.e., fish meal, marine $^{87}Sr/^{86}Sr$ = 0.70918; Hodell et al. 1989). The $^{87}Sr/^{86}Sr$ of hatchery adults (0.71080) suggests that approximately 70% of the Sr in calcified tissues is derived from diet and 30% is from the water when averaged over a salmon's entire lifetime (Fig. 3).

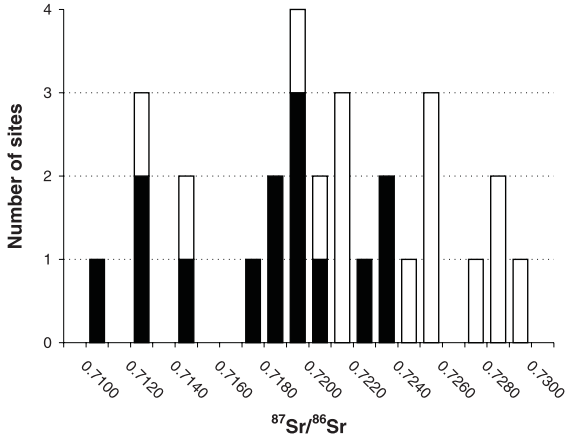
Fig. 3. Strontium isotope ratios in adult Atlantic salmon otoliths, vertebrae, and scales. The three fish are mature 4-year-old salmon maintained on the same diet and water source. Each fish is represented by a different symbol. Averaged fish isotopic values were not significantly different. All points represent a single isotopic value, except for the scale sample represented by the open circle, which is the average of three separate scale samples from the same individual. In this case, the standard deviation is plotted but is much smaller than the symbol size.



Isotopic variability of sympatric age-0 Atlantic salmon (within-stream variability)

The mean $^{87}Sr/^{86}Sr$ value for four genetically marked residents of Flood Brook is 0.714870. The standard deviation for the Flood Brook sympatric salmon is 0.00009 (SE = 0.00004, coefficient of variation = 0.013%). This standard

Fig. 4. Frequencies of $^{87}Sr/^{86}Sr$ values for 29 stream sites in this study. Black bars, sites from the White River; white bars, sites from the West River. Stacked bars roughly correspond to sites with indistinguishable Sr isotopic signatures.



deviation falls in the range of the standard deviations for the other West River and White River tributaries (SD = 0.00001–0.00039) that did not contain genetically marked fish. Compared with the 20 other sites from which replicate fish were sampled, the standard deviation of known residents in Flood Brook is greater than the standard deviation at six sites and less than that at 14 sites.

Identification of movers based upon Sr isotopic signatures

In most instances, the isotopic signals of fish from West River study sites (approximately three per site) show remarkably low variability. Standard errors of replicate fish within individual sites (n = 2–5) are between 0.000017 and 0.000139. In the West River basin, we found that <5% of the age-0 salmon (3 out of 68 fish) had moved among tributaries based upon their outlying isotopic values. In these three cases, Sr isotopic compositions far exceeded the 2 SD interval rule for classification as residents. In the White River, the isotopic signatures of all age-0 salmon are very similar to that of their stream water signature and also show remarkably low within-stream variability. Based upon the close match between tissue signatures and the isotopic signal in the water where they were collected, none of the age-0 fish caught in the White River are likely to have moved between tributaries in their first summer. In contrast, the isotopic values of 5 of the 32 age-1 fish (15%) exceeded the 2 SD confidence interval and were classified as long-distance movers. For comparison, all eight individuals classified as movers in this study had isotopic values that were at least 7 SD from the mean, and in one case the fish Sr value was more than 100 SD from the signature of the stream in which it was collected.

Distinguishing Atlantic salmon populations

There are significant differences among the stream water and fish $^{87}Sr/^{86}Sr$ ratios from these 29 sites (range = 0.71058–0.73188; $F_{21,104}$ = 762.72, p < 0.001). The West River is generally more radiogenic (higher $^{87}Sr/^{86}Sr$) than the White River; however, there is some overlap between the two watersheds (Fig. 4).

Within the White River basin, the 14 sites can be separated into eight distinct isotopic groupings. Isotopic overlap occurred in Sr ratios from four pairs of streams. In all of these instances, the overlapping streams were close geographically and drained similar geologic formations. In most cases, the overlap occurred between two sites that were part of the same river system (e.g., two sections of the White River or Bingo Brook, which drains directly into the West Branch). Within the West River, there were significant differences among the $^{87}Sr/^{86}Sr$ values for 11 of the 15 study sites. Two of the three instances of overlap involved streams that drained basins that were close geographically. In the third case, the two overlapping streams are at the completely opposite ends of the West River basin but drain similar geologic formations.

When sites from both the White River and the West River basins are considered together, the 29 streams separate into 14 distinguishable isotopic groupings (Table 1). Of these 14 isotopically distinct groupings, 5 are represented by single streams and the remaining 9 are a composite of between two and four isotopically overlapping streams. Five of these groupings include streams from both basins (i.e., the stream has a unique signature within the White River basin but is indistinguishable from a stream in the West River).

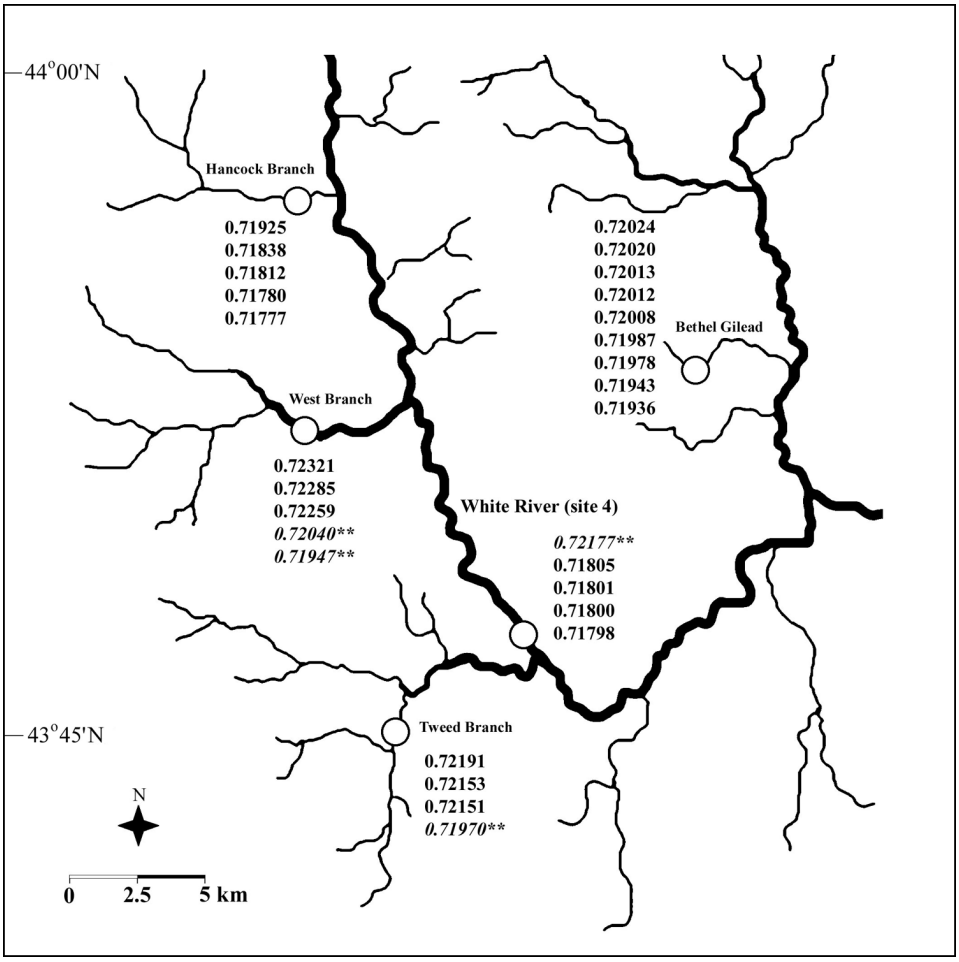
When study sites are grouped into regions, all 8 regions in the White River were separable based upon their Sr isotope signatures. In the West River, the 10 regions were grouped into 7 distinguishable signatures. When both basins were considered together, the 18 geographic regions had 11 unique isotopic signatures.

Discussion

Our work demonstrates that Sr isotope ratios in fish tissue can be used to differentiate fish from different geographical areas. Strontium isotopes in stream water are stable across seasons and years. This temporal stability produces low variance in the isotopic signature of resident fish populations. Isotopic signatures that reflect the rearing stream develop quickly in stocked fry, replacing the hatchery signal within 3 months. Based upon the variation in the basin geology of 29 river sites in the Connecticut River watershed, we identified 14 unique and distinguishable isotopic signatures. Within the White and West rivers, the number of isotopically distinct zones represents 57–73% of the number of study sites. A critical methodological feature of using Sr isotopes is that there are no differences in the Sr isotopic values among different calcified tissues. These results come from our experiments with both stream-living juveniles and hatchery-raised mature Atlantic salmon.

Isotopic signatures have several advantages over more conventional marking techniques, such as physical tags. The magnitude of some stocking programs makes the use of coded wire tags or PIT tags impractical and unfeasible. In the Connecticut River, stocked Atlantic salmon fry are too small (approximately 2 cm in length) and too numerous (>12 million) to apply tags or other physical labels, such as fin clips or thermal marks, without incurring prohibitively high financial costs and mortality levels. Genetic markers have shown promise for stock identification in some fisheries applications (Brodziak et al. 1992; O'Connell and Wright

Fig. 5. $^{87}Sr/^{86}Sr$ values of age-1 Atlantic salmon from five sites in the White River. Each $^{87}Sr/^{86}Sr$ ratio represents an individual fish. Values marked with asterisks were classified as movers based upon the dissimilarity of their isotopic signature with that of the stream. The source habitat for movers can often be inferred by comparing their mixed isotopic signatures with those of neighboring streams. For example, the moving salmon at the White River site most likely came from Tweed Branch or West Branch. Similarly, the movers from both Tweed and West branches probably spent a significant portion of time in the mainstem White River.



1997; Letcher and King 1999). However, the accuracy of identifying individual fish to specific stocks based upon DNA variation is limited when many stocks are considered or when few loci are available (Beacham et al. 1996*a*, 1996*b*). Additionally, the genetic structure of some populations such as anadromous salmon can exhibit significant temporal variability (Jordan et al. 1992; Garant et al. 2000) and can be significantly altered as a result of interactions between wild and hatchery stocks. Finally, geochemical markers based solely upon elemental concentrations are sensitive to physiological and environmental factors that often preclude a meaningful classification of individuals based upon stock or geography (Friedland et al. 1998).

In contrast, Sr isotopes are derived from an invariable geologic source that results in stable and relatively predictable stream signatures. In natural food webs, Sr isotopes do not fractionate with trophic levels (Graustein 1989; Blum et al. 2000); therefore, fish incorporate the same isotopic signature from both the dissolved Sr in stream water and the Sr in diet items. There are no major physiological or environmental effects on the fish isotopic signature, and therefore, one can develop site signatures based solely upon stream water Sr isotope ratios. Additionally, Sr isotopes are incorporated naturally into calcified tissue based upon the stream water chemistry, thereby eliminating the need for time-consuming tagging or genetic screening of individuals prior to stocking. The site-specific uptake and incorporation of isotopic signatures makes this technique useful for distinguishing fish populations in both wild and managed settings.

Geologic origins of Sr isotope markers

Harrington et al. (1998) found that watershed land use patterns predicted the differences in the N isotopic composition of streams and used these differences to distinguish fish from forested and agricultural streams. Strontium isotopic signatures differ from those of N in that they are geologically derived and are therefore not influenced by watershed changes over shorter time scales (e.g., land use change). In most cases, Sr isotopic values are consistent with geological predictions. However, some stream signatures were not predictable based upon bedrock geology alone. For example, Winhall Brook drainage in the West River is situated entirely within the undifferentiated region of Precambrian gneiss and yet has among the least radiogenic signature of any site ($^{87}Sr/^{86}Sr = 0.71233$). The headwaters of Winhall Brook lie along the western edge of the Precambrian gneiss, which

borders younger calcium carbonate rich rock further west. In this region, continental ice sheets carried entrained bedrock in a southeasterly direction over relatively short distances (<30 km) (Bailey and Hornbeck 1992) and are likely to have pushed some of the younger rocks from more western portions into headwater drainages to the east, thus contributing to lower than expected Sr isotope ratios signals in streams. The ability to predict isotopic differences across watersheds depends upon an understanding of the regional bedrock geology and the surficial geologic processes that influence the isotopic composition of the drainage.

Site stability

Our work supports the conclusion of other studies that Sr isotope ratios remain relatively constant at a given location both seasonally and annually (Fisher and Stueber 1976; Bailey et al. 1996; Yang et al. 1996). During flood periods, the $^{87}Sr/^{86}Sr$ values of streams may deviate slightly, but the short duration of these events and the small observed changes (Hogan and Blum 1999) suggest that this will not negatively influence the Sr isotope application outlined herein. Our sampling regime focused on baseflow conditions, as these represent the norm for stream hydrology and are the most important for salmonid growth.

Biological uptake and storage of Sr isotopic markers

An important finding of this study is that food contributes the majority of Sr in calcified tissues. For mature salmon, more than two thirds of the Sr in otoliths, vertebrae, and scales is derived from dietary sources. Our results contradict previous short-term radioisotope studies, which suggest that dietary Sr and Ca contribute less than 25% of the overall amount of these elements to calcified tissue (Simmons 1971; Simkiss 1974; Farrell and Campana 1996). Under natural conditions, this distinction is likely to be irrelevant, as the Sr isotopic composition of food and water sources will be identical, and therefore, relative contributions from these two sources will not be reflected as differences in the isotopic signature of the fish. However, when identifying stocked individuals that have incorporated a significant amount of Sr from the hatchery environment, the differences in Sr sources can be important. Errors in misclassifying individuals could result when trying to infer a hatchery-based geochemical signature from a hatchery water source alone (Koch et al. 1992; Ingram and Weber 1999).

Identifying Atlantic salmon movers with Sr stable isotopes

We combined isotopic and genetic approaches to develop a novel method for identifying movers in these salmon populations. This became an important goal of our study because the isotopic signatures of individuals that have incorporated Sr from movements among multiple streams can misrepresent the site signature if their value is not recognized as an isotopic blend of sites (Fig. 5).

Strontium isotopes provide a promising technique for studying long-distance movements in salmon. In the present study, our ability to resolve the specific timing or extent of fish movements is limited because we do not know the relative contributions of separate geographic regions to the mover's isotopic signal. The amount that a moving fish deviates from the sample mean is influenced not only by the difference between the isotopic values at sites where it lived, but also by the time spent in different streams. Nevertheless, we can infer possible source habitats for movers because of the extensive information that we have collected on the geographic distribution of Sr isotopic values in these rivers. The location of these possible source habitats allows us to place minimum travel distances on fish movements. For example, all three age-0 movers in the West River were captured in the mainstem after having spent some portion of time in a tributary. Based upon their isotopic signal, possible source locations were at least 1 km from where they were sampled. Of the five long-distance immigrant fish in the White River, four had spent a portion of their lives in the mainstem White River before moving up into the tributaries where they were caught. One fish made the reverse trip, spending approximately half of its growing life in one of the northwestern tributaries of the White River before being picked up in the mainstem. The minimum distances that these age-1 fish traveled to incorporate their unique isotopic signature ranges from approximately 2.5 to 5 km (Fig. 5). Separating and resolving time-specific geochemical signatures from discrete annuli in otoliths and scales represents an exciting new direction area of research that will allow for the reconstruction of the movement history of individuals.

Identifying stock composition and tracking the migratory routes of salmon has become an endeavor of considerable political, economic, and scientific interest (Ingram and Weber 1999). Applications of novel marking techniques have had a number of beneficial impacts on fisheries science. For example, the variety of promising techniques, which include genetic, chemical, electronic, and radio tags, has given us a much more precise understanding of mixed populations and the extent of fish movements (Fontaine et al. 1997; Metcalfe and Arnold 1997; Beacham and Wood 1999). However, all of these methods have their potential limitations. We have shown that Sr isotopes can provide useful information in some study systems. There are, however, limits to the discriminatory power of geochemical signatures. As the number of sample sites increases, overlap among site signatures becomes more likely. The geologic variability of a study region also dictates the spatial limitations of this method, and hence, the value of Sr isotopes as population markers will differ from place to place. It appears likely that the use of geochemical signatures as population markers may be especially valuable in conjunction with other marking techniques. For example, in the Connecticut River watershed, the incorporation of a single additional mark (genetic or physical) that could distinguish between White River and West River salmon could increase the number of unique marks from 14 to 20 of 29. Conversely, when considering genetic markers, classification accuracy greatly increases when the possible stocks of origin can be confined to a limited geographic area such as with isotopic signatures (Beacham et al. 1996*a*). The development of suitable population marking techniques will continue to make indispensable contributions to the conservation and restoration efforts of fish species in decline.

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