

Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*)

Simon R. Thorrold and Cynthia M. Jones

Applied Marine Research Laboratory, Old Dominion University, Norfolk, Virginia 23529

Steven E. Campana

Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia B2Y 4A2, Canada

James W. McLaren and Joseph W. H. Lam

Institute for National Measurements Standards, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada

Abstract

The elemental composition of fish otoliths may represent a permanent record of the environmental conditions an individual has experienced as trace elements, incorporated into the growing surface of the otolith, reflect the physical and chemical characteristics of the ambient water. We tested the utility of trace element signatures in otoliths as natural tags of the river of origin of juvenile American shad (*Alosa sapidissima*) collected from the Connecticut, Hudson and Delaware Rivers in August and October 1994. Four elements (K, Mn, Sr, and Ba) showed significant variability among sites within rivers in August, although only Mg showed a significant site effect by October. Four elements (Mg, Mn, Sr, and Ba) differed significantly among rivers in both months. Linear discriminant functions based on the trace element signatures classified fish to their natal river with ~90% accuracy in both August and October collections. The discriminant function generated from the August data was able to classify fish collected in October successfully with better than 80% accuracy. On the basis of our findings, the river of origin of adult fish could be accurately determined by quantifying the trace element composition of the juvenile portion of their otoliths.

The return of reproductive adults to the site at which they were spawned is believed to be common in many aquatic vertebrates, including marine turtles and anadromous fishes (Papi 1992). However, direct tests of spawning site fidelity in aquatic organisms are rare (Quinn 1993). Spawning site fidelity of Pacific northwest salmonids (*Oncorhynchus* spp.) is arguably the best documented example of this behavior in marine taxa. Extensive tagging studies have confirmed that most Pacific salmon return to their natal river to spawn and have provided estimates of homing and straying rates (Quinn and Fresh 1984; Quinn et al. 1991; Labelle 1992; Urwin and Quinn 1993). These studies were possible largely due to extensive hatchery releases and tag recovery programs. Although hatchery fish can be easily tagged, it is considerably more difficult and expensive to conduct tagging studies of wild fish. There has, therefore, been little comparable work in other fish species due to financial constraints and because many larvae and juveniles are hard to capture and tag without unacceptably high levels of tagging mortality.

Information on natal homing is of more than academic

interest to fisheries management, as determination of an individual's natal area also identifies the stock to which it belongs in mixed or straddling stock fisheries. This has prompted a continuing search for natural markers of natal spawning areas and population structure. Most workers have concentrated on developing genetic tags of stock structure (e.g., Beacham et al. 1985; Wood et al. 1989; Brodziak et al. 1992; Wirgin et al. 1995), although significant genetic exchange among presumed populations by larval dispersal, adult vagrancy, and deliberate stock transfers has hampered this approach in a number of anadromous and marine fishes (Nolan et al. 1991; Graves et al. 1992; Epifanio et al. 1995).

Recently, variations in the trace element chemistry of fish otoliths have been shown to reflect stock associations, often in the absence of concomitant genetic variations (Edmonds et al. 1989, 1991, 1992; Campana and Gagné 1995; Campana et al. 1995). The utility of otolith chemistry in delineating fish stocks relies upon three properties of otoliths. First, the deposition time of otolith material can be estimated by reference to concentric rings in otoliths that are routinely used in fish aging studies (Beamish and McFarlane 1987). Second, the metabolically inert nature of otoliths ensures that the aragonite mineralogy remains unaltered after deposition (Campana and Neilson 1985). Third, the calcium carbonate and trace elements that make up greater than 90% of the otolith appear to be derived mainly from water, as modified by ambient temperature (Fowler et al. 1995a; Farrell and Campana 1996). The elemental composition of the otolith will, therefore, reflect to some degree the environmental characteristics of the water in which the fish lives (Thorrold

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Table 1. Summary information from juvenile *A. sapidissima* collected in the Connecticut, Hudson, and Delaware Rivers in August and October of 1994 including number of fish analyzed (*N*), mean standard length (SL, \pm standard error), and otolith weight of both sagittae (OW, \pm standard error).

River	Site	Collection date	<i>N</i>	Mean SL	Mean OW
August					
Connecticut	Holyoake	8/10/94	19	51.1 \pm 0.9	0.34 \pm 0.03
	Salmon River	8/10/94	20	53.3 \pm 0.7	0.38 \pm 0.03
	Wilson	8/10/94	20	54.2 \pm 1.4	0.45 \pm 0.02
Hudson	River km 195	8/15/94	20	54.4 \pm 0.6	0.39 \pm 0.02
	River km 125	8/16/94	20	57.4 \pm 0.4	0.50 \pm 0.03
	River km 88	8/17/94	20	54.4 \pm 0.5	0.49 \pm 0.02
Delaware	Milford	8/09/94	20	55.7 \pm 1.1	0.41 \pm 0.03
	River Gap	8/08/94	20	60.0 \pm 0.9	0.38 \pm 0.03
	Byram	8/02/94	20	64.4 \pm 1.4	0.46 \pm 0.04
October					
Connecticut	Deep River	10/05/94	16	64.4 \pm 0.8	0.76 \pm 0.03
	Salmon River	10/05/94	19	63.5 \pm 1.1	0.78 \pm 0.04
	Wilson	10/05/94	20	65.8 \pm 1.0	0.78 \pm 0.03
Hudson	River km 125	10/15/94	29	67.5 \pm 0.6	0.83 \pm 0.03
	River km 88	10/16/94	30	69.2 \pm 0.4	0.89 \pm 0.02
Delaware	Milford	10/12/94	20	68.5 \pm 0.9	0.66 \pm 0.03
	River Gap	10/11/94	20	66.9 \pm 1.3	0.67 \pm 0.04
	Byram	10/04/94	20	69.2 \pm 0.8	0.72 \pm 0.02

et al. 1997). Because physical and chemical composition characteristics of water vary spatially, otolith microchemistry should record the water mass characteristics specific to a particular natal area.

The ability of otolith chemistry to distinguish among adjacent fish populations suggests that trace element signatures may also be useful as a natural marker of natal spawning area of individual fish. Previous studies of stock structure based on analyses of whole otoliths from adult fish have not addressed this question because it was not possible to determine at what life history stage(s) the fish were separated. In this study, we investigate the ability of trace element signatures in otoliths to act as natural tags of natal river in American shad (*Alosa sapidissima*), an anadromous clupeid. The advantage of anadromy in the current application is that larvae and juveniles remain in their natal river before out migration to coastal waters in the fall. We minimized the possibility of larval dispersal or juvenile migrations among rivers confounding the trace element signatures by collecting specimens before this out migration. The specific objectives of this study were to quantify intra and inter-river differences in the trace element chemistry of juvenile *A. sapidissima* otoliths and then to test the discriminatory abilities of these differences among the rivers as a natural tag of natal origin.

Methods

Juvenile *A. sapidissima* were collected from three rivers (the Connecticut, Hudson, and Delaware Rivers) along the northeast coast of the United States in August and October 1994, before out migration to the coastal ocean. Rivers were chosen deliberately to ensure a powerful test of our methodology. Two earlier studies were unable to find any genetic variation among juveniles from these rivers (Bentzen et al. 1989; Nolan et al. 1991), although a third study found small,

but statistically significant, genetic differences (Epifanio et al. 1995). Juveniles were collected from three sites within each river during the August and October (Table 1). One exception was the Hudson River in October, where sufficient numbers of juveniles were collected from two sites only. Samples were stored on ice in the field and frozen upon return to the laboratory. In preparation for the elemental analysis, sagittal otoliths were removed from the fish with acid-washed glass probes, placed in a drop of ultrapure (Milli-Q) water, and cleansed of adhering tissue. Otoliths were then ultrasonically cleaned for 3 min, triple-rinsed in Milli-Q water, and air dried under a Class 100 laminar flow hood. After drying for at least 24 h, otoliths were weighed to the nearest 5 μ g and stored in high-density, acid-washed polyethylene vials. Left and right otoliths were pooled for subsequent chemical analyses because of the small size of juvenile *A. sapidissima* otoliths (<1 mg). Blank vials were similarly prepared for blank corrections and to calculate limits of detection.

A preliminary study suggested eight elements (Mg, K, Mn, Cu, Zn, Sr, Ba, and Pb) were detectable by inductively coupled plasma mass spectrometry (ICPMS) in *A. sapidissima*. Isotope dilution (ID) was the preferred method of quantification due to superior accuracy and precision in otolith assays (Campana et al. 1995). However, K and Mn are monoisotopic and consequently unsuitable for ID analysis, and therefore both elements were referenced to an internal standard (^{52}Cr). Otoliths were dissolved in 300 μ l of a 10% re-distilled nitric acid solution containing the enriched isotopes of the six elements targeted for ID, along with the internal standard. The enriched isotope spike solution contained ^{25}Mg , ^{65}Cu , ^{67}Zn , ^{87}Sr , ^{137}Ba , and ^{207}Pb , while ^{24}Mg , ^{63}Cu , ^{66}Zn , ^{88}Sr , ^{138}Ba , and ^{208}Pb were monitored in the sample solutions for quantification. Otolith solutions were assayed with a Perkin-Elmer SCIEX ELAN 5000 ICPMS at

the National Research Council laboratory in Ottawa, Canada. Polyatomic interference on mass 55 from potassium oxides makes absolute concentrations of Mn difficult to determine using ICPMS—instead both K and Mn are reported as ratios to ^{52}Cr .

Sample solutions were introduced into the ICPMS with a high-efficiency pneumatic nebulizer because of the small size of the *A. sapidissima* otoliths. Limits of detection (LOD = mean + 3σ of blank values from 28 analyses of five blank vials) for each of the elements were as follows: Mg ($0.31 \mu\text{g g}^{-1}$), K ($0.25 \text{ counts:}^{52}\text{Cr}$), Mn ($0.17 \text{ counts:}^{52}\text{Cr}$), Cu ($0.32 \mu\text{g g}^{-1}$), Zn ($1.3 \mu\text{g g}^{-1}$), Sr ($0.09 \mu\text{g g}^{-1}$), Ba ($0.09 \mu\text{g g}^{-1}$), and Pb ($0.16 \mu\text{g g}^{-1}$). LODs were higher than those routinely achieved with larger otoliths (Campana et al. 1995) but still well below observed values for Mg, K, Mn, Sr, and Ba. However, Cu, Zn, and Pb were below detection limits for at least 10% of the otolith samples, and these elements were therefore dropped from the subsequent analyses.

Physicochemical data from each of the three rivers were recorded by the United States Geological Survey as part of the National Stream Quality Accounting Network (NASQAN) and are available from the USGS on CD-ROM (USGS Digital Data Series DDS-37) or at the USGS web site (<http://www.rvares.er.usgs.gov>). Dissolved metal data for each of the five elements quantified in the otoliths were available at 1–2-month intervals during 1994 from Thompsonville, Connecticut (Connecticut River), Poughkeepsie, New York (Hudson River), and Trenton, New Jersey (Delaware River). Sr and Ba data were not available from the Hudson River NASQAN site in 1994, so mean concentrations from all samples taken at the site from April to November 1993 were used instead. This was justified on the basis that although absolute concentrations of both Sr and Ba varied over time, Sr:Ca and Ba:Ca ratios were conservative. For instance, Sr:Ca and Ba:Ca ratios (mean \pm SD) were very similar between 1993 and 1994 at both the Thompsonville (Sr:Ca[1993] = $0.25 \pm 0.055 \text{ mmol mol}^{-1}$; Sr:Ca[1994] = 0.24 ± 0.23 ; Ba:Ca[1993] = $0.32 \pm 0.04 \text{ mmol mol}^{-1}$; Ba:Ca[1994] = 0.33 ± 0.01) and Trenton (Sr:Ca[1993] = $1.82 \pm 0.1 \text{ mmol mol}^{-1}$; Sr:Ca[1994] = 1.94 ± 0.19 ; Ba:Ca[1993] = $0.39 \pm 0.06 \text{ mmol mol}^{-1}$; Ba:Ca[1994] = 0.44 ± 0.07) NASQAN sites. Elemental data from river waters and otoliths were converted from concentrations to metal:Ca ratios and expressed as molar fractions. Ca concentrations in the otoliths were given a nominal value of 38%, as Ca was not measured directly in the otolith samples (Campana et al. 1997). Mean daily water temperatures were also available from the NASQAN sites at Thompsonville and Trenton and from the New York State water treatment plant at Poughkeepsie (Fig. 1).

Statistical analyses of the trace element signatures were carried out using both univariate and multivariate approaches. To test for among-river differences in otolith chemistry while accounting for spatiotemporal variation within rivers, an experimental design with collection sites nested within river systems, and with river system and collection month as orthogonal main effects, was implemented. The presence of small, but statistically significant, differences in otolith weight among the rivers (ANOVA; MS = 0.367; $F_{2,6} = 5.7$; $P = 0.0415$) meant we had to adjust for these differences

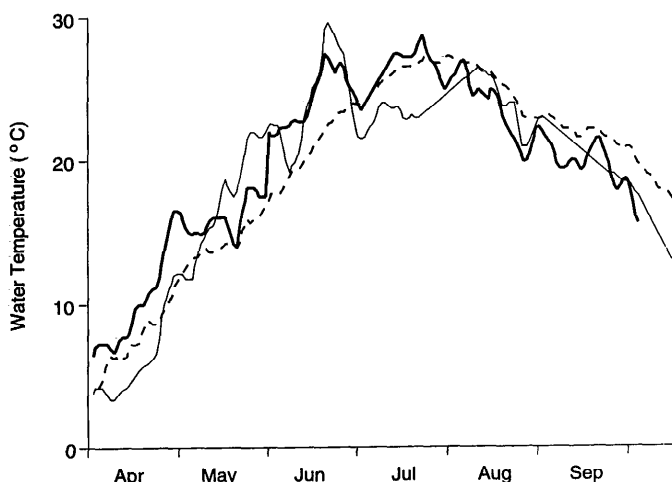


Fig. 1. Mean daily temperature records from Thompsonville, Connecticut (solid line), Poughkeepsie, New York (thin solid line), and Trenton, New Jersey (dashed line) from 1 April through 15 October 1994.

using otolith weight as a covariate in an analysis of covariance (ANCOVA). The ANCOVA approach that was used is dependent upon homogeneity of slopes in the relationship between otolith weight and element concentration among the treatment groups, which was tested for each element in both months. When the ANCOVA identified significant differences among rivers, pairwise comparisons of least-squares means (LSMs) were used as an a posteriori test of contrasts among rivers, after lowering α to account for the number of paired comparisons ($\alpha' = 0.0167$). LSMs, which are sometimes referred to as adjusted treatment means, are designed to remove any biases due to differences in the covariate (otolith weight) among treatment groups (Littell et al. 1991). Assumptions of homogeneity of slopes were tested for each element within both sampling months, while residual analysis was used to test for homogeneity of variances and normal distribution of errors (Winer 1971). To meet these assumptions it was necessary to \log_{10} -transform the Mg, Sr, and Ba data.

Linear discriminant function analysis (LDFA) evaluated the ability of trace element signatures to determine accurately the river of origin of juvenile *A. sapidissima*. The effect of otolith weight was removed from the data before running the LDFA using the pooled within-group slope from otolith weight (independent variable) regressed upon element concentration (dependent variable) for each of the elements. Only those elements that showed significant differences among the rivers, either from the ANCOVA or individual pairwise LSM comparisons, were entered into the LDFA. The cross-validation algorithm in the SAS Inst. (1990) DISCRIM procedure, which uses a jackknife technique, was used to determine classification accuracy within each of the sampling months, while the robustness of the technique to account for intra-annual variations was determined by classifying the October samples according to the discriminant function determined from the August data set.

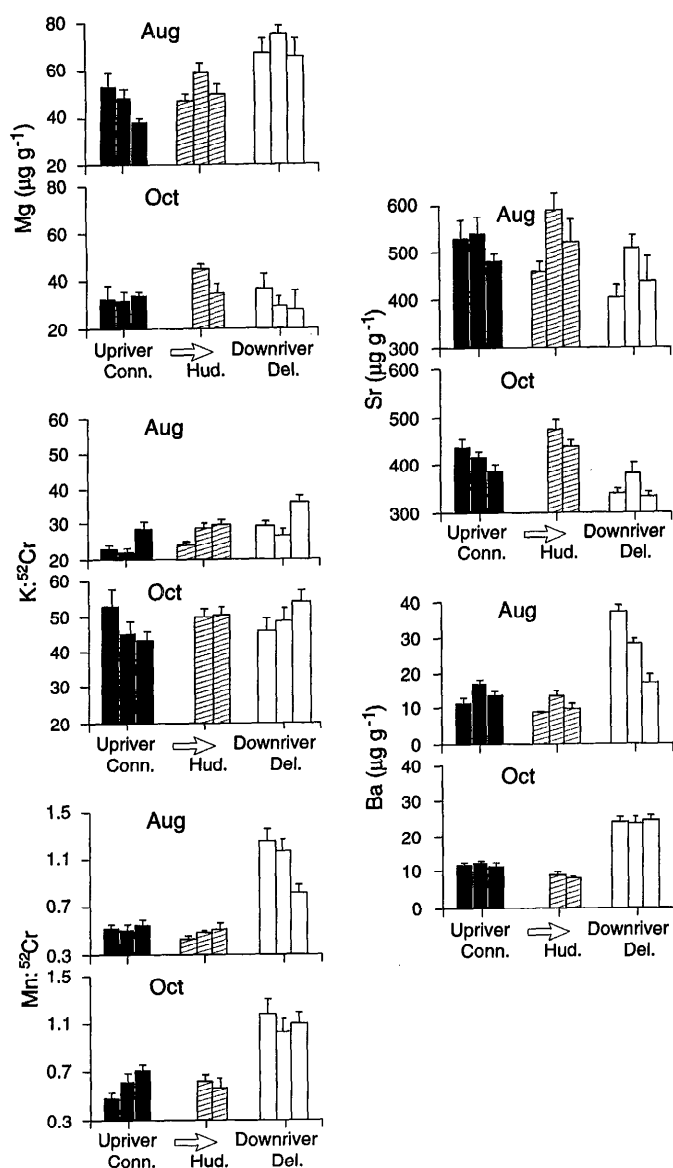


Fig. 2. Mean concentrations ($\mu\text{g g}^{-1} \pm \text{SE}$) of five elements in the otoliths of juvenile *A. sapidissima* collected at three sites on the Connecticut (Conn.), Hudson (Hud.), and Delaware (Del.) Rivers in August (top panel) and October (bottom panel).

Results

Trace element concentrations in juvenile *A. sapidissima* otoliths were significantly different among sites within rivers, among rivers, and between sampling occasions (Fig. 2). Three elements (Mg, Sr, and Ba) were found in higher concentrations in August, while K and Mn were higher in October. Univariate ANCOVAs from collections in August and October confirmed that otolith weight was a significant covariate in the statistical model for most elements (Table 2). Homogeneity of slopes was confirmed in 6 of the 10 tests ($\alpha = 0.05$), while probabilities (P) for the remaining four tests (K in August, Mn in both months, and Sr in October) were all $0.05 > P > 0.01$ (Table 2). Slopes for K and Mn

in both months were not sufficiently different to generate significant differences in planned pairwise comparisons among the rivers. However, the slope of the Sr:otolith weight relationship in October from Hudson River fish was significantly different from that of fish from the Delaware and Connecticut Rivers. This difference was generated by relatively high Sr values in six otoliths from Hudson River fish. If these fish were removed, there are no significant pairwise contrasts among rivers. Differences in slopes were unlikely to compromise the ANCOVAs and subsequent LDFA analyses, and while the six fish with high Sr values were not removed from any subsequent analysis, classification accuracy from the LDFAs was slightly higher if these otoliths were rejected.

Otolith chemistry was significantly different both within and among rivers (Table 2). In August, K, Mn, Sr, and Ba showed significant site-within-river effects. Of more importance to this study was the observation that despite variability at the site level, four elements (Mg, Mn, Sr, and Ba) differed significantly among rivers (Table 2). Pairwise comparisons of LSMs, which are adjusted to account for the effect of otolith weight, revealed significant differences in Mg, Sr, and Ba concentrations among all three rivers, while Mn: ^{52}Cr ratios in otoliths from the Delaware River differed significantly from those in the Connecticut and Hudson Rivers (Fig. 3). Mg concentrations decreased from high values in the Delaware River to low values in the Connecticut River, with the Hudson River intermediate between the two. Otoliths from the Delaware River were also high in Mn, although in this instance there were no significant differences between the Connecticut and Hudson Rivers. Otolith Sr and Ba concentrations were negatively correlated among rivers. Sr was found in highest concentrations and Ba in lowest concentrations in otoliths from the Hudson River, the Connecticut River had intermediate values for both elements, and otoliths from the Delaware were characterized by comparatively low Sr values and comparatively high Ba values.

There was considerably less evidence for intrariver variations in otolith chemistry from October relative to the August samples. Mg was the only element with significant variation among sites within rivers (Table 2). The same elements that showed significant variability among rivers in August (Mg, Mn, Sr, and Ba) were again significantly different in October. Pairwise comparisons of LSMs were significant among all rivers for Mg, Sr, and Ba and between the Delaware and both the Connecticut and Hudson Rivers for Mn: ^{52}Cr ratios. Mn, Sr, and Ba showed almost identical patterns of variation among rivers in both August and October (Fig. 3). Although there were significant variations in levels of Mg in otoliths from each of the rivers in both months, there was also an obvious river \times month interaction. Otoliths from the Hudson River had the highest mean value of Mg of any river in August, but by October the samples from the Hudson were characterized by comparatively low Mg concentrations.

Trace element signatures among rivers were positively correlated with variations in metal:Ca ratios in the river waters for some, but not all, of the elements examined (Fig. 4). Both Mg:Ca ($n = 6$, $r = 0.87$, $P = 0.023$) and Ba:Ca ratios ($n = 6$, $r = 0.98$, $P = 0.0005$) in the otoliths were

Table 2. ANCOVA table of five elements quantified in juvenile *A. sapidissima* otoliths. Otolith weight (OW, mg) was used as an independent covariate to test for effects of river (R) and site. The otolith weight \times river interaction (OW \times R) tested for homogeneity of slopes prior to the ANCOVA. Pairwise comparisons of LSM, adjusted for the influence of otolith weight, between each of the three river combinations are also presented. Note that $\alpha' = 0.0167$ to maintain an experimentwise error rate of $\alpha = 0.05$ for all pairwise comparisons.

Source	DF	SS	MS	F value	P > F	Pairwise LSM probability, P > T			
						LSM (±SE)	Conn	Hud	
Mg—August						Mg—August			
OW	1	1.79	1.79	256.2	0.0001	Conn	1.63±0.01	—	—
River	2	1.0	0.5	89.7	0.0001	Hud	1.74±0.01	0.0001	—
Site	6	0.033	0.0056	0.8	0.574	Del	1.81±0.01	0.0001	0.0001
OW × R	2	0.0026	0.0013	0.18	0.832				
Mg—October						Mg—October			
OW	1	1.79	1.79	256.2	0.0001	Conn	1.51±0.01	—	—
River	2	1.0	0.5	89.7	0.0001	Hud	1.60±0.01	0.0001	—
Site	5	0.033	0.0056	0.8	0.574	Del	1.45±0.01	0.0001	0.0001
OW × R	2	0.0026	0.0013	0.18	0.832				
K: ⁵² Cr—August						K: ⁵² Cr—August			
OW	1	1,081.3	1,081.3	28.8	0.0001	Conn	25.3±0.81	—	—
River	2	987.53	493.77	3.1	0.1191	Hud	26.6±0.81	0.2423	—
Site	6	956.29	159.38	4.24	0.0005	Del	30.8±0.80	0.0001	0.0003
OW × R	2	274.34	137.17	3.38	0.037				
K: ⁵² Cr—October						K: ⁵² Cr—October			
OW	1	2,968.4	2,968.4	18.94	0.0001	Conn	47.6±1.76	—	—
River	2	689.4	349.2	1.25	0.3627	Hud	47.5±1.81	0.9500	—
Site	5	1,395.7	279.1	1.78	0.1200	Del	52.5±1.86	0.0598	0.0706
OW × R	2	174.0	87.0	0.54	0.5848				
Mn: ⁵² Cr—August						Mn: ⁵² Cr—August			
OW	1	1.17	1.17	18.7	0.0001	Conn	0.55±0.03	—	—
River	2	13.55	6.78	15.12	0.005	Hud	0.45±0.03	0.035	—
Site	6	2.69	0.45	7.15	0.0001	Del	1.08±0.03	0.0001	0.0001
OW × R	2	0.6114	0.3057	4.17	0.017				
Mn: ⁵² Cr—October						Mn: ⁵² Cr—October			
OW	1	1.47	1.47	12.28	0.0006	Conn	0.61±0.05	—	—
River	2	10.39	5.193	43.58	0.0007	Hud	0.54±0.05	0.3015	—
Site	5	0.596	0.119	0.99	0.423	Del	1.17±0.05	0.0001	0.0001
OW × R	2	0.997	0.498	4.34	0.015				
Sr—August						Sr—August			
OW	1	1.672	1.672	396.6	0.0001	Conn	2.67±0.009	—	—
River	2	0.344	0.172	8.33	0.005	Hud	2.73±0.008	0.0001	—
Site	6	0.124	0.02	4.89	0.0001	Del	2.66±0.008	0.0001	0.0001
OW × R	2	0.0204	0.010	2.17	0.117				
Sr—October						Sr—October			
OW	1	0.245	0.245	51.44	0.0001	Conn	2.61±0.009	—	—
River	2	0.664	0.332	37.95	0.0009	Hud	2.68±0.01	0.0068	—
Site	5	0.044	0.009	1.84	0.108	Del	2.51±0.01	0.0001	0.0001
OW × R	2	0.045	0.023	4.86	0.010				
Ba—August						Ba—August			
OW	1					Conn	1.11±0.015	—	—
River	2	4.11	2.06	6.97	0.027	Hud	1.03±0.015	0.0001	—
Site	6	1.77	0.30	21.75	0.0001	Del	1.39±0.015	0.0001	0.0001
OW × R	2	0.10	0.05	2.24	0.109				
Ba—October						Ba—October			
OW	1	0.15	0.15	11.68	0.0008	Conn	1.07±0.015	—	—
River	2	4.21	2.11	222.2	0.0001	Hud	0.93±0.016	0.0001	—
Site	5	0.05	0.01	0.75	0.587	Del	1.35±0.016	0.0001	0.0001
OW × R	2	0.04	0.19	1.49	0.228				

significantly correlated with their respective metal:Ca ratios in ambient waters, suggesting that these elements are deposited on the otolith in proportion to the metal:Ca ratio of the water in which the fish had lived. Sr:Ca ratios were positively related to ambient Sr:Ca concentrations in August

and October, although there was enough difference in Sr:Ca ratios in the otoliths between months to generate a nonsignificant correlation coefficient ($n = 6$, $r = 0.63$, $P = 0.18$). Both K:Ca ($n = 6$, $r = 0.02$, $P = 0.96$) and Mn:Ca ratios ($n = 6$, $r = -0.43$, $P = 0.40$) in juvenile *A. sapidissima*

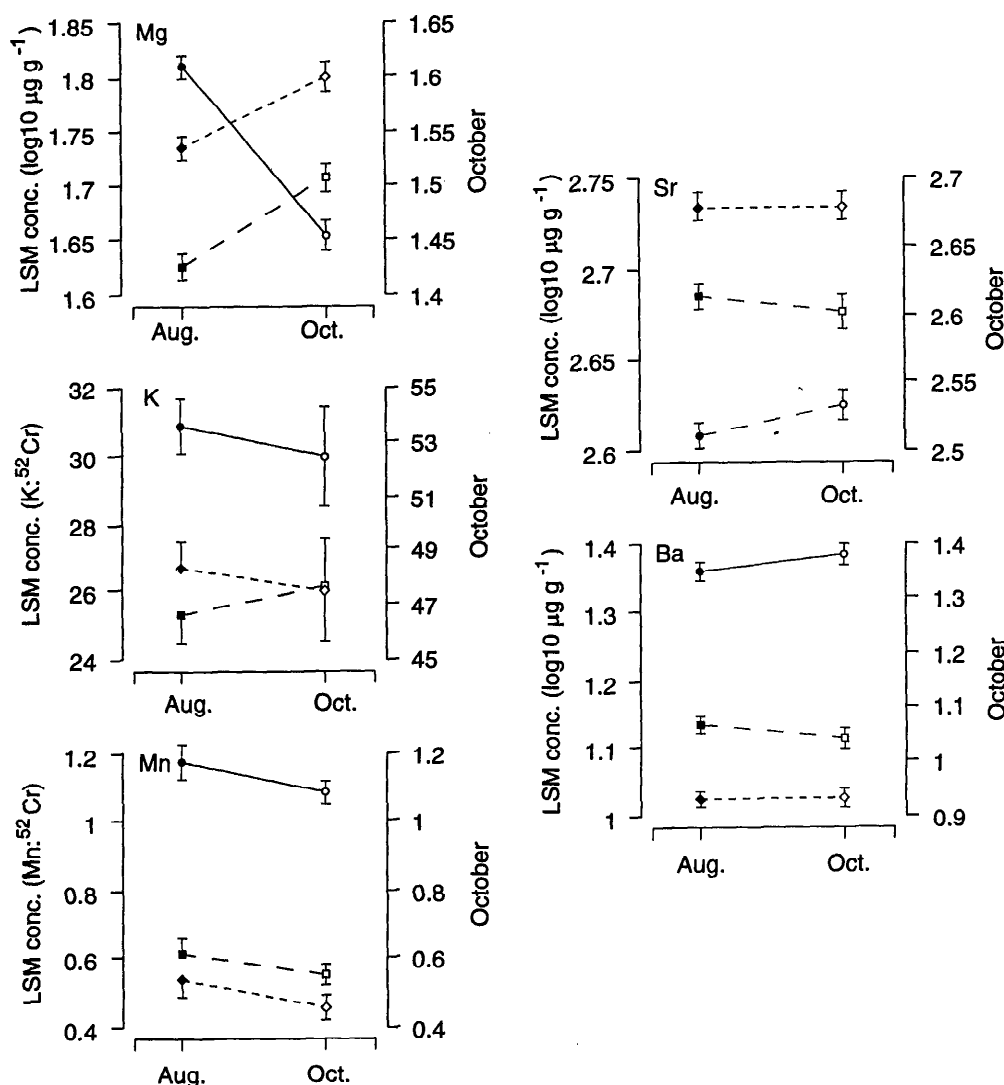


Fig. 3. Mean concentrations, adjusted for the effect of otolith weight, of the five elements in juvenile *A. sapidissima* otoliths (LSM \pm SE) from the Connecticut (■), Hudson (◆), and Delaware (●) Rivers in August (left axis, closed symbols) and October (right axis, open symbols).

otoliths were, however, uncorrelated with the corresponding metal:Ca ratios in the ambient water.

Cross-validated classification success of the LDFA derived from trace element signatures that included Mg, K, Mn, Sr, and Ba, adjusted for the effect of otolith weight, ranged between 88 and 93% for fish collected in August (Table 3). The multivariate separation of the elemental signatures was reduced to two dimensions using canonical discriminant analysis (CDA). Plots of the first and second canonical variates showed that the Delaware River samples separated clearly from the Connecticut and Hudson Rivers along the first canonical variate, while the Connecticut and Hudson Rivers separated almost completely along the second canonical variate (Fig. 5).

Four elements (Mg, Mn, Sr, and Ba) were used to construct trace element signatures from fish collected in October. River groupings were tighter than in August and concentrated along the first canonical variate (Fig. 6). Cross-vali-

dated classification success rates were greater than 90% for all three rivers (Table 3). As a final, and most conservative, test of the ability of otolith elemental fingerprints to resolve the river of origin, fish from August were used as a training data set to derive discriminant functions that were then used to classify the fish from October. In this instance, the necessary criteria for an element to be entered into the LDFA was the presence of significant variability among rivers in both months, without a river \times month interaction. Although only three elements (Mn, Sr, and Ba) met this criterion, classification success rates for the October samples based on discriminant functions from the August samples remained high (Table 3). Fish from the Connecticut River proved to be the most difficult to classify accurately with these data (80% accuracy), while October samples from the Hudson and Delaware Rivers were both classified with over 90% success using the discriminant functions generated from August collections.

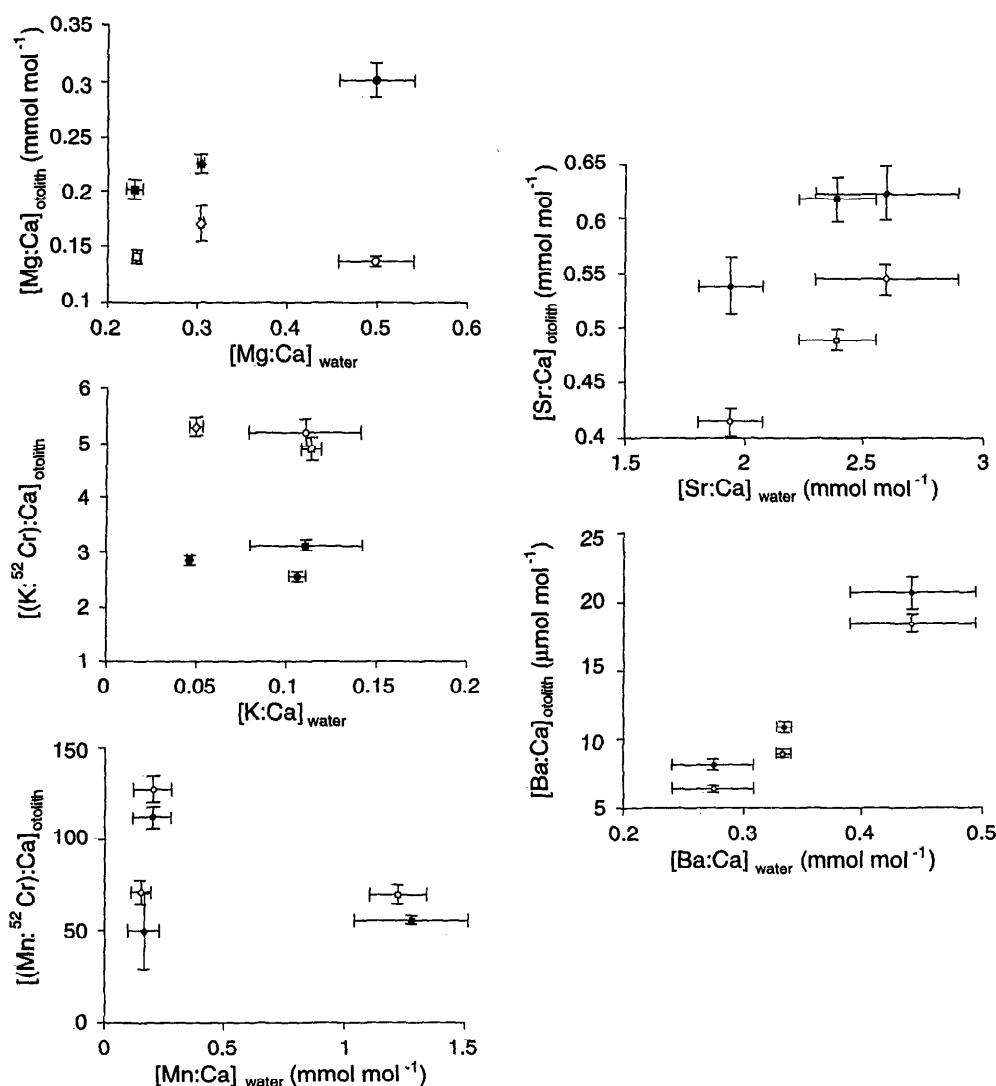


Fig. 4. Relationship between metal:Ca ratios in river water (mean \pm SE) and in juvenile *A. sapidissima* otoliths (mean \pm SE) from the Connecticut (■), Hudson (◆), and Delaware Rivers (●) in August (closed symbols) and October (open symbols) of 1994.

Discussion

The elemental composition of juvenile *A. sapidissima* otoliths varied considerably within and among rivers and between sampling dates. Although demonstration of such variability is necessary if trace element signatures in otoliths are to be useful markers of natal river, it does not ensure that individual fish of unknown natal origin can be identified with reasonable accuracy. The most important finding in the present study was that trace element signatures from each river appeared to be distinct enough to be used as a natural mark of the river in which juvenile *A. sapidissima* were spawned. High classification success rates (generally >90%) of the discriminant functions derived from trace element signatures in the otoliths confirmed their use as an effective tag of the natal river of juvenile *A. sapidissima*. More impressively, the temporal stability of the signatures allowed juveniles from

October to be assigned accurately to their natal river using the LDFA functions generated from juveniles in August.

A number of studies have documented differences in otolith composition among geographically separated fish populations (e.g., Edmonds et al. 1989; Campana et al. 1995). However, the mechanisms generating differences in trace element composition of otolith aragonite among geographically separated locations have rarely been addressed (Fowler et al. 1995a). There are insufficient data on the metal exposures of juvenile *A. sapidissima* in each of the rivers to draw definitive conclusions regarding the influence of the physicochemical characteristics of the ambient water on otolith composition. Indeed, for highly mobile aquatic organisms such as *A. sapidissima* this would be difficult to achieve outside of a laboratory study. However, our data do suggest that Mg, Sr, and Ba concentrations in otoliths are largely determined by the respective metal:Ca ratios in the ambient

Table 3. Results of LDFAs for classifying individual juvenile *A. sapidissima* to the Connecticut, Hudson, or Delaware Rivers based on trace element signatures in otoliths. All data were first adjusted for the effect of otolith weight.

	From river		
	Connecticut	Hudson	Delaware
August—cross-validation accuracy (%) with Mg, K, Mn, Sr, and Ba as dependent variables			
Connecticut ($n = 59$)	88.1	11.9	0
Hudson ($n = 60$)	11.7	88.3	0
Delaware ($n = 58$)	3.4	3.5	93.1
October—cross-validation accuracy (%) with Mg, Mn, Sr, and Ba as dependent variables			
Connecticut ($n = 52$)	96.2	3.8	0
Hudson ($n = 56$)	7.1	92.9	0
Delaware ($n = 54$)	7.4	0	92.6
Classification results with August as training set and October as test set with Mn, Sr, and Ba as dependent variables			
Connecticut ($n = 52$)	79.9	7.7	13.4
Hudson ($n = 56$)	5.4	92.9	1.7
Delaware ($n = 54$)	0	1.8	98.2

water. Of these elements, only otolith Sr showed a consistent difference between months that could not be accounted for by changes in the Sr:Ca ratios in the rivers. We do not have accurate thermal histories for individual fish to determine if this was due to a temperature dependence of the Sr:Ca partition coefficient in otoliths. However, Sr:Ca ratios are temperature dependent in at least some biogenic carbonates (Beck et al. 1992), and there were considerable differences in water temperatures among the rivers and through time (Fig. 1). There also may be ontogenetic changes in Sr uptake by the fish through larval and juvenile development. Fowler et al. (1995b) noted ontogenetic effects on Sr deposition in the otoliths of juvenile *Micropogonias undulatus* reared under constant physicochemical conditions in the laboratory. Such effects, if present, apparently do not alias the relationship between Sr:Ca levels in otoliths and in the ambient water if the fish to be analyzed are at a similar developmental state and/or age.

Differences in otolith Mn and K levels could not be explained by variations in element concentrations among the three rivers. Other possible contributing factors may include the influence of diet or genetics on trace element incorporation in otoliths. Limburg (1995) found that Sr:Ca levels in the otoliths of juvenile *A. sapidissima* were sensitive to Sr:Ca levels in the diet, although there is no similar information available for either Mn or K. There is a small, but statistically significant, genetic variability among each of the three rivers (Epifanio et al. 1995), although a convincing link between genetic variability and otolith elemental composition has yet to be demonstrated. More work is obviously needed to identify the mechanisms that regulate the differences in trace element signatures among rivers. We stress, however, that it is not necessary to understand fully these mechanisms before using empirically derived signatures as natural tags of spawning location.

Although most of the variance in trace element signatures

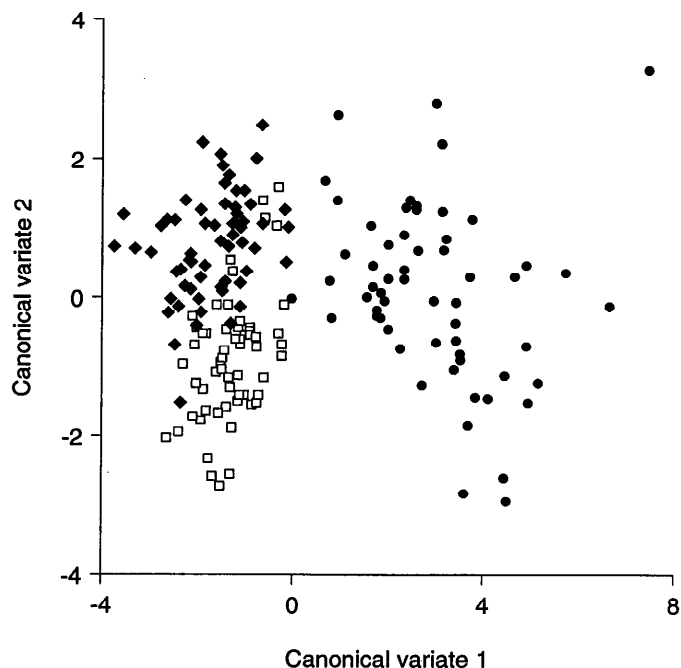


Fig. 5. Plot of first two canonical variates, summarizing variations in trace element signatures of juvenile *A. sapidissima* otoliths collected from the Connecticut (\square), Hudson (\blacklozenge), and Delaware (\bullet) Rivers in August 1994.

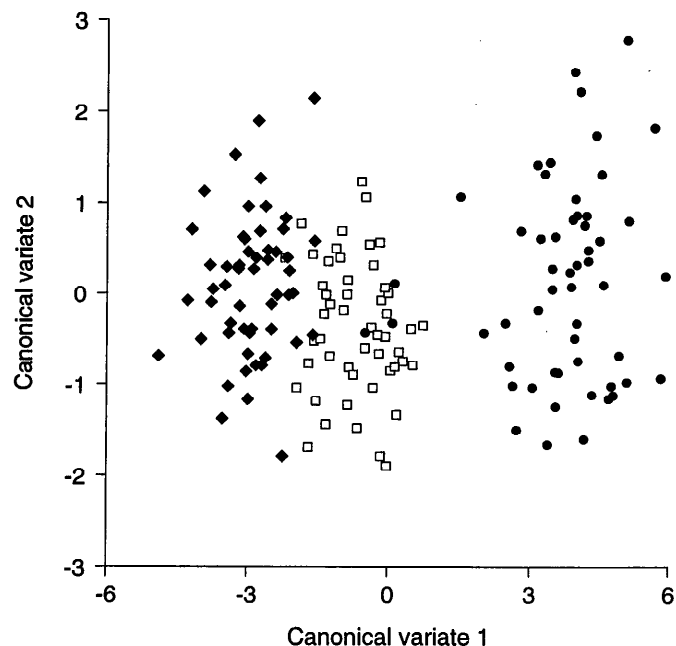


Fig. 6. Plot of first two canonical variates summarizing variations in trace element signatures of juvenile *A. sapidissima* otoliths collected from the Connecticut (\square), Hudson (\blacklozenge), and Delaware (\bullet) Rivers in October 1994.

was concentrated among rivers, we also found significant differences among sites within the three rivers, predominantly in the August collections. These data suggest that the physicochemical characteristics of specific sections of the rivers may vary enough to generate the differences in otolith chemistry that we observed within each river. Such an interpretation also presupposes that there is little exchange of larvae and early juveniles among different sections of the rivers, at least during early to mid summer. Limburg (1996) used an anomalous mark in the microstructure of larval and juvenile *A. sapidissima* from the Albany region of the Hudson river to estimate that the average loss of *A. sapidissima* from this section of the river to lower regions was on the order of 1% d⁻¹. This figure suggests that more than 50% of the larval and juvenile *A. sapidissima* within the upper reaches of the Hudson River would remain in that section after a 2-month period. The absence of site effects for all but one element in the October collections probably reflects significant down-river migrations of juvenile *A. sapidissima*, as very few juveniles are found in any of these rivers by November. Although we did not have enough data from within each river to assign the October fish to the section of the river that they may have resided in before down migration, increased sampling effort within the rivers may make this possible in the future.

The use of trace element signatures in otoliths as natural tags has both advantages and disadvantages over more traditional mark-recapture approaches. Mark-recapture studies undoubtedly provide unambiguous descriptions of individual migration patterns and spawning site fidelity. However, to be effective, such studies must tag sufficiently large numbers of fish to ensure that adequate numbers of tagged fish survive to be recaptured at a later stage. Although this is possible in hatchery situations, large-scale tagging of wild larvae or early-stage juveniles is much more difficult (but see Labelle 1992). Mark-recapture studies also make the assumption that recaptured fish are a representative sample of marked fish. This is problematic in recovery programs that rely upon returns from commercial or recreational fisheries, especially if the tag is visible (Paulik 1961; Green et al. 1983). The use of internal tags such as coded wire tags avoids problems of deliberate nonreporting, but extensive tag recovery programs must still be financed. The power of a natural tag such as trace element signatures in the otolith is that every fish from an area is invisibly tagged and capture of a single fish spawned in that area represents a recovery. Thus, the element signatures have considerable application in the management of all anadromous species where it is becoming increasingly necessary to identify naturally produced fish and the rivers in which they were spawned.

In summary, we have shown that trace element signatures in otoliths are specific to the river of origin in an anadromous clupeid, *A. sapidissima*. Accordingly, we should be able to track movement patterns of *A. sapidissima* after out migration from natal rivers and calculate individual stock exploitation rates in the coastal intercept fisheries by sectioning adult otoliths and using laser ablation ICPMS to probe the juvenile portion of the otolith (Campana et al. 1994; Thorrold et al. 1997). The ability to assign individual fish accurately to their natal river or stock has considerable implica-

tions for the fisheries management of *A. sapidissima* along the Atlantic coast of the U.S. and Canada, as juveniles and adults are targeted in mixed stock fisheries while resident in coastal waters. If misclassification rates can be reduced further by assaying more elements or by more precise measurements of isotopic ratios (Kennedy et al. 1997), it will also be possible to examine the question of spawning site fidelity in natural fish populations. An answer to that question would address a longstanding enigma in fish biology, one that has never been adequately tested outside the realm of hatchery releases.

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