Geochemical Signatures in Otoliths Record Natal Origins of American Shad

BENJAMIN D. WALTHER*[1] AND SIMON R. THORROLD

Biology Department, MS 50, Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543, USA

JOHN E. OLNEY

School of Marine Science, Virginia Institute of Marine Science, College of William and Mary,
Gloucester Point, Virginia 23062, USA

Abstract.—Population connectivity is a critical component in the life history dynamics of anadromous fishes and in the persistence of local populations. We used geochemical signatures in the otoliths of American shad *Alosa sapidissima* to determine natal origins and estimate rates of straying among river-specific populations along the U.S. Atlantic coast. Stable isotope ($\delta^{13}C$, $\delta^{18}O$ and $^{87}Sr:^{86}Sr$) and elemental (Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) signatures in otoliths of juvenile American shad from rivers from Georgia to New Hampshire varied significantly, allowing for an average of 91% cross-validated accuracy when classifying individual fish to their natal rivers. We also found significant interannual variability in the geochemical signatures from several rivers, due largely to differences in $\delta^{18}O$ values among years. We then used the ground-truthed geochemical signatures in the otoliths of juvenile American shad to identify the natal origins of spawning adults in the York River system in Virginia. Approximately 6% of the spawning adults collected in the York River were strays from other rivers. Of the remaining fish, 79% were spawned in the Mattaponi River and 21% in the Pamunkey River. The combined results of this and other recent studies suggest that although most American shad spawning in the York River were homing to their natal river, there was much less fidelity to individual tributaries. Small-scale straying could allow fish spawned in the Mattaponi River to subsidize spawning in the Pamunkey River, which has experienced persistent recruitment failure.

Anadromous fishes often display complicated migration patterns that present challenges to investigators seeking to understand the relationships among movements, life history traits, and population dynamics. Unresolved questions include the degree of homing to natal rivers and the effects of fishing pressure directed at small, tributary-specific stock components. Although significant work has gone into addressing these questions, direct tests of hypotheses concerning natal origin and migratory behavior are difficult with traditional tagging techniques (Dingle 1996; Thorrold et al. 2002). Most information on anadromous migrations comes from mark–recapture studies that apply a tag to a fish and attempt to reconstruct a route once that tag is recovered (Dadswell et al. 1987; Hendry et al. 2004). Although the tags employed are becoming increasingly sophisticated (e.g., Block et al. 2005), this approach can only yield information about movements subsequent to tag application after the fish

reaches some minimum size (Webster et al. 2002). As a result, traditional tags are unable to provide data about early life history movements and spawning origins of fishes, both of which are crucial aspects of population dynamics (Metcalfe et al. 2002).

The use of natural geochemical tags in animal tissues and hard parts provides an alternative marking technique for species that are difficult to tag using conventional approaches (Rubenstein and Hobson 2004). Recently, fish otoliths have been shown to be particularly useful natural tags (e.g., Thorrold et al. 2001). Otoliths are paired calcareous structures in the inner ear of fishes that are formed by the sequential addition of inert layers of calcium carbonate, usually in the form of aragonite, from birth to death (Campana and Nielson 1985; Campana 1999). The composition of otolith aragonite reflects, at least to some degree, the chemistry of ambient waters at the time of deposition (Bath et al. 2000; Walther and Thorrold 2006). Thus, otoliths from fish spawned in chemically distinct waters will record unique signatures reflective of those habitats and continue to record movements between distinct waters over their lifetimes.

The combined use of isotope ratios and elemental concentrations can allow fine-scale geographic discrimination of freshwater habitats. A pronounced

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* Corresponding author: benjamin.walther@adelaide.edu.au
[1] Present address: Southern Seas Ecology Laboratories, School of Earth and Environmental Science, Darling Building, University of Adelaide, Adelaide, South Australia 5005, Australia.

latitudinal gradient of the isotope ratio $\delta^{18}O$ in surface waters of eastern North America exists, primarily because of the preferential retention of ^{18}O in liquid form and the variation of $\delta^{18}O$ with mean annual temperature (Bowen 1988). As a result, $\delta^{18}O$ of local precipitation becomes isotopically lighter poleward, an affect known as the Rayleigh distillation (Dansgaard 1964; Poage and Chamberlain 2001). Fish incorporate $\delta^{18}O$ ratios in their otoliths without metabolic or kinetic fractionation (Thorrold et al. 1997; Høie et al. 2004), and therefore, freshwater natal $\delta^{18}O$ otolith signatures should reliably indicate spawning latitude. Environmental $^{87}Sr:^{86}Sr$ ratios are also highly location-specific, although instead of varying along any uniform gradient, they reflect the underlying geology of each stream bed (Faure and Powell 1972; Bricker and Jones 1995; Capo et al. 1998; Beard and Johnson 2000). These geographically distinct $^{87}Sr:^{86}Sr$ ratios are reliable markers recorded in otoliths and have proved useful in determining natal origins of salmonids (Kennedy et al. 1997; Kennedy et al. 2000). Finally, trace elemental compositions, expressed as ratios to calcium, recorded in otoliths have similarly allowed separation of fish according to the river in which they were born (Thorrold et al. 1998). A suite of these elemental and isotope signatures should provide a powerful marker of the natal origins of anadromous fishes.

American shad *Alosa sapidissima* are an excellent species with which to apply analyses of otolith geochemistry because there is a pressing need to understand the migratory pathways of individuals during ocean residency and when returning to spawn in rivers. Most populations along the Atlantic coast are fully exploited or under moratorium (Olney and Hoenig 2001), and all are at a fraction of their historical abundances (Limburg et al. 2003). American shad, anadromous alosine clupeids native to the East Coast of North America, spawn from spring to early summer in freshwater habitats from the St. Johns River in Florida to the St. Lawrence River in Quebec (Limburg et al. 2003). After developing in freshwater, juveniles migrate to the coastal ocean where they spend 3–7 years before reaching maturity and returning to spawn in freshwater (Maki et al. 2001; Collette and Klein-MacPhee 2002). Adult American shad are presumed to return to their natal river to spawn. However, this hypothesis has only been tested using methods including traditional tagging and genetic approaches (Melvin et al. 1986; Nolan et al. 1991; Waters et al. 2000). Estimates of straying rates based on tagged adults were only able to assess interannual fidelity to a river of previous spawning, under the assumption that the spawning river was their natal one

(Melvin et al. 1986). Because of their sensitivity to low exchange rates of individuals among populations, genetic analyses cannot quantify actual rates of philopatry but can only determine whether there is either some unknown yet significant degree of straying or negligible straying. The use of otolith chemistry as an alternative method to estimate natal homing rates could avoid these limitations and shed light on the migratory dynamics of American shad.

Previous work using otolith chemistry has shown that elemental signatures in juvenile American shad from three large rivers along the northeast Atlantic coast of the United States were highly distinct (Thorrold et al. 1998). Our study had three aims: (1) to expand on the work of Thorrold et al. (1998) in both geographical coverage and the suite of analyzed chemical signatures and thereby detect distinct signatures from rivers across the native spawning range of American shad, (2) to examine the interannual variability in river-specific signatures for those rivers where juveniles were collected over multiple years, and (3) to use these juvenile signatures to estimate the natal origins of adults spawning in the York River system and thus to determine homing on both a river and tributary scale.

Methods

Sample Collections

Juvenile American shad were collected in 2000–2002 from 12 rivers from New Hampshire to Georgia (Figure 1a; Table 1). Juveniles were obtained from three of these rivers over consecutive years: the Hudson River in 2000 and 2001 and the Mattaponi and Pamunkey rivers in 2000, 2001, and 2002. The Mattaponi and Pamunkey rivers are the two tributaries that join to form the York River at West Point, Virginia (Figure 1b). The remaining nine rivers were sampled in one year only. To ensure that individuals were collected from their natal rivers, juveniles were sampled while in freshwater or the upper estuarine environment before migration to marine habitats. Collections occurred in summer, fall, or winter months, depending on spawning latitude, and were timed to coincide with high juvenile abundance in each river following spawning migrations. Push nets and beach seines were used to obtain representative samples, and specimens were subsequently transported to the laboratory and frozen whole. For each year and river, we analyzed an average of 25 juveniles (range, 18–29; Table 1).

Adult American shad were collected from the York River during their upriver spawning migration between late February and early June in 2002. Fish were collected in staked gill nets and pound nets located in
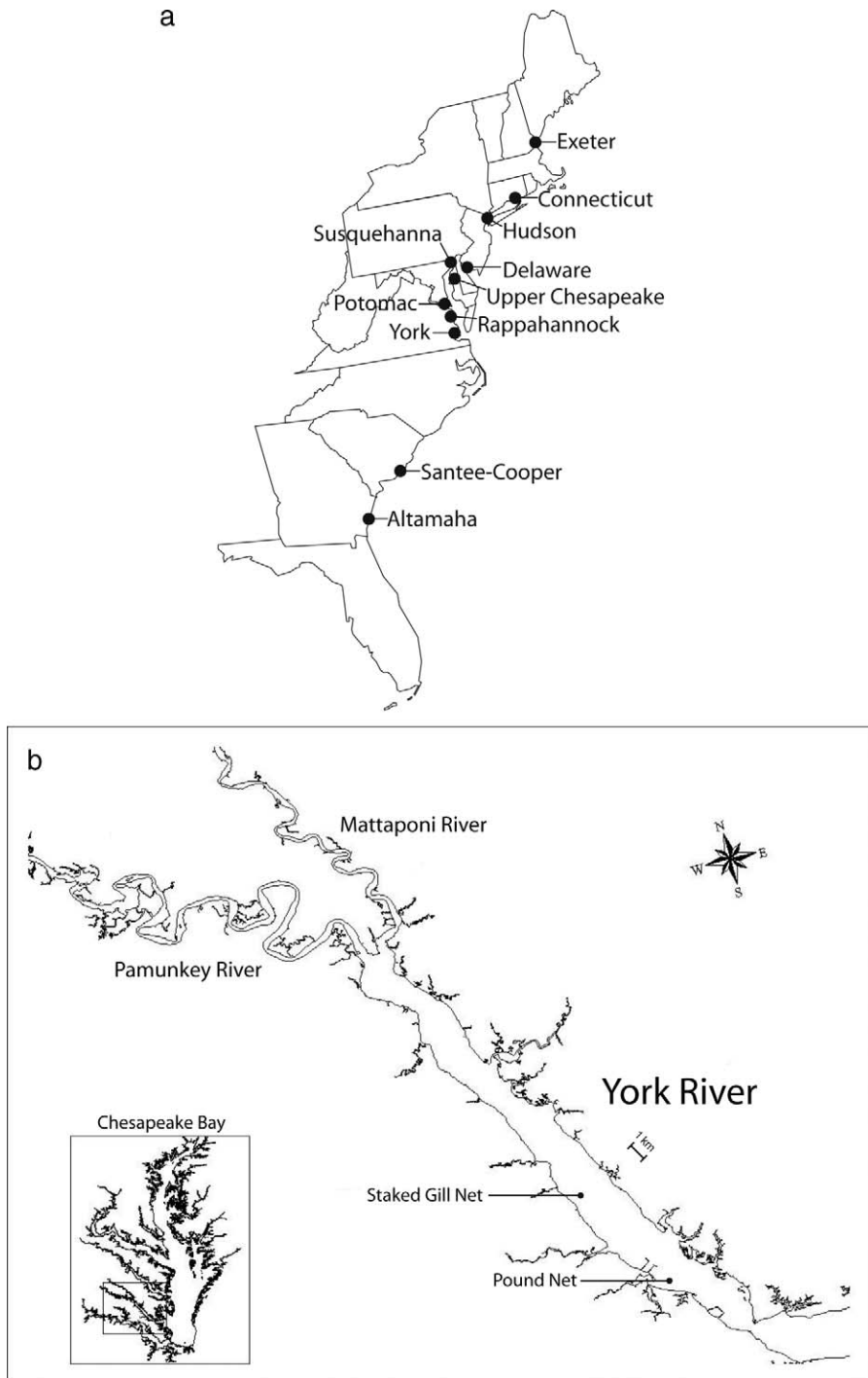
FIGURE 1.—Panel **(a)** shows the river mouths for all systems in which juvenile American shad were collected for analysis of otolith signatures. Juvenile collections in the York River system were made in both the Mattaponi and Pamunkey rivers, the two tributaries of that system. Panel **(b)** shows the York River system in more detail, indicating the locations of spawning adult collections at the staked gill and pound nets downstream of the confluence of the Mattaponi and Pamunkey rivers.

TABLE 1.—Data for juvenile American shad collected in 12 U.S. Atlantic rivers and tributaries (Figure 2); juvenile otoliths were used to ground-truth geochemical signatures in each spawning habitat. Fork lengths for the Santee-Cooper River were unavailable.

River	n	Year collected	Fork length (mean ± SD; mm)
Exeter	28	2001	92 ± 8
Connecticut	28	2001	72 ± 7
Hudson	27	2000	52 ± 8
Hudson	28	2001	60 ± 4
Delaware	21	2000	56 ± 14
Susquehanna	20	2000	141 ± 9
Upper Chesapeake Bay	29	2000	71 ± 4
Potomac	23	2000	70 ± 3
Rappahannock	21	2000	70 ± 13
Mattaponi	27	2000	53 ± 5
Mattaponi	28	2001	57 ± 6
Mattaponi	24	2002	54 ± 5
Pamunkey	18	2000	51 ± 8
Pamunkey	29	2001	58 ± 6
Pamunkey	19	2002	48 ± 10
Santee–Cooper	26	2000	
Altamaha	24	2000	63 ± 12

the middle reaches of the river below the confluence of the Mattaponi and Pamunkey tributaries (Figure 1b). Scales from a midlateral location on the left side posterior to the pectoral fin base were removed from each adult and retained dry in paper envelopes for estimating age. Sagittal otoliths were removed and stored in numbered tissue culture trays for subsequent chemical analyses. From 719 individuals we selected the otoliths of 78 male and female adults for geochemical analyses. Subsampled individuals were randomly selected in proportion to the total catch in each week of fishing.

Otolith and Scale Preparation

Frozen fish were thawed, measured (fork length ±1 mm), and dissected to remove sagittal otolith pairs. Once removed, otoliths were rinsed in distilled water, dried, and mounted on petrographic glass slides with cyanoacrylic glue. One otolith of each pair was ground to the midplane using 30-µm and 3-µm lapping film for elemental and Sr isotope analyses. Once ground, the otolith was sonicated for 2 min in ultrapure water, triple-rinsed with ultrapure water and air-dried under a laminar flow hood for 12–24 h. All cleaning took place in a class-100 clean room. The second otolith of the same pair was ground to just above the midplane to leave the required amount of otolith material for C and O isotope analyses. Adult otoliths were mounted, ground to the midplane, and cleaned using similar methods. Adult scales were cleaned with a dilute bleach solution, mounted by pressing on acetate sheets,

viewed on a microfilm projector, and aged following the methods of Cating (1953).

Geochemical Analyses

Laser ablation inductively coupled plasma mass spectrometry.—Juvenile otolith pairs were analyzed for a suite of elemental and isotopic ratios to produce a combined river-specific geochemical signature. The first otolith of each pair was analyzed with inductively coupled plasma mass spectrometry (ICP–MS) on a Thermo Finnigan Element2 single collector ICP–MS coupled to a New Wave Research UP213 (213 nm) Nd:YAG laser ablation system. The laser software was used to trace a 200-µm × 200-µm ablation raster centered on the nucleus and extending toward the posterior lobe of each otolith. Ablated material was carried by a He gas stream from the laser cell to the ICP–MS where it was mixed with Ar sample gas and a wet aerosol (2% HNO_3) supplied by a self-aspirating (20-µm/min) PFA nebulizer in the concentric region of the quartz dual-inlet spray chamber.

We quantified the Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios in the juvenile otoliths by monitoring ^{25}Mg, ^{48}Ca, ^{55}Mn, ^{86}Sr, and ^{138}Ba. Instrument blanks (2% HNO_3) and standards were analyzed at the beginning, middle, and end of each block of 10 otoliths. A dissolved otolith certified reference material (CRM; Yoshinaga et al. 2000), diluted to a Ca concentration of 40 µg/g of sample, was used to correct for instrument mass bias following the approach of Rosenthal et al. (1999). Measurement precision was assessed by running a 40-µg/g solution of an internal laboratory standard consisting of powdered otoliths. External precision (relative standard deviations) for the laboratory standard ($N = 92$) was Mg:Ca = 12%, Mn:Ca: = 3%, Sr:Ca = 0.3%, and Ba:Ca = 0.6%.

Strontium isotope ratios (^{87}Sr:^{86}Sr) were analyzed in the same otolith used for elemental ratio measurements. Otoliths were assayed using a Thermo Finnigan Neptune multiple collector ICP–MS coupled to a 213-nm laser ablation system. The laser software was used to trace out a 250-µm × 200-µm raster centered on the nucleus, extending toward the posterior lobe of each otolith and adjacent to the raster ablated for elemental ratio measurements. Ablated material was carried by a He gas stream from the laser cell to the ICP–MS where it was mixed with an Ar sample gas and a wet aerosol in a spray chamber, as described above for the elemental analyses. The core regions of adult American shad otoliths were ablated and simultaneously analyzed for Sr:Ca and ^{87}Sr:^{86}Sr ratios on the multiple collector ICP–MS. During each ablation pass, the instrument cycled between monitoring two sets of isotopes; ^{83}Kr, ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr,

and ^{88}Sr were monitored simultaneously for 3 s, and ^{48}Ca was monitored for 1 s. By cycling through the sets of monitored isotopes, the method quantified ratios of Sr:Ca and ^{87}Sr:^{86}Sr with a single ablated raster on the core of each adult otolith (e.g., McCulloch et al. 2005).

Although there are a number of potential interferences on Sr isotopes in carbonates, including Ca dimers, Ca argides, and doubly charged Er and Yb (Woodhead et al. 2005), we have found that only Rb and Kr isotopes present significant difficulties for accurate and precise analyses of ^{87}Sr:^{86}Sr in otoliths on our multiple collector ICP–MS (Barnett-Johnson et al. 2005; Jackson and Hart 2006). We monitored ^{85}Rb and applied the mass bias correction determined from the measured ^{88}Sr:^{86}Sr ratios to these counts to remove the contribution of ^{87}Rb on ^{87}Sr intensities. We followed the strategy outlined by Jackson and Hart (2006) to correct for Kr interferences on ^{86}Sr. Briefly, Kr was subtracted from the mass 84 intensity until the ^{84}Sr:^{88}Sr value equaled the natural abundance ratio of the isotopes (0.006755). The resulting Kr value was then used to account for the ^{86}Kr contribution on ^{86}Sr. A mass bias correction was determined from the measured ^{88}Sr:^{86}Sr ratios and applied to monitored counts of ^{85}Rb to remove the contribution of ^{87}Rb on ^{87}Sr intensities. This procedure obtains the mass-bias corrected sample value ^{87}Sr:^{86}Sr$_{true}$ via an exponential relationship between the measured ^{87}Sr:^{86}Sr$_{sample}$, the measured ^{88}Sr:^{86}Sr$_{sample}$, and the known value ^{88}Sr:^{86}Sr$_{certified}$, where

$$^{87}Sr:^{86}Sr_{true} = \frac{^{87}Sr:^{86}Sr_{sample}}{\left(\frac{^{88}Sr:^{86}Sr_{sample}}{^{88}Sr:^{86}Sr_{certified}}\right)^{\beta}}.$$

The exponent β is derived from the relationship

$$\beta = \frac{\log_e\left(\frac{M_{87}}{M_{86}}\right)}{\log_e\left(\frac{M_{88}}{M_{86}}\right)},$$

and each M represents the nuclidic mass of the respective Sr isotope. Finally, data were normalized to a standard reference material (SRM) 987. The correction strategies produced accurate and precise long-term measurements of liquid and solid standards that were run throughout the otolith analyses. Daily laser sampling ($N = 18$) of the aragonitic skeleton from a marine sclerosponge produced a mean ^{87}Sr:^{86}Sr value of 0.70918 (SD, 0.00001); solutions of SRM987 ($N = 40$) and the otolith CRM ($N = 38$) produced values of 0.71025 (0.00002) and 0.70915 (0.00002), respectively. These numbers compare favorably with the global marine ^{87}Sr:^{86}Sr ratio of 0.70917 and the generally accepted ^{87}Sr:^{86}Sr value of 0.71024 for SRM987.

Isotope ratio mass spectrometry.—The second otolith from each juvenile was analyzed for δ^{18}O and δ^{13}C using isotope ratio mass spectrometry (IR–MS). The core of each otolith was removed using a computer-controlled mill to trace out a 400-μm × 400-μm raster with a 75-μm drilling depth adjacent to the nucleus and extending toward the posterior lobe. The mean mass of 420 samples of the milled otolith powder was 43 μg (SD, 12). Samples were analyzed on a Thermo Finnigan MAT252 equipped with a Kiel III carbonate device following methods outlined by Ostermann and Curry (2000). Isotopic values were reported relative to Vienna Pee Dee belemnite (VPDB) and expressed in standard δ notation. The long-term precision estimates of the mass spectrometer based on analyses of NBS19 are ±0.07 for δ^{18}O and ±0.03 for δ^{13}C (Ostermann and Curry 2000).

Statistical Analyses

Juvenile American shad.—Laser ablation ICP–MS and IR–MS analyses produced a total of seven variables for each juvenile: Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, ^{87}Sr:^{86}Sr, δ^{18}O, and δ^{13}C. Each variable was tested for assumptions of normality and equality of variance–covariance matrices. Normal probability plots, residual analysis, and Box's M-tests indicated that distributions were nonnormal and the variance–covariance matrices were not equal. However, because departures from the assumptions were modest and log transformations of the data failed to significantly alter the distributions or the results of the Box's M-tests, raw data were used in all analyses. Geographic differences in multivariate signatures among locations and years were visualized using canonical discriminant analysis (CDA). Canonical variate coefficients provided a useful way to measure the relative importance of each variable to the observed separation among rivers and years. Finally, we employed a quadratic discriminant function analysis (DFA) to determine the accuracy with which individual American shad could be assigned to their natal river. A quadratic DFA was used because this procedure does not assume homogeneity of covariance matrices and tolerates modest deviations from normality (McGarigal et al. 2000). The DFA used a jackknife cross-validation procedure to determine classification accuracy.

Adult American shad.—We compared the ^{87}Sr:^{86}Sr and Sr:Ca values obtained from cores of adult otoliths from the York River in 2002 with the ^{87}Sr:^{86}Sr and Sr:Ca signatures from juveniles caught in the Mattaponi and Pamunkey rivers in 2000, 2001, and 2002. The maximum likelihood estimation (MLE) program HISEA (Millar 1990) determined the proportion of returning adults hatched in the Mattaponi or Pamunkey
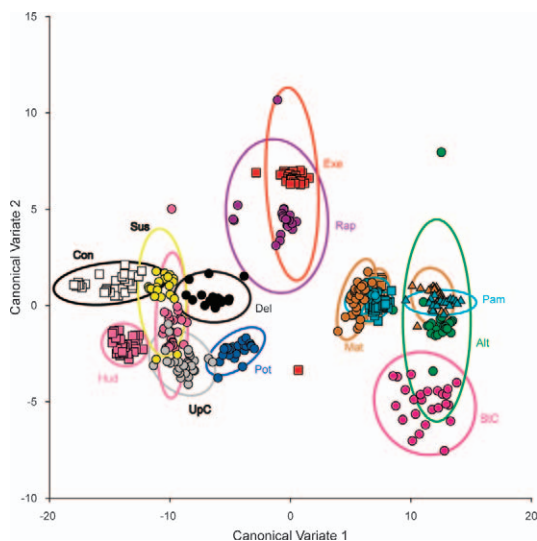
FIGURE 2.—Canonical discriminant analysis of juvenile American shad otolith signatures from all fish collected in 2000 (circles), 2001 (squares) and 2002 (triangles) grouped by river of origin and year-class. Symbols represent individual fish, and ellipses are 95% confidence intervals around each group. River codes are as follows: Exe = Exeter River, New Hampshire; Con = Connecticut River, Connecticut; Hud = Hudson River, New York; Del = Delaware River, New Jersey; Sus = Susquehanna River, Maryland; UpC = Upper Chesapeake Bay, Maryland; Pot = Potomac River, Maryland; Rap = Rappahannock River, Virginia; Mat = Mattaponi River, Virginia; Pam = Pamunkey River, Virginia; StC = Santee–Cooper River, South Carolina; and Alt = Altamaha River, Georgia.

River. We used ground-truthed ^{87}Sr:^{86}Sr and Sr:Ca signatures from these juveniles pooled over the 2000, 2001, and 2002 year-classes to parameterize the MLE algorithm. Pooling the three year-classes allowed us to account for interannual variability in the signatures. The program calculated standard deviations on the contribution of each tributary in the adult samples by resampling the mixed stock data 1,000 times with replacement.

Results

Juvenile American Shad

We found strong geographical separation of juveniles based on the geochemical signatures in otoliths (Figure 2; Table 2). Individuals from different rivers were generally separated on the first two canonical variates, with the exception of the Mattaponi and Pamunkey tributaries of the York River. However, when the CDA was restricted to these two tributaries, the signatures were mostly distinct between locations for a given year (Figure 3). Interannual variations in signatures were also apparent from the CDAs. Juvenile signatures from the Hudson River were distinct and nonoverlapping between 2000 and 2001 (Figure 2). Mattaponi and Pamunkey juvenile signatures occupied similar canonical space in 2000 and 2001 but shifted substantially in 2002 (Figure 3).

The magnitude of the total canonical structure coefficients reflected the importance of the geochemical variables used to generate the multivariate geochemical signatures (Table 3). Oxygen isotopes loaded highly on the first canonical variate, the latitudinal gradient in $\delta^{18}O$ accounting for differences among river-specific signatures (Figure 4). Separation

TABLE 2.—Juvenile American shad otolith elemental and isotopic signatures (mean ± SD) for 12 U.S. Atlantic rivers by collection year; these data were used in canonical discrimination analyses.

| River	Year	$\delta^{18}O$	$\delta^{13}C$	Mg:Ca (mmol/mol)	Mn:Ca (µmol/mol)	Sr:Ca (mmol/mol)	Ba:Ca (µmol/mol)	^{87}Sr:^{86}Sr
Exeter	2001	−8.12 ± 0.28	−17.21 ± 0.71	0.134 ± 0.047	5.63 ± 1.09	0.470 ± 0.025	5.65 ± 1.58	0.71676 ± 0.00143
Connecticut	2001	−11.42 ± 0.42	−13.93 ± 0.78	0.098 ± 0.022	3.58 ± 0.62	0.531 ± 0.069	10.51 ± 2.51	0.71323 ± 0.00023
Hudson	2000	−10.37 ± 0.15	−15.49 ± 0.56	0.095 ± 0.040	2.62 ± 0.85	0.443 ± 0.041	6.43 ± 1.10	0.71133 ± 0.00102
Hudson	2001	−11.26 ± 0.21	−14.92 ± 0.49	0.139 ± 0.056	2.35 ± 0.50	0.463 ± 0.045	5.05 ± 1.02	0.71075 ± 0.00024
Delaware	2000	−9.32 ± 0.26	−14.38 ± 0.69	0.127 ± 0.075	2.36 ± 0.70	0.355 ± 0.041	8.02 ± 2.26	0.71256 ± 0.00041
Susquehanna	2000	−10.36 ± 0.32	−14.30 ± 0.87	0.068 ± 0.009	3.78 ± 1.34	0.265 ± 0.120	9.26 ± 2.60	0.71236 ± 0.00073
Upper Chesapeake Bay	2000	−9.77 ± 0.25	−13.11 ± 0.66	0.261 ± 0.147	2.94 ± 0.85	0.660 ± 0.131	4.02 ± 1.42	0.71086 ± 0.00038
Potomac	2000	−8.57 ± 0.18	−12.95 ± 1.10	0.181 ± 0.071	2.21 ± 0.36	0.400 ± 0.065	4.82 ± 0.85	0.71088 ± 0.00015
Rappahannock	2000	−8.17 ± 0.39	−15.33 ± 0.44	0.100 ± 0.034	2.55 ± 0.63	0.509 ± 0.048	15.42 ± 6.04	0.71579 ± 0.00137
Mattaponi	2000	−7.01 ± 0.16	−17.51 ± 0.74	0.041 ± 0.011	5.70 ± 0.79	0.756 ± 0.067	18.34 ± 4.03	0.71212 ± 0.00047
Mattaponi	2001	−6.65 ± 0.13	−17.94 ± 0.64	0.092 ± 0.031	3.66 ± 0.97	0.572 ± 0.043	13.91 ± 2.92	0.71226 ± 0.00029
Mattaponi	2002	−5.58 ± 0.18	−18.60 ± 0.43	0.092 ± 0.017	4.72 ± 1.73	0.704 ± 0.085	16.01 ± 2.06	0.71224 ± 0.00035
Pamunkey	2000	−6.60 ± 0.19	−16.72 ± 0.35	0.050 ± 0.034	4.15 ± 1.01	0.571 ± 0.074	17.35 ± 4.23	0.71228 ± 0.00021
Pamunkey	2001	−6.33 ± 0.16	−16.68 ± 0.46	0.171 ± 0.059	2.82 ± 0.64	0.492 ± 0.057	12.81 ± 2.11	0.71213 ± 0.00016
Pamunkey	2002	−5.13 ± 0.27	−17.57 ± 0.62	0.101 ± 0.019	4.82 ± 0.98	0.469 ± 0.079	8.60 ± 1.29	0.71199 ± 0.00014
Santee–Cooper	2000	−5.52 ± 0.36	−16.14 ± 0.99	0.125 ± 0.055	2.70 ± 0.85	1.507 ± 0.510	26.44 ± 11.13	0.70954 ± 0.00014
Altamaha	2000	−5.08 ± 0.22	−16.57 ± 0.93	0.112 ± 0.058	2.84 ± 1.02	0.419 ± 0.180	14.32 ± 6.29	0.71138 ± 0.00145
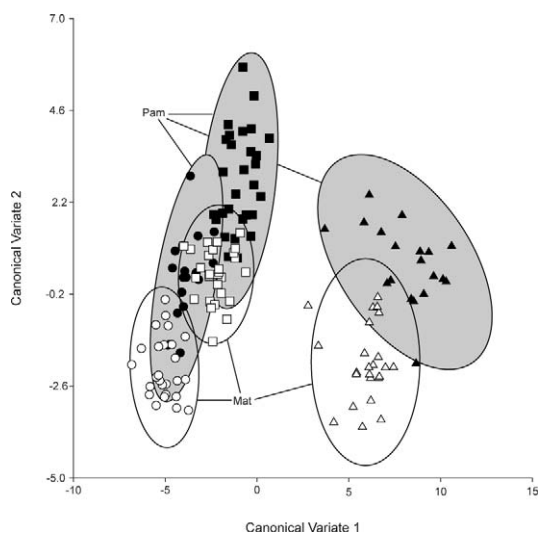
FIGURE 3.—Canonical discriminant analysis of juvenile American shad otolith signatures from the Mattaponi (Mat; filled symbols) and Pamunkey (Pam; open symbols) rivers of Virginia for the years 2000 (circles), 2001 (squares), and 2002 (triangles); 95% confidence ellipses surround each group.
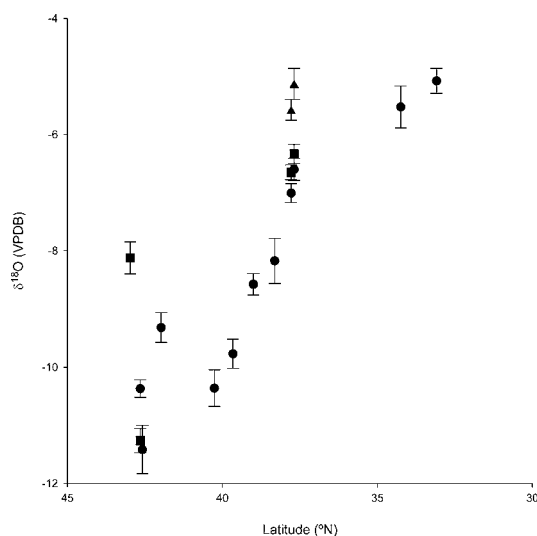

FIGURE 4.—Variation in $\delta^{18}O$ signatures recorded in otoliths of juvenile American shad with latitude of spawning habitat from all fish collected in 2000 (circles), 2001 (squares), and 2002 (triangles). Isotopic values are means $\pm$ SDs (relative to Vienna Pee Dee belemnite [VPDB]) of fish grouped by river of origin and year-class.

of signatures along the second canonical variate was primarily driven by variations in $^{87}Sr:^{86}Sr$ values. Interannual variations in $\delta^{18}O$ were most responsible for differences in geochemical signatures in the Hudson, Mattaponi, and Pamunkey juvenile otoliths across years on the first canonical variate (Table 4).

The cross-validation classification accuracies of juveniles based on geochemical signatures in otoliths ranged from 72% to 100% and averaged 91% (Table 5). Errors were almost exclusively restricted to a single fish from a given river. The exception to this pattern was among fish from the Mattaponi and Pamunkey rivers, which showed moderate error rates between tributaries and among years. However, nearly all of

TABLE 3.—Total canonical structure coefficients for canonical discriminant analysis performed on elemental and isotope ratios in otoliths of juvenile American shad collected in 12 U.S. Atlantic rivers over 3 years. The absolute value of each coefficient indicates the relative importance of the ratio in driving combined geochemical signature separation along that variate.

Ratio	Variate 1	Variate 2
$\delta^{18}O$	0.995	−0.027
$\delta^{13}C$	−0.730	−0.317
Ba:Ca	0.562	−0.144
Sr:Ca	0.360	−0.442
Mn:Ca	0.275	0.441
Mg:Ca	−0.168	−0.245
$^{87}Sr:^{86}Sr$	−0.049	0.974

these misclassifications were to the adjacent tributary; classification to the York River combined was high. There were no misclassifications between year-classes from the Hudson River, indicating strong interannual differences in geochemical signatures of juvenile otoliths between the 2000 and 2001 year-classes.

Adult American Shad

Otoliths were collected from a total of 78 adults during their spawning migration into the York River. Adults ranged from 4 to 8 years old and were predominately from the 1995–1997 year-classes (ages 5–7). Significant interannual variability meant that we could not use the full suite of elemental and isotope ratios from the 2000–2002 ground-truthed juvenile signatures to determine natal origins of the York River adults. However, York River juveniles separated clearly from all other rivers based on $^{87}Sr:^{86}Sr$ and Sr:Ca values only (Figure 5a). Further, the $^{87}Sr:^{86}Sr$ and Sr:Ca ratios did not vary significantly among years in the York River tributaries or in the Hudson River. We therefore assumed that adults collected in the York River could be divided into fish that were homing to their natal river and those that were spawned in a different river system based on the $^{87}Sr:^{86}Sr$ and Sr:Ca ratios in otolith cores. Moreover, consistent ratio differences between the Mattaponi and Pamunkey rivers meant that we were able to determine the natal

TABLE 4.—Total canonical structure coefficients for canonical discriminant analyses of otolith chemistry for juvenile American shad from the Hudson, Mattaponi, and Pamunkey rivers. The coefficients, derived from analyses restricted to one river across two or three collection years, were used to assess the relative importance of each ratio in driving interannual variability.

Ratio	Variate 1	Variate 2
Hudson River, 2000 and 2001		
$\delta^{18}O$	0.966	0.120
Ba:Ca	0.576	0.356
$\delta^{13}C$	−0.506	0.463
Mg:Ca	−0.430	−0.101
$^{87}Sr:^{86}Sr$	0.386	−0.463
Mn:Ca	0.205	−0.187
Sr:Ca	−0.241	0.834
Mattaponi River, 2000–2002		
$\delta^{18}O$	0.985	−0.014
$\delta^{13}C$	−0.580	0.126
Mg:Ca	0.519	−0.659
Ba:Ca	−0.135	0.591
Mn:Ca	−0.126	0.678
$^{87}Sr:^{86}Sr$	0.098	−0.164
Sr:Ca	0.002	0.921
Pamunkey River, 2000–2002		
$\delta^{18}O$	−0.972	−0.051
Ba:Ca	0.743	−0.336
$\delta^{13}C$	0.634	0.201
$^{87}Sr:^{86}Sr$	0.529	−0.231
Mn:Ca	−0.454	−0.669
Sr:Ca	0.404	−0.394
Mg:Ca	−0.012	0.896

tributary of those adults spawned in the York River. This consistency was assessed by conducting a separate DFA based only on the Sr:Ca and $^{87}Sr:^{86}Sr$ ratios in juveniles collected only from the Mattaponi and Pamunkey rivers pooled across years. High classification accuracies for the Mattaponi (67%) and Pamunkey (89%) rivers supported the assumption of consistent differences in these two ratios between the York River tributaries. Five adults (6%) were outside the range of $^{87}Sr:^{86}Sr$ and Sr:Ca ratios from the juvenile otoliths collected in the York River and were therefore classified as strays that were spawned in a different river system (Figure 5b). Of the remaining 73 adults whose $^{87}Sr:^{86}Sr$ and Sr:Ca signatures matched those of York juveniles, the MLE analysis found that an estimated 79% (SD, 8) were spawned in the Mattaponi River and 21% (8) in the Pamunkey River.

Discussion

The geochemical signatures in juvenile American shad otoliths collected over a wide geographical range were highly distinct and specific to their river of origin. Thorrold et al. (1998) used elemental signatures in American shad otoliths to distinguish juveniles from the Delaware, Hudson, and Connecticut rivers. Our data demonstrate that the approach can be extended to determine the natal origins of American shad spawned throughout their range by also assaying stable C, O, and Sr isotopes. Moreover, signatures are significantly different among rivers, allowing an average of 91% of fish to be accurately classified to their natal river. Natural geochemical tags in otoliths of anadromous fishes will be particularly useful for determining population affinities of individuals during ocean residency, whereas this is currently only possible for hatchery fish that can be marked before release (e.g., Volk et al. 1999).

The source of variability in otolith geochemical signatures among rivers depends on the elemental or isotopic ratio under consideration. We observed a strong latitudinal cline in $\delta^{18}O$, enriched (more positive) values occurring in the south and depleted (more negative) values in the north. The cline mirrors latitudinal trends in $\delta^{18}O$ values of precipitation that arise from the Raleigh distillation process (Dansgaard 1964; Bowen 1988). Experimental work has demonstrated neither kinetic nor metabolic fractionation of $\delta^{18}O$ between otolith aragonite and ambient water, suggesting that otolith $\delta^{18}O$ values directly reflect water $\delta^{18}O$ values, as modified by temperature (Thorrold et al. 1997; Høie et al. 2003). Juvenile American shad therefore record $\delta^{18}O$ signatures that reflect the latitude of the watershed in which they were spawned.

Strontium isotopes in otoliths provided a powerful addition to the suite of variables used to determine the natal origins of juvenile American shad. The composition of rocks within a watershed determines the $^{87}Sr:^{86}Sr$ ratio of dissolved inorganic Sr in river water. Otolith Sr is, in turn, isotopically equilibrated with the ambient water (Kennedy et al. 2000). Differences in bedrock geology among river drainages therefore generate predictable variations in otolith $^{87}Sr:^{86}Sr$ values that are likely to be stable over ecological time scales. Because American shad spawn in river systems across such a large geographical range, their natal habitats drain significantly diverse lithologies that produce unique local dissolved $^{87}Sr:^{86}Sr$ values. In general, older rock types are enriched in ^{87}Sr because of radiogenic decay of ^{87}Rb. Thus, based simply on rock age, early Paleozoic schists and granites of the northeastern United States should yield relatively higher $^{87}Sr:^{86}Sr$ values than the less-radiogenic Cenozoic sedimentary rocks that are common in southern states (Beard and Johnson 2000). However, most of the rivers included in this study drain basins with highly heterogeneous surficial rock formations of varying ages and compositions, each of which influence the downstream dissolved $^{87}Sr:^{86}Sr$ values that are incor-

TABLE 5.—Cross-validation summary from the quadratic discrimination function analysis run on the complete set of juvenile American shad geochemical signatures ($N = 420$). Groups are categorized by river of origin (codes listed in Figure 2) and collection year. Reported values are percent classifications and numbers of individuals (in parentheses) assigned to each location and year indicated by columns. Accurate classifications to group of origin are shown on the diagonal; accuracies sum to 100% across a row for a given source group. Blank spaces indicate no classifications.

River and year	Exe 2001	Con 2001	Hud 2000	Hud 2001	Del 2000	Sus 2000	UpC 2000	Pot 2000	Rap 2000	Mat 2000	Mat 2001	Mat 2002	Pam 2000	Pam 2001	Pam 2002	StC 2000	Alt 2000
Exe 2001	96 (27)						4 (1)										
Con 2001		100 (28)															
Hud 2000			92 (25)				4 (1)	4 (1)									
Hud 2001				100 (28)													
Del 2000			5 (1)		85 (18)	5 (1)		5 (1)									
Sus 2000			5 (1)			95 (19)											
UpC 2000							100 (29)										
Pot 2000								100 (23)									
Rap 2000	5 (1)								95 (20)								
Mat 2000										89 (24)			11 (3)				
Mat 2001										4 (1)	78 (22)		7 (2)	11 (3)			
Mat 2002											4 (1)	88 (21)		4 (1)			4 (1)
Pam 2000											17 (3)	11 (2)	72 (13)				
Pam 2001												7 (2)		93 (27)			
Pam 2002												5 (1)			84 (16)		11 (2)
StC 2000																96 (25)	4 (1)
Alt 2000	4 (1)															4 (1)	92 (22)

porated into otoliths (Kennedy et al. 2000). Thus, local heterogeneity of streambed geology exerts strong control on American shad ^{87}Sr:^{86}Sr signatures recorded during their freshwater residency.

Multivariate geochemical signatures in the otoliths of juveniles from the York and Hudson rivers differed significantly among years. This variability was primarily driven by fluctuations in $\delta^{18}O$ values. Because otolith oxygen isotopes are deposited in equilibrium with ambient water (Kalish 1991; Thorrold et al. 1997; Høie et al. 2003), the variability is probably derived from environmental factors that altered the source or amount of water vapor that fell as rain in the watersheds. For instance, tropical storms can import isotopically heavy $\delta^{18}O$ water from low latitude source regions to higher latitudes (Cole et al. 1999). Alternatively, fluctuations in mean annual temperature could alter $\delta^{18}O$ values through altered evaporation rates. The potential for $\delta^{18}O$ to be affected by various stochastic environmental effects highlights the importance of ground-truthing juvenile signatures from each cohort of interest when $\delta^{18}O$ is included in the classifying signature. Because $\delta^{18}O$ was the most important classifying signature along the first canonical variate when discriminating rivers across the large latitudinal range considered in this study, it will be a vital component of future efforts to classify origins of migrating fish. The second most important classifying signature was ^{87}Sr:^{86}Sr values, further demonstrating the value of combining analyses of stable isotope ratios with elemental concentrations to obtain unique multivariate signatures along the Atlantic coast.

Interannual variability in the multivariate signatures limited our ability to use the full suite of elemental and isotope ratios in classifying adult American shad spawning in the York River. Because we determined river-specific signatures from juveniles spawned several years after the spawning adults, we were unable to match cohorts of the known-origin juveniles and spawning adults. As a result, we classified adults using only the temporally stable values of ^{87}Sr:^{86}Sr and Sr:Ca. Because dissolved ambient ^{87}Sr:^{86}Sr ratios depend on the lithology of the drainage basin, these values showed little variation between 2000 and 2002 and were assumed to be relatively constant between 1995 and 1997, when the returning adults were spawned. Although Sr:Ca values are also strongly controlled by streambed geology, otolith Sr:Ca has a greater potential for interannual variability because accreted concentrations can depend on temperature as well as ambient availability (Thorrold et al. 1998; Elsdon and Gillanders 2003). We observed variation of up to 0.2 mmol/mol in mean yearly otolith Sr:Ca values within the Mattaponi and Pamunkey rivers between 2000 and 2002. In addition, Thorrold et al. (1998) reported otolith Sr:Ca values from American shad juveniles collected in 1994 from the Connecticut, Hudson, and Delaware rivers that were within 0.2 mmol/mol of otolith values from juveniles collected in those same rivers in 2000 and 2001. Thus, the magnitude of long-term interannual variation in Sr:Ca appears to be matched by the variation observed in the York River between 2000 and 2002. We accounted for this interannual variability in Sr:Ca values by pooling
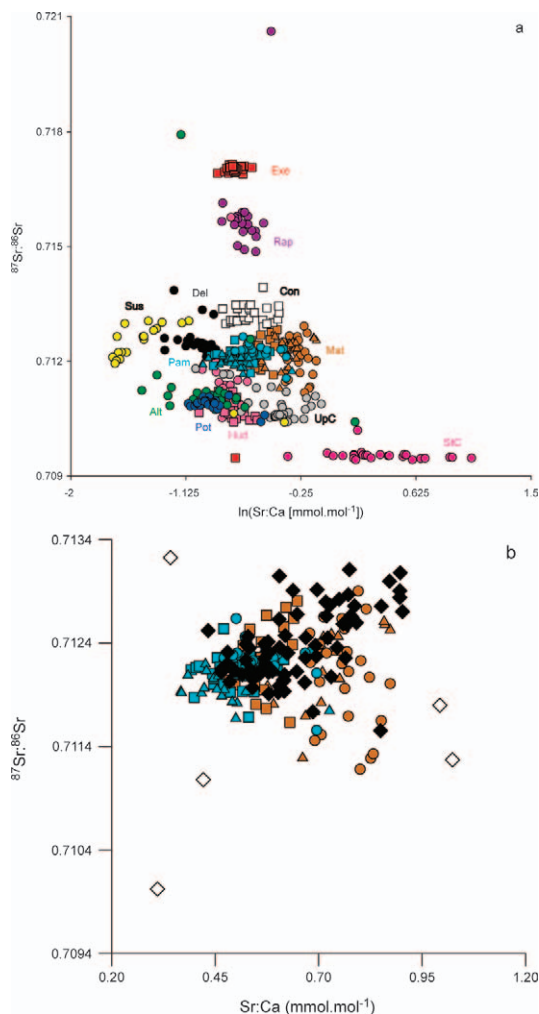
FIGURE 5.—Panel **(a)** shows juvenile American shad otolith signatures for ^{87}Sr:^{86}Sr and Sr:Ca ratios for all fish collected in 2000 (circles), 2001 (squares), and 2002 (triangles) grouped by year-class and river of origin (codes given in Figure 2). Panel **(b)** shows signatures from otolith cores of York River adults of York River origin (filled diamonds) or strays from other rivers (open diamonds) plotted over juvenile signatures from the Mattaponi (brown symbols) and Pamunkey (blue symbols) rivers; the year-class symbols are the same as in panel (a).

Mattaponi and Pamunkey juveniles from 2000 to 2002 to classify adults spawned between 1995 and 1997, thereby encompassing the probable range of Sr:Ca values accreted by the older cohorts.

Our estimate that approximately 6% of spawning adults in the York River originated from other rivers supports previous estimates of mixing among populations. Several extensive tagging studies reported returns of tagged adult American shad to the river of previous spawning (Talbot and Sykes 1958; Nichols 1960; Melvin et al. 1986). Although these studies were unable to test homing to natal rivers, Melvin et al. (1986) suggested that 3% of returning spawners were possible strays. Three previous studies involved releasing tagged juveniles in a stream to determine natal homing. Hollis (1948) reported tag recaptures within 16 km of the release site, but the conclusions were limited by a sample size of just three recaptured fish. Tetracycline marks in otoliths of hatchery-reared larvae allowed Olney et al. (2003) to estimate that an average of 4% of marked fish caught in the James River between 1999 and 2001 were strays from the Pautuxent, Pamunkey, Juniata, and Susquehanna rivers. McBride et al. (2005) also estimated that the probability of fish straying from the Susquehanna River to the Delaware River was negligible. Homing behavior of American shad was also inferred based on significant meristic, morphological, and life history variations among populations (Carscadden and Leggett 1975a, 1975b; Melvin et al. 1992). Finally, subtle but significant genetic differences in mitochondrial and microsatellite DNA sequences suggested reproductive isolation among some populations (Nolan et al. 1991; Waters et al. 2000). Our results add further support to the hypothesis that most American shad home to their natal river to spawn.

The predominance of adults spawned in the Mattaponi River among returning York River adults corresponded with long-term juvenile production trends in the York River (Wilhite et al. 2003). Our sample was predominated by the 1995–1997 year-classes (ages 5–7). Juvenile abundance indices in the river basin indicated consistently low recruitment in the Pamunkey River and relatively high recruitment in the Mattaponi River for all year-classes of the returning adult spawners (Wilhite et al. 2003). Because of consistent differences in abundances of American shad eggs and larvae in the two tributaries of the York River, several researchers have hypothesized that the York River system is predominated by fish spawned in the Mattaponi River (Bilkovic et al. 2002; Wilhite et al. 2003). The results of our natal classifications based on otolith chemistry suggest that the predominance of the Mattaponi River population remained strong once fish matured. As a result, marine mortality of migrants did not appear to alter the relative abundance of these two populations, and the effects of year-class strength was evident during spawning events, despite extensive migrations during their years at sea.

Although most mature York River adults were apparently returning to their natal river to spawn, the combined results of this study and a recent investiga-

tion of York River spawning behavior (Olney at al. 2006) suggested that this homing tendency was not preserved at the level of tributaries within a river. Subsequent to our collections of spawning adults in 2002, river migrants collected in the same location were used in an acoustic tagging study to determine their ultimate spawning location (Olney et al. 2006). Although there was evidence of significant handling effects, most released fish ultimately migrated to either the Mattaponi or Pamunkey river to spawn. Of these migrants, 57% spawned in the Mattaponi River and 43% spawned in the Pamunkey River (Olney et al. 2006). If the natal origins of the tagged migrants in 2003 reflected the composition of the adult sample in 2002 (79% Mattaponi River origin and 21% Pamunkey River origin), our data suggest that a large proportion of tagged American shad migrating to the Pamunkey River were of Mattaponi River origin. The combined results of our study and that of Olney et al. (2006) indicate that although migrants home to the York River system, they do not discriminate between the two tributaries when selecting a spawning habitat. This behavior may act to subsidize the Pamunkey River population with spawners hatched in the Mattaponi River, ensuring population persistence despite recruitment failure. Coupling data derived from otolith chemistry analyses and tagging approaches presents new opportunities to validate tributary-specific juvenile abundance indices, even after extensive marine migrations. Such information is critical to effective management of these exploited and recovering populations.

Acknowledgments

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