2008

**Novel use of a Natural Isotope Signature to Track Recruitment and Evaluate Age Determination Methods for the 2002 Year Class of American Shad in the York River**

Sally A. Upton

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[https://dx.doi.org/doi:10.25773/v5-c7hp-wc09](https://dx.doi.org/doi:10.25773/v5-c7hp-wc09)

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Novel Use of a Natural Isotope Signature to Track Recruitment and Evaluate Age Determination Methods for the 2002 Year Class of American Shad in the York River

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William and Mary

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science

by
Sally A. Upton
2008
APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science

Approved July 2008

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ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. John Olney, for his unwavering belief in this study and in my abilities, for his commitment to my education, his constant encouragement, and his contagious laugh. I thank Dr. Elizabeth Canuel, Dr. Mary Fabrizio, and Dr. Robert Latour for serving on my committee and offering helpful comments.

This thesis would not have been possible without the contributions of Dr. Benjamin Walther and Dr. Simon Thorrold. Dr. Walther offered continual support and advice throughout this project. Dr. Thorrold’s willingness to provide advice and suggestions and his commitment to the successful outcome of this project were invaluable.

Special thanks go to Brian Watkins, who spent numerous hours training me, reading American shad scales and otoliths for this project, and making those tasks bearable. I also thank Dorrinda Ostermann for her enthusiasm and willingness to help in any way needed to get my samples analyzed. I thank Pat Crewe for her help with a little bit of everything, and Troy Tuckey for his insight and his ability to understand the frustration of working with American shad otoliths. I would also like to thank Raymond “Kennyman” and Tony Kellum for their work at collecting the specimens used in this project.

This thesis may have taken less time to complete, but would have been much less enjoyable without the presence of a few people. I thank Justine Woodward, Andre Buchheister, Patrick Lynch, Candi Spier, Abby Lynch, Chris Magel, Kathleen McNamee, and Dan Dutton for the support, advice, stress-relief, “intellectual” lunch-time conversations, runs, email distractions, and laughs they provided me with over the past three years. Your friendship has meant the world to me.

I would like to thank my parents, Bill and Janie Upton. Their constant, unimaginable love and support of me has been a source of inspiration and comfort and has helped me in ways they can’t even imagine.

Funding for this project was provided by the U.S. Fish and Wildlife Service through the Virginia Marine Resources Commission (F116-R-9 and F116-R-10) and by the National Oceanic and Atmospheric Administration and the National Marine Fisheries Commission Grant NA07NMF4050164.
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Temporal variability of natural geochemical signatures in the otoliths of fishes may result in cohort-specific signatures that facilitate tracking of a cohort over time and investigation of its life history characteristics. A natural isotope signature in the otoliths of one year class of American shad (*Alosa sapidissima*) in the York River was used to track recruitment of the year class over two spawning events and evaluate scale-based and whole otolith-based age determination methods used for the species. $\delta^{18}O$ signatures of juveniles of the 2002 year class in the Mattaponi and Pamunkey Rivers (two tributaries that join to form the York River) were enriched relative to other year classes (2000, 2001, 2003, and 2004), and members of this year class could be identified in collections of adults returning to spawn in the York River in 2006 and 2007 based on the $\delta^{18}O$ signature in the core of their otoliths. Adults identified by isotope signatures as members of the 2002 year class exhibited expected patterns of growth for York River American shad. The percent contribution of the 2002 year class to the spawning migrations in 2006 (6%) and 2007 (19 or 20%) estimated by isotope signatures conformed to the expected recruitment pattern for the 2002 year class based on juvenile abundance indices. Agreement between isotope signature-based age determinations and scale-based (average between readers: 64% in 2006; 46% in 2007) and whole otolith-based (average between readers: 39% in 2006 and 2007) age estimates was low. Neither the Cating (1953) method of scale-based age determination nor the whole otolith-based method was suitable for aging age-4 and age-5 American shad in the York River. While natural geochemical signatures may not serve as a marker for most cohorts, cohort-specific geochemical signatures have potential to facilitate estimation of vital rates and recruitment strategies and test age determination methods. Use of a cohort-specific marker for American shad in the York River suggested that caution should be used when using scale-based and whole otolith-based age determinations in stock assessments of the species.
Novel Use of a Natural Isotope Signature to Track Recruitment and Evaluate Age Determination Methods for the 2002 Year Class of American Shad in the York River
INTRODUCTION

Natural geochemical signatures in fish otoliths are widely used in investigations of the life histories of fishes. Geochemical signatures have been used to address questions about natal origins (Kennedy et al. 1997; Thorrold et al. 1998a, b; Kennedy et al. 2000; Walther et al. 2008), philopatry (Thorrold et al. 2001), habitat use (Secor et al. 2001; Dorval et al. 2005), migration patterns (Limburg 1995; Secor et al. 2001; Kennedy et al. 2002), and population structure (Thorrold et al. 2001). Otoliths are paired calcareous structures in the inner ear of teleost fishes that function in the hearing and balance systems (reviewed in Popper and Lu 2000). Several properties of otoliths contribute to the presence of natural geochemical signatures within the structures. First, otoliths form by the accretion of daily and yearly increments of calcium carbonate that are commonly used to determine the age of fishes. Second, the material accreted onto otoliths is metabolically inert, allowing the chemical composition of the otolith to remain stable over time (Campana and Neilson 1985). Third, certain elements within otoliths, such as Sr, Ba, and O, are primarily derived from the ambient water (Thorrold et al. 1997; Bath et al. 2000; Høie et al. 2003; Walther and Thorrold 2006). As a result, each increment reveals, to some degree, the chemical composition of the water occupied at the time of accretion (Thorrold et al. 1997). The chemical composition of water varies spatially due to environmental factors (Dansgaard 1964; Bricker and Jones 1995) and analysis of discrete increments in the otolith can be used to determine the geographic origins of
fishes as well as age-specific movements between chemically distinct waters (Thorrold et al. 1997; Campana 1999).

Environmental factors also cause the chemical composition of water and otoliths to vary temporally (Dansgaard 1964; Cole et al. 1999). Several studies have investigated temporal variability in otolith geochemistry (reviewed in Gillanders 2002), but almost all have done so out of the necessity of understanding temporal variability in order to accurately interpret spatial variability (e.g. Gillanders and Kingsford 2000; Hamer et al. 2003; Rooker et al. 2003; Patterson et al. 2004; Patterson and Kingsford 2005; Feyrer et al. 2007; Patterson et al. 2008). Temporal variability in otolith geochemistry may result in cohort-specific signatures that facilitate tracking of a cohort over time, leading to the determination of various life history characteristics of the cohort, including maturity schedules, mortality, and growth. However, distinct, cohort-specific signatures likely require anomalous environmental conditions for their formation and may be rare. Anadromous fishes are ideal for the investigation of cohort-specific geochemical signatures because the developmental habitat of larval and juvenile stages of these species is susceptible to high environmental variability. As a result, cohorts of anadromous species may have distinct geochemical signatures in the cores of their otoliths. Environmental variability has been linked to patterns of larval survival and growth and ultimately to recruitment of anadromous fishes (Crecco and Savoy 1985; Rutherford and Houde 1995; Limburg et al. 1996; McGovern and Olney 1996; Aprahamian et al. 2003; Hoffman et al. 2007), and identification of specific cohorts at later stages using geochemical signatures has the potential to allow for investigations of the factors and conditions that drive survival and recruitment variability of these species.
Inter-annual variability in the geochemical signatures of juvenile American shad otoliths, an anadromous species native to the Atlantic coast of North America, was reported by Walther et al. (2008). Geochemical signatures for Hudson River juveniles were distinct and non-overlapping between 2000 and 2001, and geochemical signatures for Mattaponi and Pamunkey River (two tributaries that join to form the York River) juveniles were similar in 2000 and 2001 but distinct in 2002. The isotope ratio $\delta^{18}O$ was the driving factor behind the inter-annual variability. In the York River system, juveniles of the 2002 year class had enriched $\delta^{18}O$ signatures compared to juveniles of the 2000 and 2001 year classes. Environmental factors are believed to have caused this inter-annual variability (Walther et al. 2008). Several complex processes lead to temporal variations in the chemical composition of water; however, differences in the source or amount of precipitation in 2002 compared to other years probably gave rise to the enriched $\delta^{18}O$ values (Dansgaard 1964; Cole et al. 1999; Walther et al. 2008).

Discrete spawning stocks of American shad range from the St. John’s River, Florida to the St. Lawrence River, Quebec (Nolan et al. 1991; Limburg et al. 2003). American shad spend the majority of their lives in the marine environment, but sexually mature individuals migrate into coastal rivers to spawn in freshwater (Melvin et al. 1986; Dadswell et al. 1987). It is estimated that American shad sexually mature between the ages of three and seven (Carscadden and Leggett 1975; Maki et al. 2001), at which time they return to natal rivers to spawn (Melvin et al. 1986; Waters et al. 2000). American shad in the York River are estimated to sexually mature and begin recruiting to the spawning stock at age three. Recruitment increases to peaks at age four and five and steadily decreases thereafter (Nichols and Massmann 1963; Maki et al. 2001). Juvenile
American shad are presumed to spend their first summer in freshwater nursery regions and migrate to the ocean in the fall; however, variations in this pattern have been observed (Limburg 1995; 1996; Limburg et al. 2003; Hoffman and Olney 2005; Hoffman et al. 2008).

Many stocks of American shad are at historic lows in abundance, and uncertainty about the accuracy of many life history parameters estimated for the species continues to hamper stock assessments and management efforts (ASMFC 2007a, b). The accuracy of age estimates for American shad has been questioned by scientists and managers (McBride et al. 2005; ASMFC 2007a, b). The current standard for coast-wide monitoring of the species is scale-based age determination based on methods established by Cating (1953). The methods of Cating (1953) are based on the assumption that certain annuli consistently fall within the bounds of certain transverse grooves, “distinct grooves in the surface of the anterior, sculpted portion [of the scale], crossing it laterally”. Cating’s (1953) term ‘transverse groove’ refers to the transverse striae or radii that are characteristic of clupeoid scales (Roberts 1993). Fish from the Hudson River were used to develop the Cating (1953) method, and the method was validated by Judy (1961) with fish from the Connecticut River. However, McBride et al. (2005) failed to validate the method for fish from the Delaware River system when they found that only 31.8% of all age estimates made by experienced American shad scale readers on known-age fish from the system were accurate. The McBride et al. (2005) study concluded that the Cating (1953) method of age determination may not be applicable to all stocks and ages of American shad. The importance of confidence in age estimates was realized in the 2007 stock assessment for American shad when scientists chose not to use age data from the
Delaware River system due to the findings of McBride et al. (2005) and applied extreme caution in the use of age data from other river systems (Olney 2007).

We hypothesized that the $\delta^{18}O$ signature in otoliths of juvenile American shad hatched in 2002 was a distinct marker that could be used to identify adult members of the 2002 year class that returned to spawn in the York River. In addition to $\delta^{18}O$, the isotope ratio $\delta^{13}C$ was investigated for inter-annual variability, but high variability in this ratio was not expected since carbon isotopes are less responsive to environmental changes than oxygen isotopes (Høie et al. 2003; Thorrold et al. 1997). The objectives of this study were to: 1) further investigate the inter-annual variability of $\delta^{18}O$ and $\delta^{13}C$ signatures of juvenile American shad in the Mattaponi and Pamunkey Rivers over the years 2003 and 2004 to determine if the unique $\delta^{18}O$ signature in juveniles of the 2002 year class could be used as a marker for that year class; 2) use the $\delta^{18}O$ signature to identify adults of the 2002 year class in the York River spawning migration in 2006 and 2007 and track recruitment of the year class over two spawning events; 3) compare age as determined by isotope signatures to age estimates based on the Cating (1953) method of scale-based age determination for American shad in the York River; and 4) investigate the potential of whole otoliths as structures for age determination of American shad in the York River by comparing estimates of age based on whole otoliths to age as determined by isotope signatures.
METHODS

Specimen Collection

Juvenile American shad were collected in the Mattaponi and Pamunkey Rivers (Figure 1) in 2003 and in the Mattaponi River in 2004 following methods outlined in Walther et al. (2008). In 2003, 38 (mean 51 ± 6 (SD) mm FL) juveniles from the Mattaponi River and 28 (mean 53 ± 3 (SD) mm FL) from the Pamunkey River were collected and analyzed. In 2004, 59 (58 ± 7 (SD) mm FL) juveniles from the Mattaponi River were collected and analyzed.

Adult American shad were collected in the York River in 2006 (n = 196) and 2007 (n = 335) during their spawning migrations (late February – April) as part of the Virginia Institute of Marine Science’s (VIMS) American shad monitoring program (Olney and Hoenig 2001). Fish were collected in a staked gill net (273 m, 4 7/8” (12.4 cm) stretched-mesh monofilament netting) located in the middle reaches of the York River (Figure 1). The net is designed to select for pre-spawning, sexually mature, female American shad; however, males and post-spawning females are occasionally caught. The net was fished twice weekly over two succeeding days (two 24-hour sets), and sampling was carried out over the entire spawning run. Total weight, total length, fork length, sex, and gonad stage were recorded for each fish. Scales were collected from the mid-lateral 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 analysis.

**Stable Isotope Analyses**

**Juvenile Otolith Cores**

Cores of juvenile otoliths collected in 2003 and 2004 were analyzed for $\delta^{18}$O and $\delta^{13}$C following methods outlined in Walther et al. (2008). Juvenile and adult otoliths were analyzed following the same methods (described below). A two-factor analysis of variance (ANOVA) with an interaction term was used to test for differences in $\delta^{18}$O and $\delta^{13}$C signatures between years and between rivers. The linear model used to analyze the data was:

$$y_{ijk} = \mu + \gamma_i + \beta_j + \gamma \beta_{ij} + \epsilon_{ijk}$$

where $y_{ijk}$ was the $\delta^{18}$O or $\delta^{13}$C signature of the $k$th individual collected in the $i$th year in the $j$th river, $\mu$ was the mean $\delta^{18}$O or $\delta^{13}$C signature for all individuals in all rivers in all years, $\gamma_i$ was the effect of the $i$th year, $\beta_j$ was the effect of the $j$th river, $\gamma \beta_{ij}$ was the effect of the interaction of the $i$th year and the $j$th river, $\epsilon_{ijk}$ was the random or unexplained error associated with the $k$th individual collected in the $i$th year in the $j$th river, $i$ was 2000-2004, and $j$ was Mattaponi or Pamunkey. $\delta^{18}$O and $\delta^{13}$C signatures were tested for the assumptions of normality and homogeneity of variance. Kolmogorov-Smirnov tests ($\delta^{18}$O: $D = 0.079$, p<0.05; $\delta^{13}$C: $D = 0.062$, p<0.05), frequency plots, and quantile-quantile plots indicated that the data were non-normal, and plots of residuals indicated that the data had homogeneous variance. Data were not transformed to adjust for non-
normality because ANOVA tests are robust to violations of normality if sample sizes are balanced and variances are equal (Quinn and Keough 2002).

**Adult Otolith Cores**

One otolith from each adult collected in 2006 and 2007 was analyzed for $\delta^{18}O$ and $\delta^{13}C$ with isotope ratio mass spectrometry (IR-MS). The otolith was rinsed in distilled water, air dried, and mounted sulcus side up on a glass slide with cyanoacrylic glue. The otolith was ground to the midplane using 3 and 30 $\mu$m lapping film to expose the core. A small area (400 (long) x 400 (wide) x 75-100 (deep) $\mu$m) within the core of the otolith adjacent to the nucleus and extending toward the posterior lobe was removed with a New Wave Research MicroMill. This was the same region that was milled in the juvenile otolith cores. Initially, samples were milled at a depth of 75 $\mu$m, but further analysis revealed that greater milling depths were needed (see results). Subsequent samples were milled at depths between 75 and 100 $\mu$m. It should be noted, however, that whereas the mill depth could be specified, the depth the otoliths were ground to was variable. Visual inspection was used during grinding to determine when the midplane of the otolith had been reached and when the otolith core was exposed; there was no way to measure the amount of material that had been ground off of the otolith. As a result, otoliths were of varying thickness when they were milled. The thickness (or height) of the otolith could be assessed with the micromill and was used to determine the appropriate milling depth.

A sample mass of no less than 15 $\mu$g was necessary for analysis, but sample masses of approximately 30-50 $\mu$g were typical. The milled material was analyzed on a Thermo Finnigan MAT252 mass spectrometer equipped with a Kiel III carbonate device following methods outlined in Ostermann and Curry (2000). Isotopic values were
reported relative to the standard Vienna Pee Dee Belemnite (VPDB) and expressed in δ notation where:

\[
\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

and \( R \) represents the ratio \(^{18}\text{O}^{16}\text{O} \) measured in the sample and standard, respectively.

Long-term precision estimates of the mass spectrometer based on analyses of the standard NBS19 are ±0.07 for \( \delta^{18}\text{O} \) and ±0.03 for \( \delta^{13}\text{C} \) (Ostermann and Curry 2000). Every attempt was made to sample all specimens collected in 2006 and 2007. However, errors in sample processing led to the absence of isotope signatures for some specimens; isotope signatures were available for 190 out of 196 specimens from 2006 collections and 306 out of 335 specimens from 2007 collections.

Initial analyses of adult otolith cores revealed the need to determine the \( \delta^{18}\text{O} \) and \( \delta^{13}\text{C} \) signatures of marine-derived material, material that was accreted onto the otolith during the fish’s time in the marine environment (see results). To obtain these signatures, a section (400 (long) \( \times \) 400 (wide) \( \times \) 75 (deep) \( \mu \text{m} \)) on the outer portion of the otolith, outside of the core area and near the edge of the otolith, was removed and analyzed following the methods described above for otolith cores.

**Age Determination**

Two readers used scale-based and whole otolith-based methods to make blind, independent age estimates for adult American shad collected in 2006 and 2007 (Olney 2007). The same two readers were used for both methods. Every attempt was made to estimate the age of every specimen collected in 2006 and 2007 with both scale-based and whole otolith-based methods. However, for some specimens scales or otoliths were not
available or the structures were determined to be unusable by one or both of the readers. These specimens were not included in analyses. Scale-based age estimates were completed for 163 out of 196 specimens from 2006 collections and for 268 out of 335 specimens from 2007 collections. Whole otolith-based age estimates were completed for 153 out of 196 specimens from 2006 collections and 318 out of 335 specimens from 2007 collections.

Scale-based Age Determination

Scales were cleaned with a dilute bleach solution, pressed on acetate sheets, and read on a microfilm projector following the methods of Cating (1953). The criteria established by Cating (1953) for aging American shad with scales specify that the first annulus is located within the first 4-7 transverse grooves (predominantly at transverse groove number five or six), the second annulus is located between the 8th and 11th transverse grooves (predominantly at transverse groove number nine 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. Cating (1953) described the fresh-water zone (FWZ) of American shad scales as “an important false annulus (...) [that] forms at the time of transition from fresh water in the parent river to salt water in the ocean when the shad are from 3-5 months old”, and established that this zone is located within the first 1-5 transverse grooves (predominantly at transverse groove number two or three). Cating (1953) also gave criteria for determining false annuli from true annuli. False annuli are said to “not show up as clearly as do annuli, and are not usually found circling into the posterior portion of the scales”.

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The criteria established by Cating (1953) for identifying the FWZ, first, second, and third annuli, and false annuli were followed as strictly as possible. 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 one year at the edge of the scale to the total number of annuli counted on the scale to account for growth since the last annulus was laid down (Figure 2). Notes and comments regarding age assignments were recorded by each reader.

**Whole Otolith-based Age Determination**

Whole otoliths were placed in watch glasses, submerged in water, and allowed to sit for 2-3 minutes before being read under a dissecting microscope with transmitted light. An annulus was counted as one pair of opaque (dark) and hyaline (light) bands. The final age of the fish was estimated by adding one year at the edge of the otolith to the total number of annuli counted in the otolith to account for growth since the last annulus was accreted. Notes and comments regarding age assignments were recorded by each reader.

**Method Comparison**

Isotope signature-based, scale-based, and whole otolith-based methods of age determination were compared in two ways: 1) percent contribution of the 2002 year class to the spawning migration in 2006 and 2007 estimated by each method; and 2) percent
agreement on age between isotope signature-based and scale-based and isotope signature-based and whole otolith-based methods for individuals identified as members of the 2002 year class by isotope signatures. Statistics for 2006 and 2007 were calculated separately, and statistics for scale-based and whole otolith-based age estimates were calculated for each reader. Specimens that did not have scale or otolith samples or had scales or otoliths that were determined to be unusable were not included in the calculations. It should be noted, however, that even though there were no age data from scale-based or whole otolith-based methods for these specimens, isotope signatures were able to provide age estimates for some of these specimens.

Two-way chi square tests of symmetry (Hoenig et al. 1995) were used to evaluate systematic biases exhibited by the scale-based and whole otolith-based methods compared to the isotope signature-based method.

**Evaluation of the Scale-based Age Determination Method**

**Scale Morphology of the 2002 Year Class**

Transverse groove and annuli counts were tabulated for fish identified by isotope signatures as members of the 2002 year class ($n = 3$ in 2006, $n = 17$ in 2007). The descriptions of transverse grooves and annuli given by Cating (1953) were used to identify these morphological features on the scales. Cating (1953) described annuli as "lines seen on the surface of the scale following the contour of the periphery through both the anterior and posterior portions", and transverse grooves as "distinct grooves in the surface of the anterior, sculptured portion, crossing it [the scale] laterally". Scales were not evaluated if they were determined to be unusable by one or both of the readers
or if there was disagreement between readers on the age of the fish. Transverse grooves and annuli were not tabulated for scales that had a false annulus \( (n = 4 \text{ in } 2006, n = 7 \text{ in } 2007) \). The FWZ on American shad scales was assumed to be a false annulus, as described by Cating (1953); thus, the first visible annulus-like mark on the scale was identified as the FWZ and was not counted as the first annulus. All marks that were interpreted as the FWZ or annuli were counted and their location was recorded by transverse groove number. If an annulus was located between two transverse grooves it was recorded as being located at the higher numbered transverse groove (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).

**Methodological Sources of Agreement and Disagreement of Age Estimates**

Methodological sources of agreement and disagreement between isotope signature-based and scale-based age estimates were evaluated. Specimens were divided into three categories for evaluation: 1) specimens that had agreement or disagreement on the designation of an annulus as false or true between scale-based and isotope signature-based methods \( (n = 6 \text{ in } 2006, n = 16 \text{ in } 2007) \); 2) specimens that had agreement on age between scale-based and isotope signature-based methods \( (n = 3 \text{ in } 2006, n = 8 \text{ in } 2007) \); and 3) specimens that had disagreement on age between scale-based and isotope signature-based methods \( (n = 23 \text{ in } 2006, n = 37 \text{ in } 2007) \). Included in the third category were specimens that were identified by isotope signatures as members of the 2002 year class but were not aged as such by the scale-based method \( (n = 0 \text{ in } 2006, n = 7 \text{ in } 2007) \) and specimens that were aged as members of the 2002 year class by the scale-based method but were not identified as such by isotope signatures \( (n = 23 \text{ in } 2006 \text{ and } n = 30 \text{ in } 2007) \).
in 2007). Transverse groove and annuli counts were tabulated following the methods described above for fish included in categories two and three. The transverse grooves and annuli of scales in category one were not tabulated. Scales were not evaluated if they were determined to be unusable by one or both of the readers or if there was disagreement between readers on the age of the fish. In total, 32 scales were evaluated from 2006 collections and 61 scales were evaluated from 2007 collections.

_Evaluation of the Whole Otolith-based Age Determination Method_

There was no systematic method of evaluating whole otolith-based age estimates since there were no established criteria or landmarks to follow for the method. Age estimates made with the whole otolith-based method were evaluated based on comments recorded by the readers (see discussion).
RESULTS

Isotope Signatures of Juvenile American Shad

δ\textsuperscript{18}O signatures of juveniles collected in 2002 were enriched (more positive) and distinct from δ\textsuperscript{18}O signatures of juveniles collected in 2000-2001 (Walther et al. 2008) and 2003-2004 (Table 1, Figures 3 and 4). Ranges of δ\textsuperscript{18}O signatures of Mattaponi River juveniles collected in 2002 were -6.03 to -5.32\% and ranges of δ\textsuperscript{18}O signatures of Pamunkey River juveniles collected in 2002 were -5.91 to -4.66\% (Table 1). Pamunkey and Mattaponi River juveniles of the 2000 and 2001 year classes were indistinguishable (Walther et al. 2008) as were juveniles collected in the Pamunkey River in 2003 and the Mattaponi River in 2004 (ranges reported in Table 1, Figures 3 and 4). However, δ\textsuperscript{18}O signatures of Mattaponi River juveniles of the 2003 year class were depleted (more negative) and distinct from δ\textsuperscript{18}O signatures of juveniles in all other year classes (Table 1, Figures 3 and 4). The range of δ\textsuperscript{18}O signatures of Mattaponi River juveniles collected in 2003 was -8.35 to -7.53\%. All year classes had similar δ\textsuperscript{13}C signatures (range, -19.44 to -15.16\%; Table 1, Figures 3 and 4). The mean δ\textsuperscript{18}O and δ\textsuperscript{13}C signatures for all juveniles collected in the Mattaponi and Pamunkey Rivers in 2000-2004 (n = 270) were -6.88\% (SD = 0.86) and -17.41\% (SD = 0.90), respectively.

ANOVA tests indicated a significant interaction between year and river (δ\textsuperscript{18}O: F = 9.73, df = 3, p<0.05; δ\textsuperscript{13}C: F = 15.67, df = 3, p<0.05). Year and river had significant affects on δ\textsuperscript{18}O (year: F = 1677.39, df = 4, p<0.05; river: F = 407.81, df = 1, p < 0.05) and
δ¹³C (year: F = 18.10, df = 4, p < 0.05; river: F = 46.48, df = 1, p < 0.05) signatures, but the significance of the interaction between the two factors made interpretation of the individual affects of year and river difficult.

Isotope signatures of Adult American Shad

The range of δ¹⁸O and δ¹³C signatures of adults collected in the York River in 2006 and 2007 was large (Figure 5). δ¹⁸O signatures of adults collected in 2006 ranged from -11.86 to -0.22‰, and their δ¹³C signatures ranged from -17.70 to -3.42‰. δ¹⁸O signatures of adults collected in 2007 ranged from -11.84 to -1.36‰, and their δ¹³C signatures ranged from -18.48 to -1.58‰. The mean δ¹⁸O signature for all adults analyzed in 2006 (n = 190) was -5.66‰ (SD = 1.18), and for all adults analyzed in 2007 (n = 306) it was -5.74‰ (SD = 1.32). The mean δ¹³C signature for all adults collected in 2006 was -14.39‰ (SD = 1.97), and for all adults collected in 2007 it was -15.39‰ (SD = 1.97). These δ¹⁸O and δ¹³C signatures were enriched relative to those of juveniles.

Material from the outer portion of the otolith, which was presumably accreted in the marine environment, had enriched mean δ¹⁸O and δ¹³C signatures compared to those of otolith cores (Figure 5a). The mean δ¹⁸O signature of marine-derived material (n = 10) was -0.15‰ (SD = 0.83), and the mean δ¹³C signature was -3.91‰ (SD = 0.95). The range of δ¹⁸O signatures for this material was -5.21 to -2.63‰, and the range of δ¹³C signatures was -1.25 to 0.73‰.

Distinct groupings of adults based on δ¹⁸O and δ¹³C signatures were visible in 2006 and 2007 collections (Figure 5). However, these groupings were scattered in a direction towards the δ¹⁸O and δ¹³C signature of marine-derived material. Sequential
milling of adult otolith cores in increments of 75 μm showed that shallower mill depths (0-75 μm) resulted in enriched δ¹⁸O and δ¹³C values compared to deeper mill depths (75-150 μm and 150-225 μm). Mill depths of 75-150 and 150-225 μm resulted in depleted δ¹⁸O and δ¹³C values relative to mill depths of 0-75 μm (Figure 6). δ¹⁸O and δ¹³C signatures for different mill depths of the same otolith plotted linearly in the direction of the δ¹⁸O and δ¹³C signature of marine-derived material. Variability in the δ¹⁸O and δ¹³C values of samples milled at the same depth (0-75, 75-150, or 150-225 μm) was likely due to variability in the initial thickness of the otolith prior to milling. These results suggest that variation in mill depth caused the scatter and shifting of adult δ¹⁸O and δ¹³C signatures in the direction of the δ¹⁸O and δ¹³C signature of marine-derived material. Shallower mill depths likely incorporated a larger proportion of material into the samples that was accreted onto the otolith after the fish left the freshwater nursery regions and migrated into higher salinity waters. Inclusion of this material likely caused the δ¹⁸O and δ¹³C values of adult otolith cores to shift towards δ¹⁸O and δ¹³C values characteristic of marine waters. Samples taken at greater depths in the otolith core likely included less of this material and the δ¹⁸O and δ¹³C values of these samples were more characteristic of the freshwater, juvenile signature in the adult otolith.

Identification of the 2002 Year Class of Adult American Shad

The δ¹⁸O and δ¹³C signatures of some adults did not match those of juveniles because they had enriched δ¹⁸O and δ¹³C values (Figure 7). Adult δ¹⁸O and δ¹³C signatures were scattered between the freshwater, juvenile signatures and the marine signature depending on the proportion of marine-derived material incorporated into each

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sample. Despite the mismatch in isotope signatures of some adult samples, members of the 2002 year class were identifiable in collections of adults based on the distinct $\delta^{18}O$ signature of their otolith cores. In 2006, 12 individuals separated out from the rest of the adults and had $\delta^{18}O$ signatures in agreement with those of the 2002 juvenile year class (Figure 7a). Using these data, we estimated that 6% of the specimens analyzed in 2006 were age-4 fish (Table 2). In 2007, a group of 57 individuals separated out from the rest of the adults and had $\delta^{18}O$ signatures in agreement with those of the 2002 juvenile year class (Figure 7b). An additional four adults had enriched signatures, but were more difficult to identify as members of the 2002 year class since they exhibited greater shifts in the direction of the marine signature (Figure 7b). Thus, we estimated that either 57 or 61 adults in 2007 were members of the 2002 year class, and between 19 and 20% of the specimens analyzed in 2007 were age-5 fish (Table 2). The four adult specimens that could not be confidently identified as members of the 2002 year class were not used in subsequent analyses.

Individuals identified as members of the 2002 year class by isotope signatures in 2006 and 2007 conformed to expected growth patterns for age-4 and age-5 American shad in the York River. Fork lengths and weights of individuals identified by isotope signatures as age-4 fish in 2006 varied from 384-424 mm and 1002.6-1238.3 g, respectively (Figures 8 and 9). Fork lengths and weights of individuals identified by isotope signatures as age-5 fish in 2007 varied from 398-450 mm and 978.1-1712.1 g (Figures 8 and 9).
Method Comparison

There was disagreement on the percent contribution of the 2002 year class to the spawning migrations in 2006 and 2007 between isotope signature-based, scale-based, and whole otolith-based age determination methods. Isotope signatures estimated a lower percent contribution of the 2002 year class to the spawning migration in 2006 and 2007 than either scale-based or whole otolith-based age determination methods (Table 2). The whole otolith-based method estimated a lower percent contribution of the 2002 year class to the spawning migration in 2006 and 2007 compared to the scale-based method (Table 2). For those individuals identified as members of the 2002 year class by isotope signatures, agreement on age between isotope signature-based and scale-based age estimates and isotope signature-based and whole otolith-based age estimates was low. Agreement to within ±1 year was high for both isotope signature-based and scale-based age estimates and isotope signature-based and whole otolith-based age estimates (Tables 3 and 4). In 2006, scale and whole otolith-based age determination methods did not exhibit any systematic bias (were symmetrical) compared to the isotope signature-based method (test statistics for chi square tests of symmetry are reported in Tables 3 and 4, Figure 10). However, in 2007 both methods systematically underestimated age (were asymmetrical) compared to the isotope signature-based method (test statistics for chi square tests of symmetry are reported in Tables 3 and 4, Figure 10). The degrees of freedom of the chi square tests of symmetry were low (df = 1-4) since there was only one age estimated each year by the isotope signature-based method; however, age distributions for each method show the trends described above (Figure 10).
Evaluation of the Scale-based Age Determination Method

Scale Morphology of the 2002 Year Class

Transverse groove and annuli counts for individuals identified by isotope signatures as age-4 in 2006 agreed with the criteria established by Cating (1953) for locating the FWZ and the first, second, and third annuli. The only exception was one specimen that had the third annulus located outside the range of transverse grooves specified for that annulus by Cating (1953) (Table 5).

Transverse groove and annuli counts for individuals identified by isotope signatures as age-5 in 2007 agreed with the criteria established by Cating (1953) for locating the FWZ and the first and fourth annuli. The only exceptions were three specimens that had the fourth annulus located outside the range of transverse grooves specified for that annulus by Cating (1953) (Table 5). A substantial proportion of the individuals identified by isotope signatures as age-5 in 2007 had transverse groove and annuli counts that did not agree with the Cating (1953) criteria for locating the second and third annuli. For these specimens, the second and third annuli were located at transverse groove numbers lower than those specified for the annuli by Cating (1953) (Table 5).

Methodological Sources of Agreement of Age Estimates

Transverse Groove and Annuli Counts

For the majority of specimens identified as age-4 in 2006 and age-5 in 2007 by both isotope signature-based and scale-based methods, transverse groove and annuli counts agreed with the criteria established by Cating (1953) (Table 6 and see Figure 2). There were some exceptions, however, as a small proportion of specimens had annuli
located at transverse groove numbers lower than those specified for the annulus by Cating (1953). This applied to the second annulus of two specimens from 2007 collections, the third annulus of one specimen from 2006 collections and four specimens from 2007 collections, and the fourth annulus of one specimen from 2007 collections (Table 6).

*Designation of False and True Annuli*

For two specimens identified as age-4 in 2006 by both isotope signature-based and scale-based methods and three specimens identified as age-5 in 2007 by both methods, readers determined an annulus to be false and that designation was supported by the isotope signature-based method (e.g. Figure 11).

*Methodological Sources of Disagreement of Age Estimates*

*Transverse Groove and Annuli Counts*

For the majority of specimens that had disagreement between isotope signature-based and scale-based age estimates, transverse groove and annuli counts did not agree with the criteria established by Cating (1953) for locating the second, third, and fourth annuli. The second, third, and fourth annuli were located at transverse groove numbers lower than those specified for the annuli by Cating (1953) (e.g. Figure 12). There were exceptions: the second annulus of four fish in 2006 collections and nine fish in 2007 collections was located within the range of transverse grooves specified for the annulus by Cating (1953); the third annulus of two fish in 2007 collections was located within the range of transverse grooves specified for the annulus by Cating (1953); and the fourth annulus of 11 fish in 2006 collections and 22 fish in 2007 collections was located within the range of transverse grooves specified for the annulus by Cating (1953) (Table 7).
For the majority of specimens that had disagreement on age between methods, transverse groove and annuli counts agreed with the criteria established by Cating (1953) for locating the FWZ and the first and fifth annuli. Only seven specimens in 2006 and two specimens in 2007 had the first annulus located outside the range of transverse grooves specified for the annulus by Cating (1953) (Table 7).

Designation of False and True Annuli

For two specimens in 2006 collections that had disagreement between isotope signature-based and scale-based age estimates, readers determined an annulus to be true but the isotope signature-based method indicated that the annulus was false. For two specimens in 2006 collections and 13 specimens in 2007 collections, readers determined an annulus to be false but the isotope signature-based method indicated that the annulus was true and should be counted (e.g. Figure 13).
DISCUSSION

The 2002 year class of American shad could be identified in collections of adults returning to spawn in the York River in 2006 and 2007 using the $\delta^{18}O$ signature in the core of their otoliths. This is the first such use of a natural geochemical signature to identify a cohort of an anadromous species and track its recruitment and relative abundance over time. The 2002 year class was predicted to be a weak year class by juvenile abundance indices (JAI) generated from seine surveys in the York River system (Figure 14). Adults from this year class were expected to have minor contributions to subsequent spawning runs. The results of this study supported this expected recruitment pattern as only a small percent of adults that returned to spawn in 2006 (6%) and 2007 (19-20%) had isotope signatures identifying them as members of the 2002 year class. Error in sample processing associated with milling depths of otolith cores appeared to be the main factor affecting precision of the isotope signature-based age determination method. As a result, four specimens in 2007 could not be confidently placed in the 2002 year class due to the presence of a strong marine-derived signal in otolith core samples (see below). Regardless, the results tend to support the usefulness of the JAI seine survey to identify years of recruitment failure of American shad in the York River (Wilhite et al. 2003; ASMFC 2007a).

The $\delta^{18}O$ and $\delta^{13}C$ signatures of most adult otolith cores were enriched compared to those of juvenile otolith cores, and varied with the depth at which the samples were
milled. Shallower mill depths resulted in $\delta^{18}O$ and $\delta^{13}C$ values in adult otolith cores that were enriched compared to those of juveniles, and greater mill depths resulted in depleted $\delta^{18}O$ and $\delta^{13}C$ values that were more characteristic of juvenile signatures. As a result, incorporation of material that was accreted onto the otolith after the fish left the freshwater nursery regions and migrated into higher salinity waters caused signatures in otolith cores of adult American shad to be shifted towards $\delta^{18}O$ and $\delta^{13}C$ values characteristic of marine waters.

Oxygen and carbon isotope ratios in otoliths have been shown to have a positive relationship with salinity (Elsdon and Gillanders 2002), and $\delta^{18}O$ and $\delta^{13}C$ are known to be depleted in freshwater compared to seawater (Epstein and Mayeda 1953; Hoefs 1980; Tan et al. 1983; Garvey 1990; Sharp 2007). Patterns of variable $\delta^{18}O$ and $\delta^{13}C$ signatures in otoliths similar to those observed in this study have been reported by Elsdon and Gillanders (2002) and Thorrold et al. (1998a). In a laboratory study, Elsdon and Gillanders (2002) found that $\delta^{18}O$ and $\delta^{13}C$ values in juvenile black bream ($Acanthopagrus butcheri$) otoliths became more enriched with increasing salinity. Thorrold et al. (1998a) reported a linear relationship between $\delta^{18}O$ and $\delta^{13}C$ values in otoliths of juvenile weakfish ($Cynoscion regalis$) collected in Delaware Bay, and attributed the linearly increasing $\delta^{18}O$ and $\delta^{13}C$ values to inclusion of fish that had resided in different areas along the estuarine gradient from lower estuary (salinity approximately 30‰) to the tidal reaches of the Delaware River (salinity < 10‰). Juvenile weakfish with depleted isotope ratios were from locations where the fish had been exposed to significant amounts of freshwater, and juveniles with enriched isotope ratios were assumed to have come from higher salinity waters. These studies, along with the known
relationship of oxygen and carbon isotope ratios and salinity, support the conclusion that incorporation into the samples of material that was accreted onto the otolith after the fish left the freshwater nursery regions and migrated into higher salinity waters caused signatures of adult American shad to be shifted towards δ¹⁸O and δ¹³C values characteristic of marine waters. However, the nature of carbon incorporation into the otolith adds complexity to the interpretation of the enriched adult signatures. Carbon is incorporated into otoliths from both dissolved inorganic carbon in the ambient water and from metabolic sources of carbon (Kalish 1991; Thorrold et al. 1997). Otolith δ¹³C signatures could therefore reflect either variations in the δ¹³C of the environment or differences in incorporation of metabolic carbon. The relative contributions of the two sources to otolith composition are not well understood, and it is possible that metabolic effects on δ¹³C contributed to the enriched isotope signatures of adults compared to juveniles.

Temporal variability in geochemical signatures does not always produce a signature that is sufficiently distinct to serve as a marker for a cohort. The δ¹⁸O signature in the otolith cores of juvenile American shad of the 2003 year class did not allow for identification of that year class at later stages. The contrasting δ¹⁸O signatures in otoliths of juvenile American shad spawned in 2002 and 2003 were likely caused by differences in source or amount of precipitation. The Mattaponi and Pamunkey Rivers experienced drought conditions in 2002, with below average flows, while above average flows characterized 2003 (USGS 2005). Although the exact causes of these isotopic patterns are unknown, the data suggest that river flow might index the potential for natural, cohort-specific markers in American shad and other anadromous fishes. It has been suggested
that anomalous or prolonged environmental conditions, such as El Nino conditions, storm
events, or periods of above or below average precipitation or temperature, are optimal for
creating significant differences in geochemical signatures of cohorts (Cole et al. 1999;
recommended that libraries of juvenile geochemical signatures be collected over time for
accurate classifications of natal origins of adults. Geochemical analyses of otoliths can be
time consuming and costly, but information on 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.

Cating’s (1953) method was not suitable for aging scales of all age-4 and age-5
American shad in the York River in 2006 and 2007. This conclusion was reached because
of the lack of concordance between isotope signature-based and scale-based age
estimates. Only 64% (average: reader 1 = 78%; reader 2 = 50%) of the specimens
identified as age-4 in 2006 by isotope signatures were aged as age-4 fish by scale readers.
Agreement on age between the methods was lower for age-5 fish. Only 46% (average:
reader 1 = 39%; reader 2 = 53%) of the specimens identified as age-5 in 2007 by isotope
signatures were aged as age-5 fish by scale readers. An asymmetrical bias was evident for
age-5 fish; age was underestimated in these specimens, predominantly by one year.
McBride et al. (2005) also found that age estimates for American shad in the Delaware
River system were inaccurate and that the age of age-5 fish tended to be underestimated
by one year. Accuracies of age estimates in this study were slightly higher than those
reported by McBride et al. (2005) for age-4 and age-5 American shad from the Delaware
River system, but were much lower than those reported by Judy (1961) for age-4 and age-
5 fish from the Connecticut River. It should be noted, however, that Judy (1961) did not describe a protocol that McBride et al. (2005) considered a blind trial.

In their test of the validity of Cating’s (1953) method, McBride et al. (2005) suggested that process and observational error were the underlying causes of inaccurate age estimates. Process error occurs when a scale lacks necessary landmarks, such as when an annulus is absent or cannot be distinguished. Observational error occurs when readers interpret the landmarks on a scale incorrectly. Both process error and observational error were evident in this study; however, observational error appeared to be the main factor contributing to disagreement between isotope signature-based and scale-based methods.

For the majority of specimens that had disagreement on age between isotope signature-based and scale-based methods, transverse groove and annuli counts did not follow the criteria established by Cating (1953). This led to observational error as following the Cating (1953) method caused readers to misinterpret features of the scale. In the majority of these specimens there were two visible annuli located within the 1-5 or 4-7 transverse groove ranges. Readers often did not count the first visible annulus on the scale because it was located at transverse groove number four or five and counting this mark as the first annulus would mean the second annulus was located at transverse groove number six or seven, a location outside the range of transverse grooves specified for the second annulus (e.g. see Figure 12). In addition, sometimes the first annulus was faint and hard to see or was difficult to distinguish from the FWZ. As a result, the first annulus was often either skipped or considered to be the FWZ. The FWZ was also often hard to distinguish. Hammer (1942) reported that 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. Cating (1953) does not provide any guidance for situations when multiple annuli are located within the same range of transverse grooves. Not counting the first annulus resulted in scale-based age estimates that underestimated age compared to isotope signature-based age estimates. As an alternative to following the Cating (1953) criteria, identification of the FWZ and simple counts of the visible annuli, without relying on transverse groove counts, could result in agreement on age between the methods (e.g. see Figure 12).

Scale-based age determination of other *Alosa* species such as allis shad (*Alosa alosa*), twaite shad (*Alosa fallax 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. The Cating (1953) method has been applied to hickory shad (*Alosa mediocris*) (Street and Adams 1969; Street 1970; Williams et al. 1975; Harris et al. 2007). Marcy (1969) recorded transverse groove and annuli counts for alewife and blueback herring, but indicated that 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 alewife from different geographical areas. This concern was indicated by McBride et al. (2005) when they stated that “it is possible that natural variability in the formation of transverse grooves, annuli, and spawning marks confounds the application of Cating’s [1953] method to other river systems”. 

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The FWZ of anadromous fish scales was first identified by Snyder (1931) on the scales of salmon, and it was described for American shad scales by Hammer (1942). Scale-based age determination methods for allis shad and twaite shad do not identify a FWZ, and count what we have identified in this study as the FWZ as the first annulus (A. Lochet, personal communication). Marcy (1969) identified a FWZ in alewife 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. Marcy (1969) also suggested that certain stocks may not have a FWZ on their scales if juvenile residence time in freshwater was extremely short. It is likely that stocks of American shad vary in the size and even presence/absence of the FWZ due to differences in the amount of time that juveniles reside in freshwater nursery areas. Application of the transverse groove counts specified for this zone by Cating (1953) may not be appropriate for all stocks, and identification of the FWZ on the scales of certain stocks may be more difficult than others (Hammer 1942). Elemental analysis of scales has been used to infer past life histories of fishes, including marine and freshwater migrations, and would allow for investigation of the FWZ, as well as spawning marks, on American shad scales (Coutant and Chen 1993; Wells et al. 2000; 2003; Courtemanche et al. 2005).

Cating (1953) commented that the growth of transverse grooves seemed to be a function of age since the distance between grooves becomes proportionately less as the fish ages, but nothing has been published in the time since on the growth and deposition of transverse grooves. Further information on how these features grow and change with age is needed in order to determine how they relate to annuli deposition. A better
understanding of overall scale growth, especially in younger fish, is needed as well. A thorough investigation of the stock-specific, age-specific relationship of scale size to body size would aid scale readers in the identification of the FWZ and first and second annuli, features of scales we reported in this study as being difficult to identify. Leim (1925) and LaPointe (1958) conducted studies of the relationship of scale length to fish length for American shad. The results they reported could be used to compare annulus size to fish size in order to more clearly identify the FWZ, and first and second annuli, but modern, stock-specific relationships should be developed.

Designation of an annulus as false or true also contributed to disagreement on age between isotope signature-based and scale-based methods in this study. Readers often determined an annulus to be false but this determination was not supported by isotope signature-based age determinations. Several specimens, especially those that were identified by isotope signatures to be age-5 in 2007, had spawning marks on their scales that made annuli at the edge of the scale difficult to see. As a result, readers often failed to see annuli hidden by spawning marks or determined evidence of a hidden annulus to be false (e.g. Figure 13). Factors that distinguished a true annulus from a false annulus were not identified in this study, and the description of false annuli given by Cating (1953) did not always apply. There were numerous cases in which similar annuli on different scales were indistinguishable, even though one was supported as true by the isotope signature-based method and one was supported as false. Designation of an annulus as false or true was open to interpretation by the reader and considered as a source of observational error.
Whole otoliths were not a suitable structure for determining the age of age-4 and age-5 American shad from the York River. Only 39% (average: reader 1 = 56%, reader 2 = 22% in 2006; reader 1 = 47%, reader 2 = 31% in 2007) of the specimens identified as age-4 or age-5 in 2006 and 2007 by isotope signatures were aged as age-4 or age-5 by otolith readers. The low agreement between methods could reflect reader experience or the method of whole otolith-based age determination used. For both readers, this was the first attempt at reading American shad whole otoliths. Training was obtained before age determination commenced, however, the readers still had low confidence in their age assignments. Different agencies use different methods to read American shad whole otoliths. For example, the Pennsylvania Fish and Boat Commission read whole otoliths under emersion oil using reflected light. This method highlights the hyaline (light) bands of annuli rather than the opaque (dark) bands. The method used in this study highlighted the opaque (dark) bands of annuli. The major issues that readers in this study identified as contributing to difficulty in aging American shad with whole otoliths were: 1) variability in how well annuli in the otoliths could be seen due to variability in the amount of time otoliths were submerged in water; 2) a lack of clear distinctions between annuli; and 3) difficulty in seeing annuli close to the edge of the otolith.

In 1953, Mansuetti and Kolb commented that “great difficulty has been experienced by biologists in aging shad.” Since that time, shad biologists have not made significant advances in the field. Our study describes a new method to identify a cohort of American shad spawned during anomalous environmental conditions, but our results only amplify Mansuetti and Kolb’s (1953) lament, they do not solve it. Our data demonstrate that natural isotope signatures do not serve as a marker for most year classes of American
shad. Thus, we cannot propose the isotope-signature method as a panacea for the age
determination problem in this species (ASMFC 2007a, b). The relative objectivity that
natural geochemical signatures provide to cohort identification compared to the process
and observational errors associated with scale-based methods serves to emphasize that
other age determination approaches are required. Natural, cohort-specific geochemical
signatures are an obvious avenue to pursue. In the meantime, scientists should continue to
use caution when applying scale-based aging techniques in assessments of American
shad stocks.
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Assessment Report No. 07-01 of the Atlantic States Marine Fisheries

Commission.


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Table 1. $\delta^{18}$O and $\delta^{13}$C signatures (mean ± SD) of otolith cores of juvenile American shad collected during the summers of 2000-2004 in the freshwater nursery regions of the Mattaponi and Pamunkey Rivers (two tributaries that join to form the York River). Juveniles were not collected in the Pamunkey River in 2004.

<table>
<thead>
<tr>
<th>Year</th>
<th>River</th>
<th>$\delta^{18}$O (%)</th>
<th>$\delta^{18}$O range (%)</th>
<th>$\delta^{13}$C (%)</th>
<th>$\delta^{13}$C range (%)</th>
<th>n</th>
<th>Data Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Matt</td>
<td>-7.01 ± 0.16</td>
<td>-7.48, -6.73</td>
<td>-17.51 ± 0.74</td>
<td>-19.44, -16.42</td>
<td>27</td>
<td>Walther et al. 2008</td>
</tr>
<tr>
<td>2000</td>
<td>Pam</td>
<td>-6.60 ± 0.19</td>
<td>-7.07, -6.34</td>
<td>-16.72 ± 0.35</td>
<td>-17.78, -16.24</td>
<td>18</td>
<td>Walther et al. 2008</td>
</tr>
<tr>
<td>2001</td>
<td>Matt</td>
<td>-6.65 ± 0.13</td>
<td>-6.89, -6.38</td>
<td>-17.94 ± 0.64</td>
<td>-19.00, -16.52</td>
<td>28</td>
<td>Walther et al. 2008</td>
</tr>
<tr>
<td>2001</td>
<td>Pam</td>
<td>-6.33 ± 0.16</td>
<td>-6.63, -6.02</td>
<td>-16.68 ± 0.46</td>
<td>-17.53, -15.77</td>
<td>29</td>
<td>Walther et al. 2008</td>
</tr>
<tr>
<td>2002</td>
<td>Matt</td>
<td>-5.58 ± 0.18</td>
<td>-6.03, -5.32</td>
<td>-18.60 ± 0.43</td>
<td>-19.06, -17.09</td>
<td>24</td>
<td>Walther et al. 2008</td>
</tr>
<tr>
<td>2002</td>
<td>Pam</td>
<td>-5.13 ± 0.27</td>
<td>-5.91, -4.66</td>
<td>-17.57 ± 0.62</td>
<td>-18.61, -16.47</td>
<td>19</td>
<td>Walther et al. 2008</td>
</tr>
<tr>
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<td>Matt</td>
<td>-8.15 ± 0.16</td>
<td>-8.35, -7.53</td>
<td>-16.76 ± 0.75</td>
<td>-18.05, -15.16</td>
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<td>This study</td>
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<td>-7.73, -7.38</td>
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<td>-18.12, -16.12</td>
<td>28</td>
<td>This study</td>
</tr>
<tr>
<td>2004</td>
<td>Matt</td>
<td>-7.24 ± 0.12</td>
<td>-7.52, -6.99</td>
<td>-17.69 ± 1.00</td>
<td>-19.26, -15.61</td>
<td>59</td>
<td>This study</td>
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Table 2. Number of individuals of the 2002 year class and their percent contribution to the spawning migration in the York River in 2006 and 2007 as estimated by isotope signature-based, scale-based, and whole otolith-based age determination methods. n/a = not applicable.

<table>
<thead>
<tr>
<th>Year</th>
<th>Method</th>
<th>Reader</th>
<th># of individuals of 2002 year class</th>
<th>Sample size (n)</th>
<th>% Contribution of 2002 year class to the Spawning Migration</th>
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<td>Isotope Signatures</td>
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<tr>
<td></td>
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<td>52</td>
<td>163</td>
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<td>47</td>
<td>163</td>
<td>29</td>
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<td></td>
<td>Whole otolith-based</td>
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<td>21</td>
<td>153</td>
<td>14</td>
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<tr>
<td></td>
<td></td>
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<td>153</td>
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<td>Whole otolith-based</td>
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<td>2</td>
<td>109</td>
<td>318</td>
<td>34</td>
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Table 3. Agreement between isotope signature-based and scale-based age estimates for adult American shad collected in 2006 and 2007 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. Also reported are test statistics for chi square tests of symmetry between methods. NS = no scale sample, UN = unusable scales, df = degrees of freedom.

<table>
<thead>
<tr>
<th>Year</th>
<th>Reader</th>
<th>n</th>
<th>NS</th>
<th>UN</th>
<th>% Agreement</th>
<th>% Agreement ±1 year</th>
<th>X²</th>
<th>df</th>
<th>p</th>
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<tr>
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<td>12</td>
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<td>78 (n = 9)</td>
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<tr>
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<td>2</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>50 (n = 10)</td>
<td>100 (n = 10)</td>
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<td>3</td>
<td>0.17</td>
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<tr>
<td>2007</td>
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<td>57</td>
<td>2</td>
<td>6</td>
<td>39 (n = 49)</td>
<td>96 (n = 49)</td>
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<td>4</td>
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<td></td>
<td>2</td>
<td>57</td>
<td>2</td>
<td>12</td>
<td>53 (n = 43)</td>
<td>98 (n = 43)</td>
<td>20</td>
<td>3</td>
<td>&lt;0.05</td>
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</table>
Table 4. Agreement between isotope signature-based and whole otolith-based age estimates for adult American shad collected in 2006 and 2007 during spawning migrations in the York River. Only specimens that were identified by isotope signatures as members of the 2002 year class were included in the analysis. Also reported are test statistics for chi square tests of symmetry between methods. NS = no whole otolith sample, UN = unusable otoliths, df = degrees of freedom.

<table>
<thead>
<tr>
<th>Year</th>
<th>Reader</th>
<th>n</th>
<th>NS</th>
<th>UN</th>
<th>% Agreement</th>
<th>% Agreement ±1 year</th>
<th>X^2</th>
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<td>1</td>
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<td>85 (n = 55)</td>
<td>40</td>
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<td>&lt;0.05</td>
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Table 5. Location of the freshwater zone (FWZ) and annuli 1-4 on scales of American shad identified by isotope signatures as age-4 in 2006 and age-5 in 2007. Bold type indicates the number of specimens that had the FWZ or an annulus located outside the range of transverse grooves specified for these features by Cating (1953).

<table>
<thead>
<tr>
<th>Year</th>
<th>Age</th>
<th>n</th>
<th>FWZ/Annulus</th>
<th>Transverse Groove Number</th>
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<td>2006</td>
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<td>3</td>
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<td>17</td>
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<td>6</td>
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</tr>
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</table>
Table 6. Location of the freshwater zone (FWZ) and annuli 1-4 on scales of American shad identified by isotope signature-based and scale-based methods as age-4 in 2006 and age-5 in 2007. Bold type indicates the number of specimens that had the FWZ or an annulus located outside the range of transverse grooves specified for these features by Cating (1953).

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>FWZ/Annulus</th>
<th>Transverse Groove Number</th>
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<td>4</td>
<td>1 1 1 3 2</td>
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</table>
Table 7. Location of the freshwater zone (FWZ) and annuli 1-4 on scales of American shad that had disagreement on age between isotope signature-based and scale-based methods. Bold type indicates the number of specimens that had the FWZ or an annulus located outside the range of transverse grooves specified for these features by Cating (1953).

<table>
<thead>
<tr>
<th>Year</th>
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<th>Transverse Groove Number</th>
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<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16</td>
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<td>5 9 9 6 1</td>
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</tbody>
</table>
FIGURE CAPTIONS

Figure 1. The York River system, indicating its location within the Chesapeake Bay and the location of the staked gill net used to collect adult American shad during spawning migrations in 2006 and 2007. Juvenile American shad were collected in the Mattaponi and Pamunkey Rivers in 2000-2004.

Figure 2. Scale of an American shad collected in 2006 during the spawning migration in the York River. This specimen was identified by isotope signatures and scale readers as an age-4 fish in 2006. 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.

Figure 3. $\delta^{18}O$ signatures (mean ± SD) of otolith cores of juvenile American shad collected during the summers of 2000-2004 in the freshwater nursery regions of the Mattaponi (open symbols) and Pamunkey (filled symbols) Rivers (two tributaries that join to form the York River). Data for 2000-2002 are from Walther et al. (2008).

Figure 4. $\delta^{18}O$ and $\delta^{13}C$ signatures of otolith cores of juvenile American shad collected in the freshwater nursery regions of the Mattaponi (open symbols) and Pamunkey (filled symbols) Rivers (two tributaries that join to form the York River) during the summers of

Figure 5. $\delta^{18}$O and $\delta^{13}$C signatures of otolith cores of adult American shad (open circles) collected during spawning migrations in the York River in 2006 (a, $n = 190$) and 2007 (b, $n = 306$). Panel a also shows $\delta^{18}$O and $\delta^{13}$C signatures of material accreted onto the outer portion of 10 otoliths analyzed in 2006 (filled circles). Material on the outer portion of the otolith was presumably accreted during the fish’s time in the marine environment. Symbols represent individual fish.

Figure 6. $\delta^{18}$O and $\delta^{13}$C values for different mill depths of five adult American shad otolith cores collected during spawning migrations in the York River in 2007. One specimen is depicted by each set of symbols (black, gray with crosshair, open with crosshair, open, and gray). The first mill for each specimen (0-75 µm deep) is represented by an upward pointing triangle, the second mill (75-150 µm deep) is represented by a circle, and the third mill (150-225 µm deep) is represented by a square. Three of the specimens only have two data points (0-75 and 75-150 µm).

Figure 7. A composite plot of $\delta^{18}$O and $\delta^{13}$C signatures of otolith cores of juvenile (symbols given in Figure 4) and adult (open circles) American shad. Juveniles were collected in the freshwater nursery regions of the Mattaponi and Pamunkey Rivers (two tributaries that join to form the York River) in the summers of 2000-2004. Adults were
collected during spawning migrations in the York River in 2006 (a) and 2007 (b). Panel a also shows $\delta^{18}$O and $\delta^{13}$C signatures of material accreted onto the outer portion of 10 otoliths analyzed in 2006 (filled circles). Material on the outer portion of the otolith was presumably accreted during the fish’s time in the marine environment. Symbols represent individual fish. Solid ellipses highlight adults that separated out and had $\delta^{18}$O signatures in agreement with the 2002 juvenile year class. The dashed ellipse highlights four adults that were identified as possible members of the 2002 juvenile year class based on their $\delta^{18}$O signatures, but confidence in this identification was low. Data for 2000-2001 juveniles are from Walther et al. (2008).

Figure 8. Size distribution (fork length in 10 mm bins) for all American shad collected during spawning migrations in the York River in 2006 and 2007 ($n = 498$; gray bars). Individuals that were identified by isotope signatures as age-4 fish in 2006 ($n = 12$; white bars) and age-5 fish in 2007 ($n = 56$; black bars) are indicated. Only lengths for pre-spawning fish are shown.

Figure 9. Total weight (g) and fork length (mm) for all American shad collected during spawning migrations in the York River in 2006 and 2007 ($n = 498$; open circles). Individuals that were identified by isotope signatures as age-4 fish in 2006 ($n = 12$; black circles) and age-5 fish in 2007 ($n = 56$; gray circles) are indicated. Only weights and lengths for pre-spawning fish are shown.
Figure 10. Distributions of age estimates based on scale-based and whole otolith-based age determination methods for those adults collected during spawning migrations in 2006 (a) and 2007 (b) that were identified by isotope signatures as members of the 2002 year class of American shad in the York River (n = 12 in 2006 and n = 57 in 2007). Solid bars represent scale-based methods, striped bars represent whole otolith-based methods, black bars represent reader 1, and gray bars represent reader 2. NS = no sample, UN = unusable scale or otolith.

Figure 11. Scale of an American shad collected in 2006 during the spawning migration in the York River. This specimen was identified by isotope signatures and scale readers as an age-4 fish. The Cating (1953) method of age determination is depicted on the scale, but transverse groove and annuli counts for this scale do not follow the method. Transverse grooves are indicated by Arabic numerals and annuli are indicated by Roman numerals and arrows. The freshwater zone is indicated by FWZ. The star indicates a false annulus. This mark was designated as a false annulus by scale readers and this designation was supported by the isotope signature-based age determination method. In order for the scale-based age estimate to agree with the isotope signature-based age determination, this mark must be a false annulus.

Figure 12. Scale of an American shad collected in 2006 during the spawning migration in the York River. This specimen was not identified by isotope signatures as an age-4 fish in 2006, but was aged as age-4 by scale readers. The Cating (1953) method of age determination is depicted on the left side of the scale, and simple counts of the visible
annuli, without use of transverse groove counts, are depicted on the right side of 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.

Figure 13. Scale of an American shad collected in 2007 during the spawning migration in the York River. This specimen was not identified by isotope signatures as an age-5 fish in 2007, but was aged as age-5 by scale readers. 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. The star indicates evidence of an annulus that has been hidden by a spawning mark. This evidence of an annulus was designated false by scale readers, but this designation was not supported by the isotope signature-based age determination method. In order for the scale-based age estimate to agree with the isotope signature-based age determination, this mark must be a true annulus.

Figure 14. Juvenile abundance indices (JAI + SD) for American shad in the Mattaponi (black bars), Pamunkey (white bars) and York (main stem; striped bars) Rivers in 2000-2004. Data were collected by the Virginia Institute of Marine Science’s (VIMS) seine survey (see Hewitt et al. 2007 for a description of sampling methods). Indices were calculated as the geometric mean catch per seine haul.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.
Figure 8.
Figure 9.
Figure 10.
Figure 14.
VITA

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