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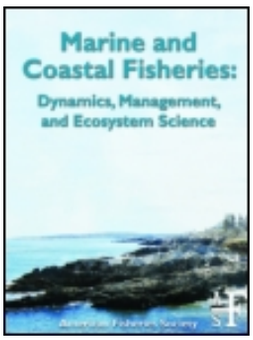

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SPECIAL SECTION: AMERICAN SHAD AND RIVER HERRING

Use of a Natural Isotopic Signature in Otoliths to Evaluate Scale-Based Age Determination for American Shad

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Abstract

We used $\delta^{18}O$ signatures in otoliths as a natural tag for hatch year to evaluate the scale-based age determination method used for adult American shad *Alosa sapidissima* in the York River, Virginia. Juveniles of the 2002 year-class exhibited high $\delta^{18}O$ values in otolith cores that identified adult members of the cohort as they returned to spawn. Recruitment of the 2002 cohort was monitored for three consecutive years, identifying age-4, age-5, and age-6 individuals of the York River stock. The scale-based age determination method was not suitable for aging age-4, age-5, or age-6 American shad in the York River. On average, 50% of the individuals from the 2002 year-class were aged incorrectly using the scale-based method. These results suggest that the standard age determination method used for American shad is not applicable to the York River stock. Scientists and managers should use caution when applying scale-based age estimates to stock assessments for American shad in the York River and throughout their range, as the applicability of the scale-based method likely varies for each stock. This study highlights a promising new direction for otolith geochemistry to provide cohort-specific markers, and it identifies several factors that should be considered when applying the technique in the future.

Accurate age determination is critical to the assessment and management of fishes. Age-specific data allow for estimates of mortality, growth, maturity schedules, and production and are used to develop models of population dynamics (Beamish and McFarlane 1983, 1995; Campana 2001). Inaccurate age estimates can lead to incorrect assumptions about these life history parameters, including inflated estimates of natural mortality (Eklund et al. 2000; Beamish and McFarlane 1983, 1995), skewed maturity schedules (Maki et al. 2001), inaccurate production estimates (Beamish and McFarlane 1995; Campana and Thorrold 2001; Boreman and Friedland 2003), and discrepancies between juvenile and adult indices of abundance due to

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incorrect classification of year-class strengths (MacLellan and Saunders 1995). Despite their importance, however, age determination methods are rarely evaluated, especially for all age-classes and populations of a species (Beamish and McFarlane 1983; Campana 2001). One reason for this is that the known-age specimens necessary for evaluation are often difficult to obtain.

Natural geochemical markers in otoliths have potential as cohort-specific markers that would aid in the evaluation of aging methods. Certain chemical markers in otoliths are strongly determined by their ambient water values (Thorrold et al. 1997; Høie et al. 2003). This means that fish residing in a chemically distinct body of water for their first year of life will record location-specific chemical signatures in the central region of their otoliths (Campana and Thorrold 2001). However, environmental processes may alter the composition of ambient signatures in a particular nursery habitat in a given year. For instance, ambient oxygen isotope ratios ($\delta^{18}O$) are sensitive to the amount of precipitation, annual temperature, groundwater input, and water vapor source region (Cole et al. 1999; Kendall and Coplen 2001). Temporal variability in otolith geochemistry may therefore result in cohort-specific signatures that facilitate tracking that cohort over time. Several studies have investigated the temporal variability in otolith geochemistry (reviewed in Gillanders 2002), but almost all have done so out of the need to understand it in order to determine the interannual stability of spatial patterns (e.g., Gillanders and Kingsford 2000; Hamer et al. 2003; Rooker et al. 2003). Anadromous fishes are ideal for the investigation of cohort-specific geochemical signatures because the freshwater habitat of the larval and juvenile stages of these species is characterized by high environmental variability. As a result, cohorts of anadromous species may have distinct geochemical signatures in the cores of their otoliths.

Walther and Thorrold (2009) reported significant interannual variability in the geochemical signatures of juvenile American shad *Alosa sapidissima*, an anadromous clupeid native to the Atlantic coast of North America, from the Hudson River and the Mattaponi and Pamunkey rivers (two tributaries that join to form the York River; Figure 1). Of the isotope signatures investigated by Walther and Thorrold (2009), $\delta^{18}O$ exhibited the greatest variation among years. Walther and Thorrold (2009) found that Mattaponi and Pamunkey River juveniles of the 2002 year-class had higher $\delta^{18}O$ values than juveniles of the surrounding 2000,

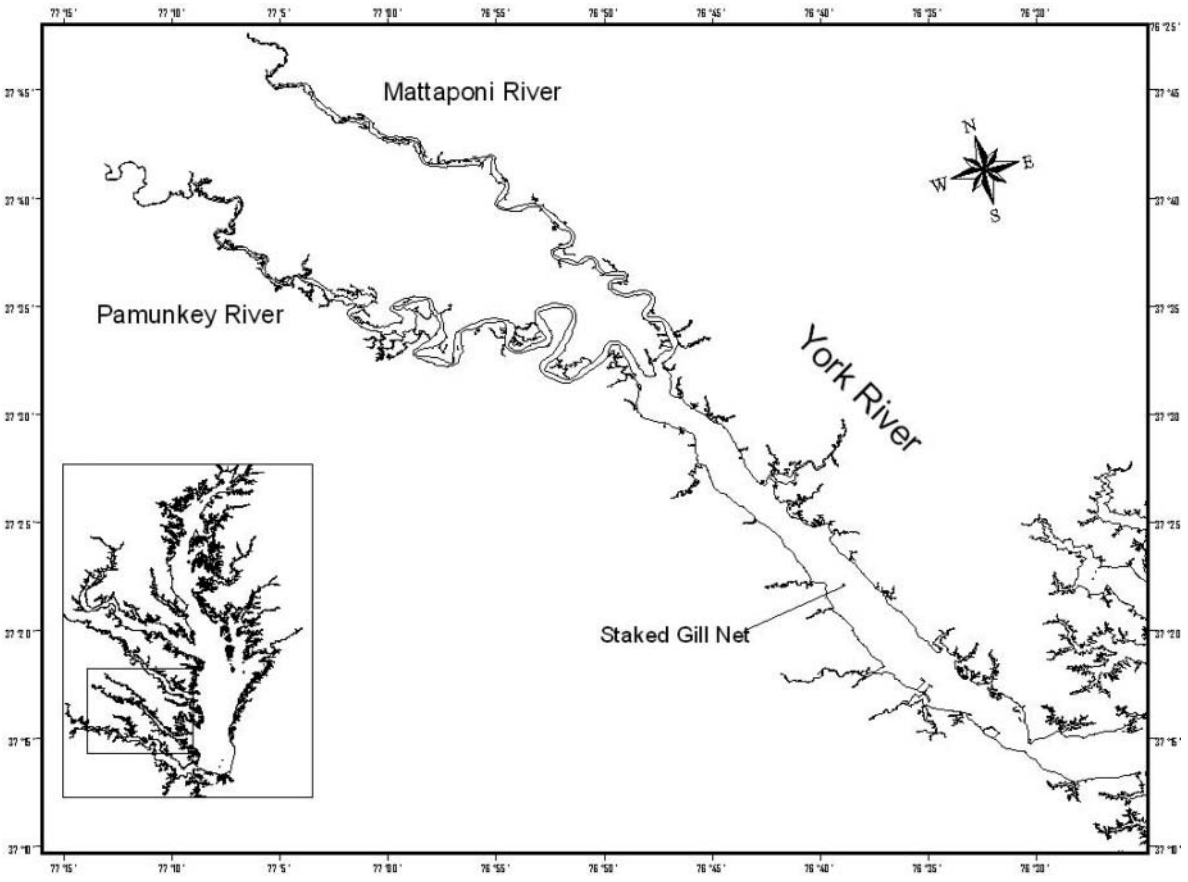

FIGURE 1. Map of the York River system showing its location within the Chesapeake Bay and the location of the staked gill net used to collect adult American shad during their spawning migrations in 2006, 2007, and 2008. Juvenile American shad were collected in the Mattaponi and Pamunkey rivers in 2003.

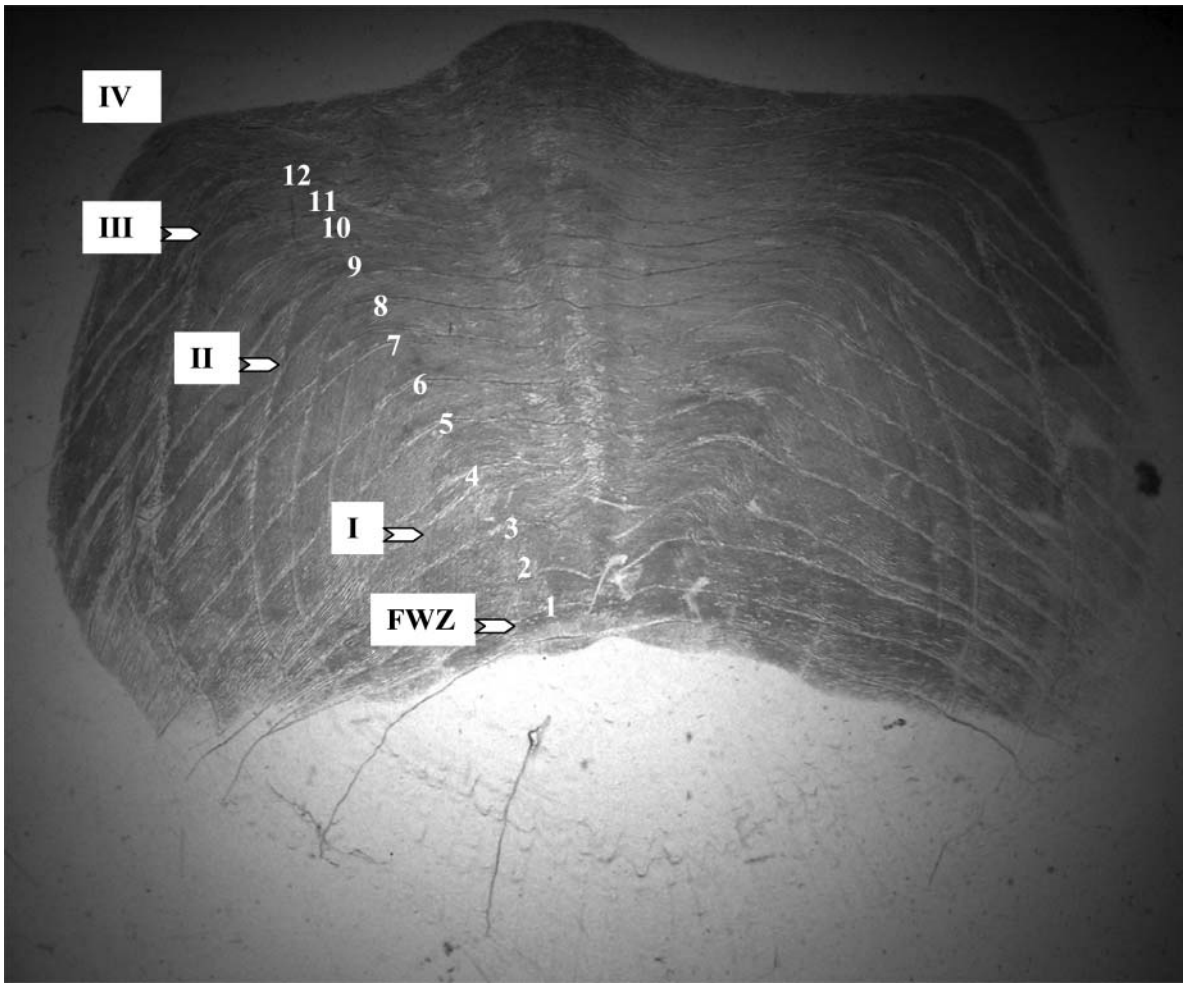

FIGURE 2. Scale of an adult American shad collected in 2006 during the spawning migration in the York River. The Cating (1953) method of age determination is depicted on the scale. Transverse grooves are indicated by Arabic numerals and annuli are indicated by Roman numerals and arrows. The freshwater zone is indicated by FWZ. This fish was identified as a member of the 2002 year-class by its isotope signatures (δ^{18}O: –4.67‰; δ^{13}C: –16.20‰) and was aged as such by both scale readers.

2001, and 2004 year-classes. This pattern was hypothesized to be due to interannual variation in total river flows and drought intensity (USGS 2005). Given this finding, we hypothesized that the δ^{18}O values in the otoliths of juvenile American shad from the 2002 year-class in the York River system were a distinct marker that could be used to identify adult members of the year-class that returned to spawn in the York River.

This unique, cohort-specific marker offered the opportunity to evaluate age determination methods for American shad, as the accuracy of age estimates for this species has been repeatedly called into question by scientists and managers (McBride et al. 2005; ASMFC 2007a, 2007b). The current standard for coast-wide monitoring of the species is scale-based age determination using methods established by Cating (1953). The methods described by Cating (1953) rely on the assumption that certain annuli consistently fall within the bounds of certain transverse grooves. Cating's (1953) term "transverse groove" refers to the transverse striae or radii that cross the scale laterally and that are characteristic of clupeoid scales (Roberts 1993; Figure 2). Scales from Hudson River fish were used to develop the Cating (1953) method, and the approach was validated for fish from the Connecticut River (Judy 1961). However, McBride et al. (2005) found that the method failed to provide accurate ages for fish from the Delaware River system and concluded that the Cating (1953) method may not be applicable to all stocks and ages of American shad. As a result of these findings, the Atlantic States Marine Fisheries Commission recommended that all states conduct stock-specific age validation studies for the species. McBride et al. (2005) used hatchery-marked individuals of known age as a basis for their evaluation. However, similar known-age material is not available for most stocks, and alternative approaches to age validation that are more widely available are needed. American shad are an ideal species in which to pursue the use of a natural geochemical marker as they return

to natal riverine habitats to spawn (Melvin et al. 1986; Waters et al. 2000). Natal river fidelity among American shad creates discrete, river-specific spawning stocks along the Atlantic coast (Walther et al. 2008), providing assurance that fish analyzed in this study were from the York River stock.

This study uses an otolith geochemical signature to identify a specific cohort of American shad and evaluate the scale-based age determination method used for the species in the York River system. The objectives of this study were to (1) expand upon the work of Walther and Thorrold (2009) by analyzing the $\delta^{18}O$ signature in the otoliths of juvenile American shad from the 2003 year-class in the York River system, which would enable us to determine whether the $\delta^{18}O$ signature from juveniles of the 2002 year-class were a distinct marker for that cohort; (2) use otolith $\delta^{18}O$ signatures to identify adults of the 2002 year-class that returned to spawn in the York River over three consecutive years; and (3) use ages determined from $\delta^{18}O$ signatures to evaluate the Cating (1953) method of age determination used for American shad in the York River.

METHODS

Specimen collection.—Juvenile American shad were collected in 2003 and processed to complete a 5-year time series (2000–2004) of juvenile otolith isotope signatures (Walther et al. 2008; Walther and Thorrold 2009). As in collections from other years, juveniles were collected in the freshwater nursery regions of the Mattaponi ($n = 38$; fork length [FL] = 51 $\pm$ 6 mm [mean $\pm$ SD]) and Pamunkey rivers ($n = 28$; 53 $\pm$ 3 mm) (Figure 1) in late summer of 2003 using push nets and beach seines. Juveniles were collected before their emigration to marine habitats, ensuring that all otolith material was accreted in freshwater. Sagittal otoliths were dissected, rinsed, and stored dry until analysis.

Adult American shad were collected in the York River in 2006 ($n = 196$), 2007 ($n = 335$), and 2008 ($n = 215$) during their spawning migrations (late February–April) as part of the Virginia Institute of Marine Science's American shad monitoring program (Olney and Hoenig 2001). Fish were collected in a staked gill net (273 m, 12.4-cm stretched-mesh monofilament netting) located in the middle reaches of the York River (Figure 1). The net was fished twice weekly over two succeeding days (two 24-h sets), and sampling was carried out over the entire spawning run. Sex, gonad stage, total length, fork length, and total weight were recorded for each fish, and scales were collected from the midlateral area on the left side of the fish posterior to the pectoral fin base and stored in paper envelopes until ready to be used for age determination. Sagittal otoliths were removed and stored in tissue culture trays for further analyses.

Geochemical analyses.—One otolith from each juvenile was analyzed for $\delta^{18}O$ and $\delta^{13}C$ following the methods outlined in Walther and Thorrold (2009). Although we hypothesized that $\delta^{18}O$ would be the most variable among years, we obtained measurements for $\delta^{13}C$ simultaneously to investigate whether this marker was similarly cohort specific. The sagittal otoliths of juveniles were mounted on glass slides with cyanoacrylic glue and ground to the midplane using 30- and 3-μm lapping film. Using a micromill, otolith material was removed from a 400-μm $\times$ 400-μm square to a depth of 75 μm located adjacent to the nucleus and extending toward the posterior lobe. These dimensions were chosen because they consistently yielded enough material to meet the minimum sample size requirements for reliable stable isotope analyses. The milled material was analyzed on a Thermo Finnigan MAT253 mass spectrometer equipped with a Kiel III carbonate device following the methods outlined in Ostermann and Curry (2000). Isotopic values are reported relative to the standard Vienna Pee Dee Belemnite and are expressed in standard δ notation. Long-term precision estimates for the mass spectrometer based on analyses of the standard NBS19 (National Bureau of Standards; Coplen 1994) are $\pm 0.07‰$ for $\delta^{18}O$ and $\pm 0.03‰$ for $\delta^{13}C$ (Ostermann and Curry 2000).

One otolith each from adults collected in 2006 ($n = 190$), 2007 ($n = 306$), and 2008 ($n = 213$) was processed, milled, and analyzed using the same methods described above for juvenile otoliths. During processing, some surrounding marine-derived material was inadvertently included into the analyzed samples. Inclusion of marine-derived material could lead to higher $\delta^{18}O$ values in adult otolith samples than in juvenile otolith samples because marine material has a higher $\delta^{18}O$ value than the material accreted on the otolith in freshwater (Epstein and Mayeda 1953; Hoefs 1980). In order to determine whether the measured $\delta^{18}O$ values were altered due to the inclusion of marine material, we employed ^{87}Sr:^{86}Sr ratios as an independent chemical marker. Like $\delta^{18}O$ values, freshwater ^{87}Sr:^{86}Sr ratios are identifiably distinct from marine ^{87}Sr:^{86}Sr ratios in most tributary systems (Ingram and Sloan 1992; Capo et al. 1998). We chose ^{87}Sr:^{86}Sr ratios because the mixing curve between freshwater and marine values for the ratio is known for the York River and because otolith ^{87}Sr:^{86}Sr ratios directly reflect dissolved ambient water values (Walther and Thorrold 2008). Because this mixing curve is well defined, measurements of ^{87}Sr:^{86}Sr ratios give us accurate assessments of the degree of marine material inclusion.

To determine the degree of marine material inclusion, we randomly selected 24 individuals across all 3 years and analyzed their remaining sagittal otolith for ^{87}Sr:^{86}Sr ratios. A section of identical size and placement (400 $\times$ 400 $\times$ 75 μm, adjacent to the nucleus and extending toward the posterior lobe) was removed from the second otolith of each fish, and the milled powder was dissolved in concentrated ultrapure HNO_3 and diluted to a final Ca concentration of 40 μg/g. We then analyzed Sr isotopes in the resulting solution using a Thermo Finnigan Neptune multiple collector inductively coupled plasma mass spectrometer. Isobaric interferences of ^{87}Rb on ^{87}Sr and ^{86}Kr on ^{86}Sr were corrected for by monitoring ^{82}Kr, ^{83}Kr, and ^{85}Rb and applying a mass bias correction using an exponential relationship (Jackson and Hart 2006; Walther and Thorrold 2008). Repeated measurement of the standard NBS987 yielded

precision estimates of 25 μg/g (2 SDs). After analysis, ^{87}Sr:^{86}Sr ratios of the milled material were compared with the known ^{87}Sr:^{86}Sr mixing curve between the freshwater value for the York River (0.7122 ± 0.0003; $n = 204$; Walther et al. 2008; Thorrold, unpublished data) and the global marine ^{87}Sr:^{86}Sr ratio (0.70918; Ingram and Sloan 1992). Depending on where the ^{87}Sr:^{86}Sr ratio values of our samples fell in relation to the known freshwater and marine values, we were able to determine the degree of marine material inclusion.

Because of the presence of marine-derived material in the samples, a regression procedure was necessary to identify members of the 2002 year-class based on core $\delta^{18}O$ signatures. This regression approach (described below) required the use of the freshwater values for $\delta^{18}O$ and $\delta^{13}C$ determined from the 2000–2004 juvenile year-classes and the $\delta^{18}O$ and $\delta^{13}C$ values for marine-derived otolith material. To obtain the marine-derived values, 10 individuals were randomly selected across all years to obtain isotope values for material accreted during marine residency. A section of the same size (400 × 400 × 75 μm) was milled on the exterior edge of the otoliths to obtain these data. The milled material was processed for $\delta^{18}O$ and $\delta^{13}C$ as described above for juvenile otoliths.

Statistical analyses.—A two-factor analysis of variance (ANOVA) with an interaction term was used to test for the effects of year and river on mean $\delta^{18}O$ and $\delta^{13}C$ signatures in juvenile otoliths (SAS version 9.1; SAS Institute 2002). The ANOVA assumptions of normality and homogeneity of variance were confirmed based on residual analysis. There was a significant interaction between year and river for both $\delta^{18}O$ ($F = 9.73$, $P < 0.001$) and $\delta^{13}C$ ($F = 15.67$, $P < 0.001$), so the ANOVAs were rerun with year and river combinations treated as a single factor. Two planned contrasts were used to test whether the $\delta^{18}O$ and $\delta^{13}C$ signatures in 2002 were significantly different from those in all other years and whether they were different between the Mattaponi and Pamunkey rivers.

Due to the inclusion of marine-derived material, we applied linear regression to identify adults of the 2002 year-class using isotope signatures. We chose a linear regression because mixing models of $\delta^{18}O$ isotope ratios between fresh and marine values typically follow a 1:1 linear mixing line (Fry 2002). Linear regressions were performed separately for each juvenile year-class using the juvenile freshwater signatures and the marine-derived signature. The estimated parameters of each linear regression model were used to calculate the squared residual of each adult data point for each of the regression models. The minimum squared residual indicated which of the regression models best fit each adult data point. Adults whose minimum squared residual corresponded to the regression model for the 2002 year-class were identified as members of that year-class. Because the regression lines for all other year-classes (2000, 2001, 2003, and 2004) were so close to each other, membership in these year-classes could not be discerned. Adults whose $\delta^{13}C$ values were higher than –12.0‰ ($n = 18$ in 2006, $n = 15$ in 2007, and $n = 11$ in 2008) were not included in analyses because at $\delta^{13}C$ values greater than –12.0‰ all of the regression models began to converge toward the marine-derived signature.

Scale-based age determination.—Two readers used the Cating (1953) scale-based method of age determination to make blind, independent age estimates for adult American shad collected in 2006 ($n = 163$), 2007 ($n = 268$), and 2008 ($n = 182$). Reader 1 had less than two years of experience aging American shad scales and underwent extensive training prior to this study. Reader 2 had greater than 10 years of experience reading American shad scales and is the primary scale reader for the Virginia Institute of Marine Science's American shad monitoring program. Prior to the start of this study, within-reader precision (measured repeatability between the first and second age estimates of the same fish) was analyzed for each reader. Each reader made duplicate, blind, independent age estimates for 50 randomly selected scales. These scales were from fish not used in this study. Each reader aged the 50 scales, the scales were renumbered by an independent third party, the order of the scales was changed, and each reader aged the scales a second time. One day was allowed to elapse between readings. Percent precision was then determined for each reader. Between-reader agreement (measured agreement as to the age of the same fish by two or more readers) was also tested before the aging for this study commenced. Both readers made blind, independent age estimates for a second set of 50 randomly selected scales from fish not used in this study, and percent agreement was determined.

Scales were cleaned with a dilute bleach solution, pressed on acetate sheets, and read on a microfilm projector following the methods of Cating (1953; Figure 2). The descriptions of transverse grooves and annuli given by Cating (1953) were used to identify these morphological features on the scales and determine the age of each individual. Cating (1953) specifies that the first annulus is located within the first 4–7 transverse grooves (predominantly at transverse groove number 5 or 6), the second annulus is located between the 8th and 11th transverse grooves (predominantly at transverse groove number 9 or 10), and the third annulus is located between the 12th and 16th transverse grooves (predominantly at transverse groove number 13 or 14). There are no specifications for transverse groove counts beyond the third annulus besides the assumption that subsequent annuli should lie beyond the 12th transverse groove. Cating (1953) described the freshwater zone (FWZ) of American shad scales as a mark that forms when juveniles leave their freshwater nursery environments and migrate into salt water and established that this zone is located within the first 1–5 transverse grooves (predominantly at transverse groove number 2 or 3). Thus, the first visible annulus-like mark on the scale was identified by readers as the FWZ and was not counted as the first annulus. Cating (1953) also described false annuli that can occur on American shad scales. He claimed that false annuli could be distinguished from true annuli because false annuli are

not visible in the posterior portion of the scale. This description was used to help identify false annuli during age determination. Annuli that could clearly be distinguished in the posterior section were considered true annuli, whereas annuli that could not be distinguished in the posterior section were considered false. Transverse grooves that branched were counted as one and both incomplete grooves (grooves that did not meet in the middle) and complete grooves (grooves that met in the middle) were counted. Transverse grooves were counted on a diagonal line extending from the center of the scale, and counts began at the first transverse groove above the baseline (Cating 1953). The final age of the fish was estimated by adding 1 year at the edge of the scale to the total number of annuli counted to account for growth since the last annulus was laid down (Figure 2).

Method evaluation.—Isotope-based age determinations were used to evaluate the scale-based method of age determination in two ways: (1) by comparison of the percentage contributions of the 2002 year-class to the spawning migration in 2006, 2007, and 2008 estimated by each method and (2) by the percentage agreement on age between the isotope-based and scale-based methods for individuals identified as members of the 2002 year-class by their isotope signatures.

Methodological sources of disagreement between isotope-based and scale-based age estimates were evaluated by tabulating transverse groove and annuli counts for specimens where we found disagreement between the two methods ($n = 23$ in 2006, $n = 37$ in 2007, and $n = 15$ in 2008). This included specimens that were identified by their $\delta^{18}O$ signatures as members of the 2002 year-class but not aged as such by the scale-based method and specimens that were aged as members of the 2002 year-class by the scale-based method but not identified as such by their $\delta^{18}O$ signatures. Scales were not evaluated if there was disagreement between readers or if the scales were determined to have a false annulus. Evaluations were completed by counting all marks that were interpreted as the FWZ or annuli and recording their location by transverse groove number. If an annulus was located between two transverse grooves, it was recorded as being located at the transverse groove with the higher number (e.g., if the second annulus was located between the 9th and 10th transverse grooves, it was recorded as being located at the 10th transverse groove).

RESULTS

Juvenile Isotope Signatures

The $\delta^{18}O$ and $\delta^{13}C$ signatures for the juveniles of the 2002 year-class differed significantly from those for the juveniles of all other year-classes ($\delta^{18}O$: $F = 4190.66$, $P < 0.001$; $\delta^{13}C$: $F = 54.78$, $P < 0.001$; Figure 3). Juveniles of the 2002 year-class had higher $\delta^{18}O$ values than juveniles of the other year-classes (Figure 3), indicating that those values served as a unique marker for that year-class.

The $\delta^{18}O$ and $\delta^{13}C$ signatures for Mattaponi and Pamunkey River juveniles were significantly different ($\delta^{18}O$: $F = 407.81$, $P < 0.001$; $\delta^{13}C$: $F = 46.68$, $P < 0.001$). However, our analysis did not attempt to identify Mattaponi versus Pamunkey River fish but rather York River adults of the 2002 year-class. Therefore, we did not separate Mattaponi and Pamunkey River fish for further analysis and instead looked at all the fish from each year as coming from the York River system.

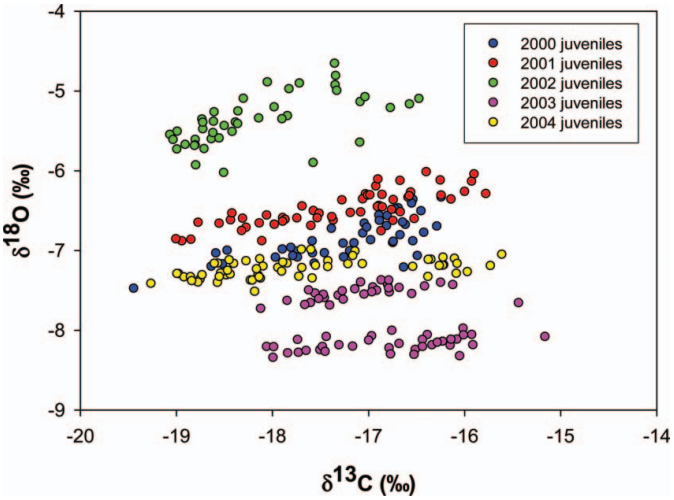

FIGURE 3. $\delta^{18}O$ and $\delta^{13}C$ signatures of otolith cores of juvenile American shad collected in the freshwater nursery regions of the York River during the summers of 2000–2004. The symbols represent individual fish. Data for 2000, 2001, 2002, and 2004 are from Walther and Thorrold (2009).

Adult Isotope Signatures

Oxygen isotope ratios in the otolith cores of adults collected in the York River in 2006, 2007, and 2008 were offset from juvenile isotope ratios and were, on average, closer to the $\delta^{18}O$ and $\delta^{13}C$ values of marine-derived material, $-0.15 \pm 0.83‰$ (mean $\pm$ SD) and $-3.91 \pm 0.95‰$ ($n = 10$), respectively (Figure 4).

Partial marine material inclusion in adult otolith samples was confirmed by analyses of ^{87}Sr:^{86}Sr ratios. The ^{87}Sr:^{86}Sr ratio for adult American shad otolith cores was 0.7111 ± 0.0007 ($n = 24$). This value was lower than the baseline, freshwater ^{87}Sr:^{86}Sr ratio of 0.7122 ± 0.0003 ($n = 204$) for the otolith cores of York River juveniles (Walther et al. 2008; Thorrold, unpublished data). Inclusion of marine material in the samples and the subsequent offset of adult and juvenile isotope signatures required the regression approach described in the Methods section to identify the 2002 year-class using $\delta^{18}O$ values.

Identification of Adult American Shad

In 2006, 2007, and 2008, 12, 63, and 22 individuals were identified as members of the 2002 year-class by isotope signatures, respectively (Table 1; Figure 4). Given these results, the isotope-based method determined that 6% of the specimens analyzed in 2006 were age-4 fish, 21% of the specimens analyzed in 2007 were age-5 fish, and 10% of the specimens analyzed in 2008 were age-6 fish (Table 1).

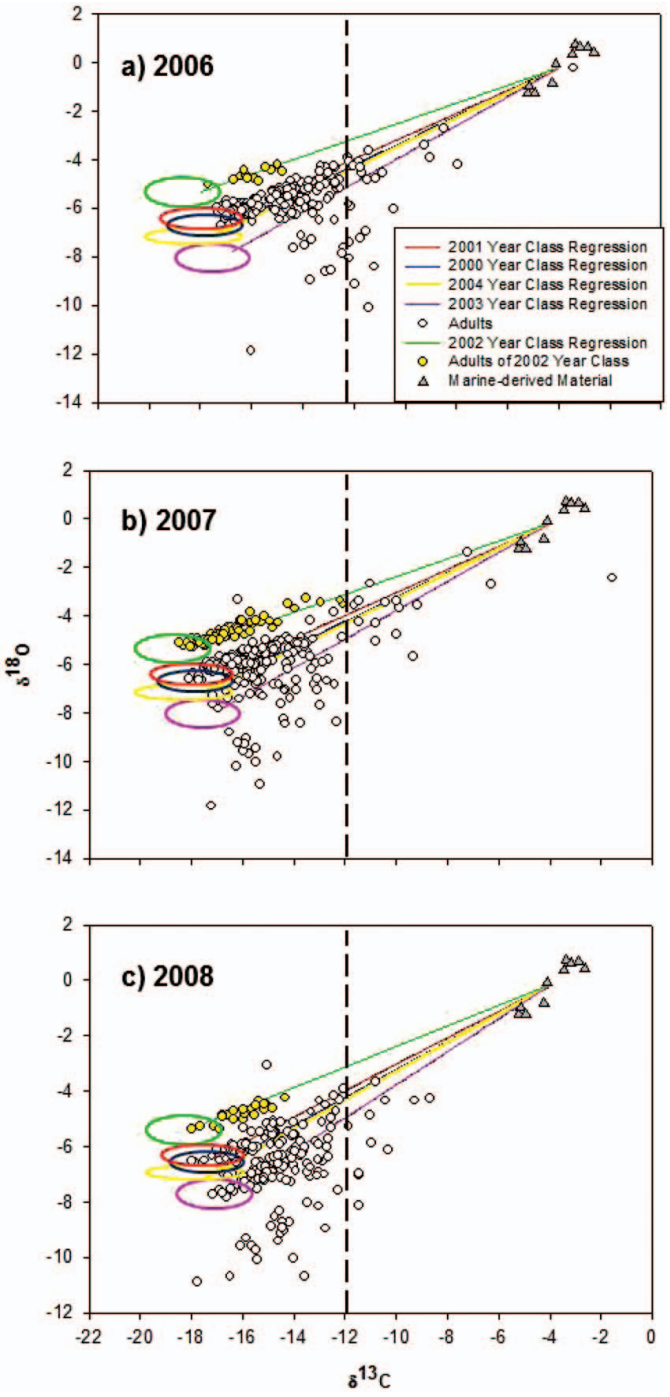

FIGURE 4. Linear regressions between the $\delta^{18}O$ and $\delta^{13}C$ signatures of juvenile otolith cores of American shad in the York River (year-classes indicated by 95% confidence ellipses) and marine-derived material from the outer portions of the otoliths. The regressions are specific to the year-classes. The $\delta^{18}O$ and $\delta^{13}C$ signatures are from fish collected during their spawning migrations in **(a)** 2006, **(b)** 2007, and **(c)** 2008; each circle represents one fish. Adults identified by their isotope signature as members of the 2002 year-class are indicated by yellow circles. The vertical dashed lines separate the plots at the $\delta^{13}C$ value of –12.0‰; adults with $\delta^{13}C$ values greater than that were not included in the analyses.

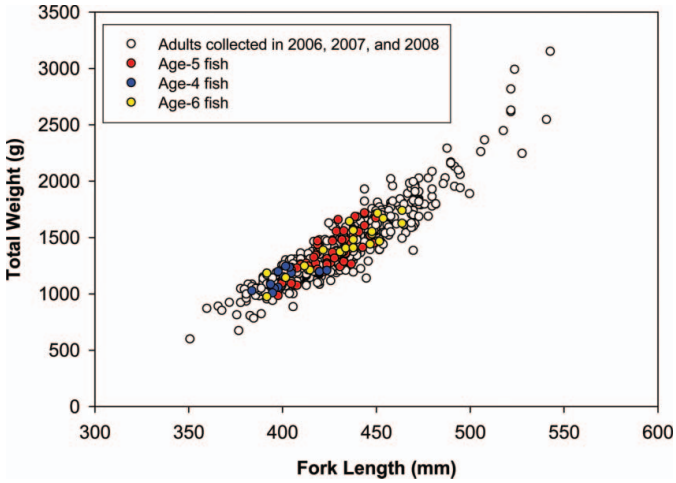

FIGURE 5. Total weight and fork length for all adult American shad collected during spawning migrations in the York River in 2006, 2007, and 2008 (n = 705). Individuals that were identified by their isotope signatures as age-4 fish in 2006 (n = 12), age-5 fish in 2007 (n = 63), and age-6 fish in 2008 (n = 22) are indicated. Only weights and lengths for prespawning fish are shown.

Individuals identified as members of the 2002 year-class by isotope signatures exhibited the allometric size relationships expected for age-4, age-5, and age-6 American shad in the York River (Figure 5). The fork lengths and weights of individuals identified by their isotope signatures as age-4 fish in 2006 varied from 384 to 424 mm and from 1,002.6 to 1,238.3 g, respectively (n = 12). The fork lengths and weights of individuals identified by their isotope signatures as age-5 fish in 2007 varied from 398 to 450 mm and from 715.9 to 1,712.1 g, respectively (n = 63). Finally, the fork lengths and weights of individuals identified by their isotope signatures as age-6 fish in 2008 varied from 392 to 464 mm and from 968.4 to 1,733.7 g, respectively (n = 22).

Method Evaluation

Prior to the commencement of scale-based age determination in this study, reader 1 demonstrated 59.0% precision and reader 2 demonstrated 72.2% precision. These values are comparable to that estimated for the group of experienced readers in the McBride et al. (2005) study (50.0–76.5%). Readers 1 and 2 also exhibited an agreement of 66.7%.

We found considerable disagreement over the percentage contributions of the 2002 year-class to the spawning migrations in 2006, 2007, and 2008 estimated by the two age determination methods. Isotope signatures estimated a lower percent contribution of the 2002 year-class to the spawning migration in all 3 years than the scale-based method (Table 1). For those individuals identified as members of the 2002 year-class by their isotope signatures, agreement on age between isotope-based and scale-based age estimates was low (Table 2). Agreement to within 1 year was high between the two methods, however (Table 2). An asymmetrical bias was evident for age-5 and age-6 fish; age was underestimated in these specimens, predominantly by 1 year (Figure 6).

TABLE 1. Number of individuals of the 2002 year-class and their percent contributions to the spawning migration in the York River in 2006, 2007, and 2008, as estimated by isotope-based and scale-based age determination methods; na = not applicable.

Year	Method	Reader	Number of individuals of the 2002 year-class	Sample size (n)	% Contribution of 2002 year-class to spawning migration
2006	Isotope-based	na	12	190	6
	Scale-based	1	52	163	32
		2	47	163	29
2007	Isotope-based	na	63	306	21
	Scale-based	1	91	268	34
		2	121	268	45
2008	Isotope-based	na	22	213	10
	Scale-based	1	32	182	18
		2	46	182	25

The scale-based method of age determination established by Cating (1953) failed to determine the correct age of the fish between 22% and 63% of the time, depending on the reader and the age of the fish (Table 2). For the majority of incorrectly aged individuals, transverse groove and annuli counts did not agree with the criteria established by Cating (1953) for identifying certain annuli (Table 3). For example, the third annulus was located below the range of transverse groove numbers specified for that annulus by Cating (1953) for 100% of the incorrectly aged individuals evaluated in 2006 ($n = 23$; Table 3). In general, the second, third, and fourth annuli of most incorrectly aged fish were located below the range of transverse groove numbers specified for the annuli by Cating (1953). This pattern held for all 3 years. These results indicate that the method of age determination established by Cating (1953) is not reliable for American shad in the York River.

DISCUSSION

This study used stable isotope ratios in otolith cores to identify the 2002 year-class of American shad in collections of adults returning to spawn in the York River over three consecutive years. This is the first such use of a natural geochemical signature to identify a cohort of an anadromous species, track its recruitment over time, and evaluate age determination methods. While we did experience processing errors in obtaining a pure, freshwater signature in adult otoliths, we were still able to confidently identify adults of the 2002 year-class using regression analysis. The percent contribution of the 2002 year-class to spawning runs determined by otolith isotope signatures exhibited patterns that were expected for this year-class. American shad in the York River are estimated to mature sexually and begin recruiting to the spawning stock at age 3, with recruitment peaking at age 4 and age 5 and steadily decreasing thereafter (Maki et al. 2001). Our isotope-based age estimates similarly identified the highest contribution of the 2002 year-class to spawning migrations at age 5 in 2007 (21%), compared with age 4 in 2006 (6%) and age 6 in 2008 (10%). In addition, the 2002 year-class was predicted to be a weak year-class by juvenile abundance indices generated from seine surveys in the York River system (Olney 2003), and adults from this year-class were not expected to dominate subsequent spawning runs. Accordingly, we identified only small proportions of the 2002 year-class in collections of returning adults in all 3 years. Adults identified as members of the 2002 year-class by their isotope signatures also followed the allometric size relationships expected for age-4, age-5, and age-6 American shad. Agreement between the expected patterns of size relationships and recruitment for the 2002 year-class in the York River and the observed patterns lends confidence to the ability of otolith isotope signatures to identify members of the 2002 cohort.

This study highlights a promising new direction for otolith geochemistry as a potential marker for cohorts of fish. However, it also identifies several factors that should be considered when applying this technique in the future. It has been suggested that anomalous or prolonged environmental conditions, such as El Niño, storm events, and periods of above- or below-average precipitation or temperature, are useful for creating significant differences in the geochemical signatures

TABLE 2. Agreement between isotope-based and scale-based age estimates for adult American shad collected in 2006, 2007, and 2008 during spawning migrations in the York River. Only specimens identified by isotope signatures as members of the 2002 year-class were included in the analysis; NS = no scale sample, UN = unusable scales.

Year	Reader	n	NS	UN	% Agreement (n)	% Agreement ± 1 year
2006	1	12	2	1	78 (9)	100 (9)
	2	12	2	0	50 (10)	100 (10)
2007	1	63	2	6	40 (55)	96 (55)
	2	63	2	12	55 (49)	98 (49)
2008	1	22	0	3	37 (19)	84 (19)
	2	22	0	5	41 (17)	100 (17)

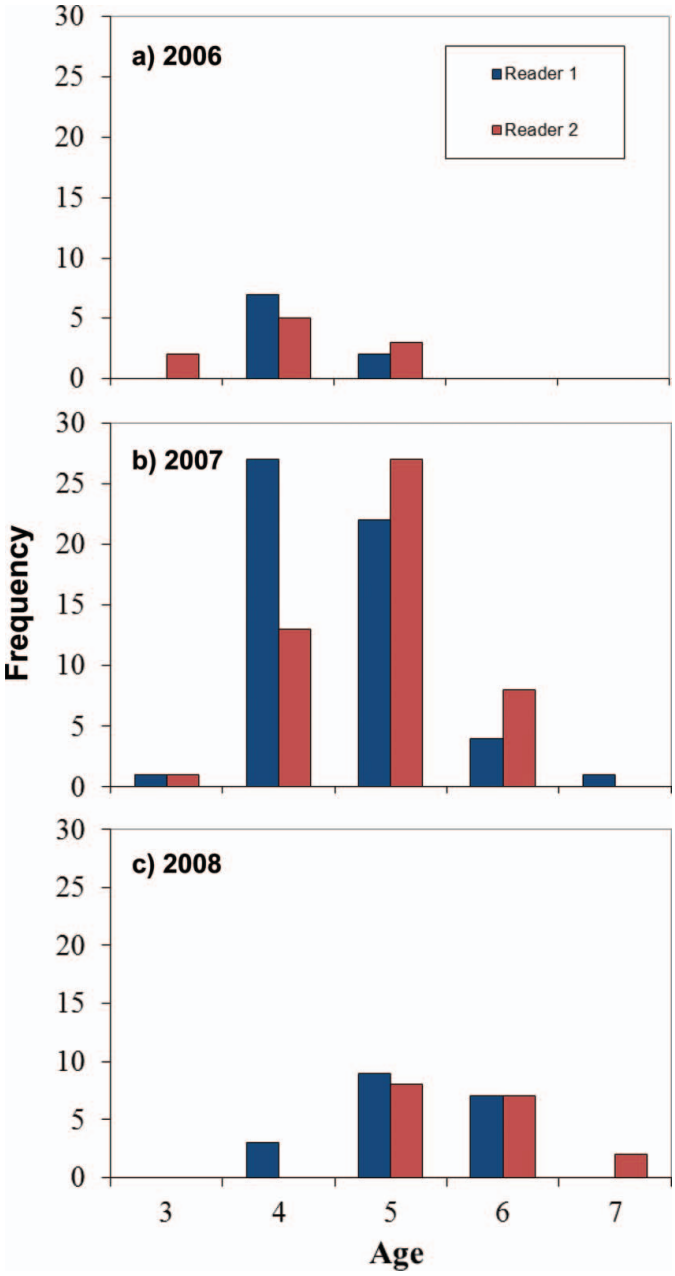

FIGURE 6. Frequency distributions of scale-based age estimates for adult American shad collected during spawning migrations in the York River in **(a)** 2006 (age-4), **(b)** 2007 (age-5), and **(c)** 2008 (age-6) and identified as members of the 2002 year-class by their isotope signatures. Specimens that did not have scale samples or whose samples were determined to be unusable were not included in this analysis.

of cohorts (Cole et al. 1999; Gillanders and Kingsford 2000; Patterson et al. 2004; 2008). This requirement for anomalous environmental conditions means that cohort-specific signatures are potentially rare. However, if libraries of juvenile otoliths were collected over time along with time series of environmental variables, such as river flow, rainfall, and temperature, time periods with high likelihoods for cohort-specific markers could be identified and the cohorts surrounding the time period could be tested for unique signatures. Geochemical analyses of otoliths can be time-consuming and costly, but information on the factors that contribute to distinct markers, such as river flow or storm events, could indicate specific time periods that should be investigated for cohort-specific markers.

The Cating (1953) method of scale-based age determination was not suitable for identifying age-4, age-5, or age-6 American shad in the York River. On average, 50% of individuals from the 2002 year-class were aged incorrectly using the Cating (1953) method. In another assessment of Cating's (1953) method, McBride et al. (2005) also found that age estimates for American shad in the Delaware River system were inaccurate and, as in the present study, that the ages of age-5 and age-6 fish tended to be underestimated by 1 year. We are confident that the disagreement between isotope-based and scale-based ages was primarily due to application of the Cating (1953) method to scale-based age estimates and not to general interpretation error by the readers. While it is to be expected that readers will sometimes misinterpret annuli, transverse grooves, and other features of the scale, the percent precisions determined for our readers (reader 1: 59.0%; reader 2: 72.2%) before this study commenced suggested that our readers were consistently interpreting scale features. McBride et al. (2005) demonstrated a range of 50.0–76.5% precision among the experienced scale readers in their study, and they concluded that their readers were aging scales with good precision. As the percent precision of our readers was well within the range reported by McBride et al. (2005), we also believe our readers were aging scales with good precision.

For the majority of the specimens for which there was disagreement between the isotope-based and scale-based methods, errors in age estimates occurred because transverse groove and annuli counts did not follow the criteria established by Cating (1953). Following the Cating (1953) method caused readers to assign an age incorrectly. In the majority of these specimens, there were two visible annuli located within the 1–5 or 4–7 transverse groove ranges. Cating (1953) does not provide any guidance for situations when multiple annuli are located within the same range of transverse grooves; thus, readers often did not count the first visible annulus on the scale because doing so would cause subsequent annuli to fall outside the range of transverse grooves specified for the annuli by Cating (1953).

An alternative method that uses identification of the FWZ and simple counts of the visible annuli, without relying on transverse groove counts, may result in better agreement on age between the methods. Scale-based age determination of other *Alosa* species, such as allis shad (also known as allice shad) *Alosa alosa*, twaite shad *Alosa fallax* (Baglinière et al. 2001), blueback herring *Alosa aestivalis* (Marcy 1969), and alewife *Alosa pseudoharengus* (Rothschild 1963; Marcy 1969) rely on counts of the visible annuli and do not use transverse groove counts. Marcy (1969) recorded transverse groove and annuli counts for alewives and blueback herring but indicated that

TABLE 3. Locations of the freshwater zones (FWZs) and annuli on scales of American shad for which there was disagreement over age between the isotope-based and scale-based age determination methods. Bold italics indicate the number of specimens that had the FWZ or an annulus located outside the range of the transverse grooves specified for these features by Cating (1953).

			Transverse groove number														
Year	*n*	FWZ/annulus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2006	23	FWZ	1	9	13													
		1			***7***	9	6	1										
		2					***1***	***10***	***8***	*4*								
		3								***3***	***15***	***4***	***1***					
		4									***1***	***4***	***7***	10			1	
2007	37	FWZ		15	19	3												
		1			***2***	21	12	2										
		2						***7***	***21***	8	1							
		3								***2***	***12***	***14***	***7***	2				
		4										***2***	***13***	12	8	2		
		5												5	9	9	6	1
2008	15	FWZ		9	6													
		1			***1***	8	5		1									
		2						***3***	***5***	3	3	1						
		3								***2***	***5***	***1***	***2***	5				
		4										***3***	***2***	3	3	1	3	
		5											2	2	5		2	2
		6											1	1	1	3		1

transverse grooves should only be used to locate an annulus that was difficult to interpret and not used as a substitute for annuli counts. Marcy (1969) also reported that transverse groove and annuli counts differed for alewives from different geographical areas. This concern was echoed for American shad by McBride et al. (2005), suggesting that Cating's (1953) method may not be applicable to all stocks.

In addition to the issues discussed above, readers noted that the first annulus and FWZ were often difficult to distinguish. This supported the observation of Hammer (1942) that the scales of American shad from the York River lacked a sharp demarcation between the FWZ and what he termed the "marine growth" portion of the scale. Scale-based age determination methods for allis and twaite shad do not identify an FWZ and count what we have identified in this study as the FWZ as the first annulus (Baglinière et al. 2001). Marcy (1969) identified an FWZ in alewives and blueback herring scales but commented that different stocks of these species differed in the size of the FWZ due to differences in the residence time of juveniles in freshwater nursery habitats (Limburg 1995; Hoffman et al. 2008). Marcy (1969) also suggested that certain stocks may not have an FWZ on their scales if the juvenile residence time in freshwater was extremely short. It is likely that stocks of American shad vary in the size and even the presence or absence of the FWZ due to differences in the amount of time that juveniles reside in freshwater nursery areas (Limburg 1995; Hoffman et al. 2008). Elemental analysis of scales has been used to infer past life histories of diadromous fishes (Coutant and Chen 1993; Courtemanche et al. 2005) and would allow for investigation of the FWZ, as well as spawning marks, on American shad scales.

In 1953, Mansueti and Kolb commented that biologists had great difficulty aging American shad. Since that time, shad biologists have not made significant advances in the field. The results of our study agree with McBride et al. (2005) that the Cating (1953) method does not appear to be applicable to all stocks of American shad. Scientists should continue to use caution when applying scale-based age estimates in stock assessments of American shad throughout their range, especially in the York and Delaware River systems. Alternative methods of age determination, such as counting annuli without reliance on transverse grooves, should be researched. However, any future investigation of age determination methods for the species should also take into account differences between stocks.

Cohort-specific geochemical markers can allow unique opportunities to validate aging methods when known-age fish are not available by traditional means. The relative objectivity that natural geochemical signatures provide to cohort identification (compared with the observational errors associated with other cohort identification methods) serves to emphasize that alternative age determination approaches would be useful. Natural, cohort-specific geochemical signatures are a promising avenue to pursue.

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