Tracking Nursery Habitat Use in the York River Estuary, Virginia, by Young American Shad Using Stable Isotopes

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Abstract.—We developed and applied a stable isotope turnover model to estimate how long age-0 American shad Alosa sapidissima reside within tidal freshwater and brackish-water habitats in the York River estuary, Virginia. The residence time was estimated by modeling the changing stable isotope ratio (either the carbon \([\delta^{13}C]\) or sulfur \([\delta^{34}S]\) stable isotope ratio) of an age-0 American shad as it migrates seaward from its present habitat to a new habitat and determining the minimum time required to acquire the isotopic signature of its new habitat. A sensitivity analysis of our turnover model indicates that the results are robust at relatively fast turnover rates, such as those experienced by young fish, but that at slow turnover rates the model can yield biologically meaningful differences with relatively small changes in variables. The average ± SE isotopic ratios for the dorsal muscle tissue of age-0 fish increased along the estuary, from \(-31.8 ± 0.3\%_{o}\) for \([\delta^{13}C]\) and \(-5.2 ± 0.7\%_{o}\) for \([\delta^{34}S]\) at the farthest upriver region to \(-21.8 ± 1.2\%_{o}\) for \([\delta^{13}C]\) and \(10.3 ± 1.7\%_{o}\) for \([\delta^{34}S]\) in the lower estuary, and were significantly different among regions. To account for these distinct signatures along the estuary, the turnover model predicts that age-0 fish remain in discrete regions (~10 river kilometers) of the tidal freshwater portion of the river for at least 15–45 d and in the lower estuary for at least 32–66 d. Juveniles, therefore, are spatially segregated, and probably migrate slowly downstream during the summer and early fall, accumulating in the lower freshwater and oligohaline portions of the estuary before their oceanic migration.

American shad Alosa sapidissima, the largest North American anadromous clupeid, migrate from their freshwater nursery habitat to the ocean in their first year of life. Investigators propose both temperature-based migration, consistent with fall migration (e.g., Leggett and Whitney 1972; Williams and Bruger 1972; O’Leary and Kynard 1986; Hoffman et al., in press) and size-based migration, migration occurring as early as June (Marcy 1976; Limburg 1996). A detailed analysis of the age-structure of Hudson River age-0 American shad suggests that age-0 fish move about 120 km downriver in about 2 weeks (Limburg 1996), implying rapid movement through their freshwater habitat. Given the coast-wide concerns regarding American shad population declines (ASMFC 1999), which are, in part, due to habitat degradation (e.g., Limburg et al. 2003), further measures of age-0 American shad habitat use, including the spatial scale, duration, and rate of seaward migration, are needed.

Stable isotopes have been applied to document movement patterns in a variety of animals (Hobson 1999). Certain stable isotope ratios of consumers, such as the carbon (C) stable isotope ratio, tend to closely resemble their prey (DeNiro and Epstein 1978; Fry and Sherr 1984). Stable isotopes, therefore, offer the potential to track habitat changes when either (1) movement is related to ontogenetic niche (diet) shifts or (2) the habitats of interest possess distinct biogeochemical properties, such as a switch from fresh to marine waters, and the rate of movement between habitats is faster than the rate of change of the isotope ratio in the animal’s tissue (Hobson 1999). Thus, the animal arrives at the new habitat with an isotopic composition indicative of its previous habitat. After settling into the new habitat, the animal’s tissues will acquire the isotopic ratio specific to that habitat.

The ability to quantify the timing of these movements (or diet shifts) is dependent on the turnover rate in the tissue being analyzed (Tieszen et al. 1983; Hobson and Clark 1992). The turnover rate, in turn, depends on growth and metabolism. Growth turnover occurs as new tissue is added, diluting the pool of older tissue derived from the previous diet. Metabolic turnover occurs as older tissue is broken down and new tissue synthesized. In both slowly and rapidly growing fish, growth dominates stable isotope turnover (Fry and Arnold 1982; Hesslein et al. 1993; Herzka and Holt 2000; MacAvoy et al. 2001; Sakano et al. 2005) and metabolism can accelerate the turnover rate beyond that exerted by growth alone (Frazier et al. 1997; Vander Zanden et al. 1998; Herzka et al. 2001; Trueman et al. 2005).
Two different models are commonly used to model turnover. Fry and Arnold (1982) proposed the following power model:

\[ R_f = R_f + (R_i - R_f) \times (W_f/W_i)^t, \quad (1) \]

where \( R_f \) is the stable isotope ratio (e.g., \( \delta^{13}C \)) of the animal or tissue at time \( t \), \( R_i \) is the stable isotope ratio at equilibrium, \( R_i \) is the initial stable isotope ratio, \( W_f \) and \( W_i \) represent the initial weight and weight at time \( t \), respectively, and \( c \) is the turnover coefficient. The model simplifies to dilution-only turnover (i.e., growth only) when \( c = -1 \) and can be used to date ecologically significant events associated with changes in isotopic ratios (Fry and Arnold 1982), such as age at settlement of fish larvae (Herzka et al. 2001).

Hesslein et al. (1993) used an exponential model with terms for a specific growth rate \( (k = \log_e(W_f/W_i)/t) \) and a metabolic rate \( (m) \), as follows:

\[ R_i = R_f + (R_i - R_f) \times e^{-(k+m)t}, \quad (2) \]

where \( i \) indicates the ratio at \( t = 0 \) and \( f \) the equilibrium ratio. The advantages of this model are that time, \( t \), is an explicit term and that \( m \) can be estimated using a curve-fitting procedure if \( k \) is known. If \( R_i \) and \( R_f \) are both known (e.g., the isotopic signatures of two habitats with distinct isotopic distributions), then the equation can be solved directly for the equilibrium time \( (t_{eq}) \) theoretically required for the isotopic signature of the population in the first habitat to resemble that in the second \( (R_f) \) upon moving into the second habitat. However, the time required for \( R_f \) to exactly equal \( R_f \) is infinite because the model has an asymptote at \( R_f \) (Figure 1a).

To resolve this problem, the turnover “half-life” (i.e., the time required to reach one-half the difference between the initial \( [R_f] \) and final \( [R_f] \) isotopic ratios; Figure 1a) is often estimated. It would be better, however, to have a biologically meaningful estimate of the \( t_{eq} \) than a convenient reference point along the transition. To achieve this, we propose a statistical method (Figure 1b). If \( R_i \) and \( R_f \) are variables with some mean and standard deviation, then we can define \( t_{eq} \) as the time at which the distribution of \( R_f \) is not significantly different from that of \( R_f \) at some probability level (e.g., \( \alpha = 0.05 \)). Now \( t_{eq} \) is not infinite. It represents both the minimum residence time required to reach approximate equilibrium and the period during which we can estimate how long ago the organism (or population) arrived in its new habitat (after which the organism will have an isotopic signature similar to that of its new habitat).

Our objective was to estimate habitat-specific residence times \( (t_{eq}) \) by applying a stable isotope turnover rate model. To evaluate the model, a sensitivity analysis was done by calculating the effect on the estimate of the time to equilibrium, \( t_{eq} \), by changing the model variables (i.e., \( R_f, R_f \) and \( k + m \)) and population variables (i.e., the number of fish sampled \( [n] \) and the standard deviations of \( R_f \) and \( R_f \)). We surveyed the \( \delta^{13}C \) of age-0 American shad during their summer residence in the upper York River estuary, Virginia, and subsequent migration to Chesapeake Bay to determine whether habitat-specific signatures were present. We then measured the \( \delta^{13}C \) and sulfur stable isotope ratios \( (\delta^{34}S) \) of age-0 American shad at selected locations and times to apply...
the model and estimate $t_{eq}$ for various regions along the estuary.

Methods

Field sampling.—Age-0 American shad were sampled in the Mattaponi River, part of the freshwater nursery grounds in the York River estuary, and the brackish York River during their migration to Chesapeake Bay. The York River is located in the southern Chesapeake Bay and is formed by the confluence of the Mattaponi and Pamunkey rivers, both of which are almost entirely fresh (Figure 2). American shad hatch from March through July in the freshwater portions of the Mattaponi and Pamunkey rivers (Bilkovic et al. 2002; Hoffman and Olney 2005).

We studied the Mattaponi River because age-0 American shad are more abundant there than in the Pamunkey River (Wilhite et al. 2003). During 2002, age-0 American shad were sampled in the tidal freshwater portion of the Mattaponi River every week from May through July with a bow-mounted push net (1.5-m × 1.5-m opening, 5.2-m-long net, 1.27-cm-stretch-mesh cod end). Twelve stations were sampled on each survey. The freshwater habitat was sectioned into three strata of equal size, and four stations were randomly chosen from each. During 2002, river kilometer (rkm) 76 was generally about the upstream limit of salt intrusion, depending on discharge and tide stage. Surveys began at the uppermost end of the nursery zone, at rkm 109 (the Mattaponi River mouth is at rkm 52 and the York River mouth at rkm 0) and proceeded down-river. Additionally, age-0 American shad were captured in the York River (rkm 0–52) from November 2002 to March 2003 by the Virginia Institute of Marine Science Juvenile Finfish and Blue Crab Trawl Survey (9.1-m semiballoon otter trawl, 6.34-mm-mesh cod end). The survey sampled the York River estuary monthly using a mixed random and fixed-station design (metadata are available at www.fisheries.vims.edu/trawlseine/methods.htm). In both surveys fish were stored on ice in the field and frozen upon arrival at the laboratory.

Stable isotope analysis.—We surveyed $\delta^{13}C$ ratios in age-0 American shad throughout the year to assess temporal variability and determine whether habitat-specific ratios were present. We analyzed fish from every other sample (i.e., bi-weekly) because we anticipated that this period was about half the time necessary for age-0 shad to reach isotopic equilibrium based on previously published growth rates (Hoffman 2005).
and Olney 2005). For $\delta^{13}C$ analysis, age-0 fish were sorted into size-classes ($<$40, 40–59.9, 60–79.9, and $>$80 mm total length [TL]) and regions (rkm 104–109, 95–100, and 74–87) by sampling date. The regions were chosen based on river geomorphology and salinity. Fish from each size-class exceeding 40 mm TL, region, and date were subsampled for stable isotope analysis by randomly selecting 10 fish of each size-class. If fewer than 10 age-0 fish were available, then all the fish were sampled. Only age-0 American shad exceeding 40 mm TL were used for two reasons: first, it is the size at which age-0 shad fully recruit to the push-net gear (Hoffman and Olney 2005), and second, this is the earliest size at which migration from the nursery zone has been documented (Limburg 1996). All American shad captured in the York River were sampled for $\delta^{13}C$.

For the modeling application, we compared turnover model results using either $\delta^{13}C$ or $\delta^{34}S$. These isotopes were chosen because trophic discrimination (i.e., the difference in isotopic composition between a consumer and its diet) is small for both C and S (Peterson and Fry 1987; Hessen et al. 1991) and both C and S have been used as a marine tracer (e.g., Hessen et al. 1991; MacAvoy et al. 2001). The same American shad were sampled for both $\delta^{34}S$ and $\delta^{13}C$ to directly compare the two stable isotopes. We analyzed a subsample of fish captured owing to the expense of analyzing both stable isotopes. We analyzed a subsample of fish exceeding 40 mm TL, region, and date were subsampled for stable isotope analysis by randomly selecting 10 fish of each size-class. If fewer than 10 age-0 fish were available, then all the fish were sampled. Only age-0 American shad exceeding 40 mm TL were used for two reasons: first, it is the size at which age-0 shad fully recruit to the push-net gear (Hoffman and Olney 2005), and second, this is the earliest size at which migration from the nursery zone has been documented (Limburg 1996). All American shad captured in the York River were sampled for $\delta^{13}C$.

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American shad were frozen until analysis because freezing has negligible effects on isotopic ratios (Bosley and Wainright 1999). After being thawed, fish were measured (±1 mm TL), weighed (±0.01 g), and rinsed thoroughly with de-ionized (DI) water. For fish selected for $\delta^{13}C$ and $\delta^{34}S$ analysis (i.e., for the model application), dorsal muscle tissue was removed, rinsed with 10% HCl (American shad have intermuscular bones that are difficult to remove), rinsed with DI water, homogenized, divided for separate analyses, and dried at 45°C for 24 h. The $\delta^{13}C$ samples (~1 mg tissue) were analyzed with an ANCA GSL elemental analyzer and Europa Hydra 20–20 isotope ratio mass spectrometer (IRMS; University of California–Davis Stable Isotope Facility). The standard deviation (SD) between replicate $\delta^{13}C$ standards was 0.04‰ and that between randomly assigned replicate tissue samples was 0.10‰. The $\delta^{34}S$ samples (~6 mg tissue) were analyzed with a Carlo Erba NC2100 elemental analyzer and Finnegan DELTA-plus Advantage IRMS (Colorado Plateau Stable Isotope Laboratory, Northern Arizona University). The SD between replicate laboratory $\delta^{34}S$ standards was 0.20‰. For fish selected for $\delta^{13}C$ analysis only (i.e., for isotopic equilibrium evaluation), the $\delta^{13}C$ samples were analyzed as previously described, with similar error.

Stable isotope ratios are reported in $\delta$X notation, where $\delta X = ([Ratiosample/Ratiostandard] - 1) \times 10^3$, and X is the stable isotope of carbon (C) or sulfur (S). Ratio is the ratio of light to heavy isotopes, and the standards are Pee Dee belemnite and Canyon Diablo trilobite for C and S, respectively. Samples were not corrected for lipid content (lipids are $\delta^{13}C$ depleted relative to carbohydrates and proteins [DeNiro and Epstein 1977]) because atomic C:N was low and similar among muscle samples from the three Mattaponi River regions (average, 3.5; one-way ANOVA between regions: df = 2, $F = 0.61, P = 0.5$), indicative of low lipid content (Sweeting et al. 2006).

Turnover model development.—We modified Hessen et al.’s (1993) turnover rate model so that it could be applied to a population by assuming that each R term represents the mean of a distribution. Thus

$$ \bar{R}_t = \bar{R}_f + \left( \bar{R}_e - \bar{R}_f \right) \times e^{-(k+m)t}. \quad (3) $$

The model describes the mean isotopic ratio of a population at time $t$ (i.e., $\bar{R}_t$) as it approaches isotopic equilibrium with the new habitat (or diet), where $\bar{R}_f$ is the mean of the population at equilibrium with the original habitat and $\bar{R}_e$ is the population’s mean once at equilibrium with its new habitat. Of course, $\bar{R}_f$, $\bar{R}_f$, and $\bar{R}_e$ each have an associated underlying distribution. An appropriate statistical test, therefore, can be applied to the distributions of $\bar{R}_f$ and $\bar{R}_e$ to determine the time, $t_{eq}$, at which the mean isotopic signature of the recently arrived population is not significantly different from
the mean isotopic signature of the new habitat (i.e., $H_{nf}$; $R_f = R_t$ at $\alpha = 0.05$; Matlab 7.0.1; see Appendix for code).

To verify that the distribution-based turnover model projected the mean isotopic ratio in the same manner as the original model, we simulated the seaward migration of 25 age-0 American shad with a typical growth rate ($k = 0.05/d$; Hoffman and Olney 2005) and no metabolic turnover (i.e., $k + m = 0.05/d$). We simulated their muscle tissue to turn over from a $\delta^{13}C$ representative of feeding and growth within a freshwater river habitat ($R_i' = -28\%$/o, SD = 1.0) to that within a marine habitat ($R_f' = -20\%$/o, SD = 1.0; Limburg 1998). The modified model yields the same projection and predicts that the fish arrive at equilibrium in about 56 d (Figure 1b), compared with an isotopic half-life of 14 d (Figure 1a).

We performed a sensitivity analysis on the model. The $t_{eq}$, defined as the first day when a Kolmogorov–Smirnov (K–S) test yielded a $P$-value greater than 0.05 (i.e., there was no longer a significant difference between the distributions), was obtained under four isotopic gradients ($\Delta R = R_f - R_i = 5, 2, 1, and 0.5$), five $(k + m)$ values (0.2, 0.1, 0.05, 0.01, and 0.001), two standard deviations (1.0 and 0.5), and two sample sizes ($n = 10$ and 100). In all trials both $R_i'$ and $R_f'$ were assumed to have the same SD and $n$. Turnover was simulated using a normal distribution to represent $R_i'$ and $R_f'$ ($R_0 = -30\%$/$^{13}C$, which was chosen arbitrarily). The K–S test was applied to $R_f'$ and $R_i'$ at each daily time step.

**Identification of habitat-specific ratios.**—We used the season-long survey of age-0 $^{13}C$ ratios (1) to quantify the stable isotope gradient across the estuary and thereby verify that habitat-specific ratios were present and (2) to ensure that habitat-specific ratios were constant in time. Our concern was that our model application would be problematic if the $R_f'$ and SD (which are estimated from field data taken on a single day) did not represent the long-term signature of each habitat. This could occur if the fish were not in equilibrium with the habitat (e.g., if they had not been resident for sufficient time). To quantify the isotopic gradient across the estuary, we estimated population mean $^{13}C$ ratios and SDs for each region within the Mattaponi River (rkm 105–109, 95–100, and 74–87) and the low-salinity (0–15%) portion of the York River (rkm 19–52). Because age-0 fish were randomly subsampled by length-class, we estimated the population’s mean (analogous to $R_f$) and SD within a region by weighting $^{13}C$ using the region-specific length-frequency distribution in the catch. We assessed whether the $^{13}C$ ratio was constant in each habitat (implying that isotopic equilibrium was reached) by comparing the population’s mean $^{13}C$ within each region to the trend in age-0 $^{13}C$ with respect to their change in weight (i.e., growth). Finally, we compared each region’s mean $^{13}C$ with typical prey $^{13}C$ ratios to determine whether the population means were isotopically representative of each region.

**Model application to field data.**—We used the distribution-based turnover model to predict the minimum residence time ($t_{eq}$) required for an age-0 American shad to isotopically equilibrate to the habitat immediately downstream. We modeled the progression of a fish’s isotopic ratio by projecting the range of values it would have through time after moving to the next region immediately downstream (e.g., those captured at rkm 104 were simulated to move to rkm 96–102) and then statistically determined whether that range was significantly different from the range of fish already in that habitat. For this we used a Monte-Carlo approach. Each fish began with its measured $^{13}C$ or $^{34}S$ ratio ($R_f$). The model was run 100 times for each of about 10 fish sampled in a region by randomly selecting 100 $R_f$ values drawn from a normal distribution having the region-specific mean and SD estimated from the $^{13}C$ and $^{34}S$ data from the next region downstream (i.e., $R_{f,96-102}$, $R_{f,74-78}$, and $R_{f,104}$). The fish was then projected through time toward each of those 100 $R_f$ values. The $R_i$ and $R_f$ values and their associated distributions were determined from independent, spatially disparate fish samples.

For each fish, an estimate of $t_{eq}$ was obtained. At each 5-d time step, a K–S test was used to determine whether $R_f = R_f$ at $\alpha = 0.05$ (i.e., whether the present isotopic ratio of fish migrating from the upstream habitat was no longer significantly different from those in the downstream habitat), where $R_f$ is the frequency distribution of the 100 $^{13}C$ or $^{34}S$ values at time $t$ and $R_f$ is the frequency distribution of the 100 randomly selected $R_f$ values (estimated from each region). As before, $t_{eq}$ was defined as the first day when $P > 0.05$. We summarized the results by averaging the $t_{eq}$ values for each region (i.e., if 10 fish were simulated, we averaged the 10 $t_{eq}$ values).

We made two assumptions about growth when applying the turnover model: first, that growth is constant ($k = 0.05/d$) and second, that growth could account entirely for isotopic turnover (i.e., $m = 0$). The first assumption is consistent with a growth variability study of juvenile American shad from the adjacent Pamunkey River (Hoffman and Olney 2005). We tested the second assumption by fitting Fry and Arnold’s (1982) model (equation 1; $W = 0.1$ g [the wet weight at metamorphosis]) to the $^{13}C$ ratios of fish captured in the upriver habitat (rkm 104–109) during a period in which a marked $^{13}C$ depletion (approximately $-6\%$/o) occurred. We used only those
age-0 fish with $W/W_i \leq 25$ (fish captured from 27 May to 15 July, or from metamorphosis at $\sim 25$ mm to $\sim 65$ mm TL). The model was fit using nonlinear regression (S-Plus 2000).

Results

Model Sensitivity

Absolute differences in $t_{eq}$ between isotopic gradients ($\Delta R$) are much greater at slow turnover rates than at rapid rates (Figure 3). For example, at a rapid rate ($k + m = 0.2$) and low power ($n = 10$, SD = 1.0), $t_{eq}$ ranges from 3 to 10 d for $\Delta R$ values of 0.5–5, respectively. For the same conditions at a slow rate ($k + m = 0.001$), $t_{eq}$ ranges from 740 to 1,861 d for $\Delta R$ values of 0.5–5, respectively. For a given turnover rate (i.e., $k + m$), $t_{eq}$ approximately doubles by increasing the power of the K–S test (i.e., 0.5 SD and $n^2$). At the lowest turnover rate ($k + m = 0.001$) and $\Delta R = 5$, $t_{eq}$ increases from about 1,861 d ($n = 10$, SD = 1.0) to 2,370 d ($n = 10$, SD = 0.5) to 2,882 d ($n = 100$, SD = 1.0) and to 3,550 d ($n = 100$, SD = 0.5). At the fastest turnover rate ($k + m = 0.2$) and $\Delta R = 5$, the $t_{eq}$ estimated with the lowest statistical power ($n = 10$, SD = 1) is about 10 d, whereas it increases to about 15 d by increasing the sample size ($n = 100$, SD = 1) or decreasing the SD ($n = 10$, SD = 0.5). It increases to 18 d by increasing the sample size and decreasing the SD ($n = 100$, SD = 0.5). With respect to the sample size chosen for the Monte-Carlo analysis ($n = 100$), the simulation implies that our results are robust on the time scale relevant to juvenile fish biology (weeks) given the fast growth rate characteristic of juvenile American shad.

![Figure 3](image-url)

**Figure 3.**—Time required to reach stable isotope equilibrium ($t_{eq}$) for various isotopic gradients ($\Delta R = R_f - R_i$) and combined growth and metabolic rates ($k + m$) when changing the power of the Kolmogorov–Smirnov test by altering the number of fish in the sample ($n$) and the standard deviations of $R_f$ and $R_i$. 
Habitat-Specific Ratios

The population means estimated from the season-long $\delta^{13}C$ survey were depleted at rkm 104–109 and increasingly enriched toward the lower estuary, implying that habitat-specific signatures are present (Table 1). The $\delta^{13}C$ of age-0 American shad was constant with respect to weight after 17 June (Figure 4). The similarity of the $\delta^{13}C$ of fish 60–79.9 mm TL captured 30 June and 15 July to the weighted average $\delta^{13}C$, as well as to each other, implies that fish in each region had obtained a reasonably stable and constant $\delta^{13}C$ value ($\pm 1\%$). Temporal variation did occur. On 27 May, the $\delta^{13}C$ of age-0 shad was similar among regions (approximately $-28\%_o$; Figure 4). Thereafter, the $\delta^{13}C$ became increasingly depleted at rkm 105–109 and rkm 95–100, stabilizing at approximately $-30$ to $-31\%$, whereas no trend was visible at rkm 74–87. In the brackish estuary (rkm 19–52) the $\delta^{13}C$ was depleted in November, indicative of a recent emigration of these age-0 fish from freshwater, and was increasingly similar to an estuarine $\delta^{13}C$ signature ($-24\%$ to $-20\%_o$; Michener and Schell 1994) as winter progressed.

Available data confirm that these equilibrium values are representative of each region. In summer, particulate organic matter $\delta^{13}C$ is enriched along the estuary axis from approximately $-29\%_o$ to $-30\%_o$ at rkm 113 to $-22\%_o$ at rkm 7 in the York River (Hoffman and Bronk 2006). Further, presuming zooplankton were the common diet items (Table 1), American shad would have a $\delta^{13}C$ from approximately $-31.5\%$ to $-32.5\%_o$ at rkm 104, $-30.4\%_o$ to $-31.3\%_o$ at rkm 96 and 102, and $-29.1\%_o$ to $-27.2\%_o$ near rkm 78, given a trophic discrimination of approximately $+1\%_o$ (Post 2002). This is similar to the measured gradient.

Model Application

The $\delta^{13}C$ and $\delta^{34}S$ of age-0 American shad vary among the regions and are increasingly enriched in fish captured further downriver (Table 2). Further, the population mean $\delta^{13}C$ ratios (Table 1) were similar to the $\bar{R}$, $\delta^{13}C$ values used in the model application (Table 2). From the upper estuary to the lower estuary, we observed a gradient of approximately $+10\%$ for $\delta^{13}C$ and $+15.5\%$ for $\delta^{34}S$. The $\delta^{13}C$ signature is significantly different between regions (ANOVA: df = 4, F = 130.8, $P < 0.001$), all regions being distinct except rkm 104 and rkm 96–102. Similarly, the $\delta^{34}S$ signature is different between regions (ANOVA: df = 4, F = 385.9, $P < 0.001$), though Tukey’s test indicates that the Mattaponi River is separated into only two distinct regions. The age-0 American shad captured in the lower Mattaponi River are slightly larger and heavier than those captured upriver, which is consistent with our assumption of downstream movement in the population. Those age-0 fish captured in January in the York River are larger still. The two stable isotopes trace proximity to marine habitat similarly (Figure 5).

To account for the observed $\delta^{13}C$ distribution, age-0 American shad would have to reside in each region of the Mattaponi River for at least 15–41 d and in the lower York River for 48–60 d; the corresponding residence times for $\delta^{34}S$ are 16–45 d and 32–66 d (Table 3). The variability by region in $t_{eq}$ is a function of both the isotopic gradients between regions and the SD of the downstream region. Owing to their similarity in tracing marine influence, $\delta^{13}C$ and $\delta^{34}S$ data result in similar estimates of $t_{eq}$. The estimates of the average $t_{eq}$ are within 7 d of each other; no consistent difference is observed. Generally the difference between $\bar{R}$ and $\bar{R}$ was small ($<0.5\%_o$), indicating a close convergence of the two populations at the $t_{eq}$. Notably, even where only small isotopic differences exist between habitats (e.g., rkm 104 and rkm 96–102), the model yields $t_{eq}$ estimates from weeks to over a month because these distributions have a small SD.

Application of the Fry and Arnold (1982) model to the $\delta^{13}C$ data from rkm 104 to rkm 109 indicated that turnover was growth dominated, supporting this assumption (Figure 6). The turnover coefficient ($c$) is
Figure 4.—The mean $\delta^{13}C$ value of age-0 American shad versus average wet weight for fish 40–59 mm (filled symbols), 60–79 mm (open symbols), and 80 mm or more TL (gray symbols) in (a–d) various river segments. In panels (a–c), age-0 fish were sampled on 27 May (circles), 17 June (squares), 30 June (diamonds), and 15 July (triangles). In panel (d), age-0 fish were sampled on 7 November (circles), 10 January (squares), and 4 March (diamonds). The error bars represent SDs. Note the difference in the scale of the y-axis in the last panel. The weighted mean $\delta^{13}C$ is indicated by the dashed line and its value is given.
within 100 d and are less sensitive (in absolute terms) to changes in the isotopic gradient. Additional problems using muscle tissue may occur where a seasonal bias is present (Perga and Gerdeaux 2005) or trophic discrimination is not constant owing to variable food quality or nutritional demands or a change in growth rate (Tieszen et al. 1983; Hobson and Clark 1992; Adams and Sterner 2000; Trueman et al. 2005), resulting in isotope shifts that are inconsistent in magnitude with diet or habitat shifts. If isotopic values are measured from the organism and its ecosystem, then these problems may be assessed. None of these problems seem to have occurred with respect to American shad; the isotopic gradient ($\delta^{13}C$) observed along the estuary was similar to the isotopic gradient in zooplankton prey, implying similar trophic discrimination.

### Model Application to Juvenile American Shad

Our assumption that the population could be modeled using growth-dominated turnover is supported by field data. It was possible to estimate the turnover coefficient ($c$) of a subset of age-0 American shad because there was a shift in the $\delta^{13}C$ during the early juvenile stage, probably due to an increased contribution from phytoplankton to the food web during May–July. In the Mattaponi River, the phytoplankton produced in situ are isotopically light ($\delta^{13}C$, $\sim31.8\%$) compared with allochthonous, terrestrially derived matter ($\delta^{13}C$ from $\sim26$ to $\sim28\%$; Hoffman and Bronk 2006). For this analysis to be applicable, we must assume that there was no emigration from or immigration into the region (rkm 104–109). This appears to be reasonable because common prey for age-0 American shad (i.e., zooplankton and macroinvertebrates; Crecco and Blake 1983; Grabe 1996) had a

### Table 2

<table>
<thead>
<tr>
<th>River</th>
<th>Location (rkm)</th>
<th>TL (mm)</th>
<th>Weight (g)</th>
<th>$\delta^{13}C$</th>
<th>$\delta^{34}S$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP</td>
<td>104</td>
<td>49.3 (5.2)</td>
<td>1.1 (0.4)</td>
<td>$-31.8$ (0.3) z</td>
<td>$-5.2$ (0.7) z</td>
<td>10</td>
</tr>
<tr>
<td>MP</td>
<td>96, 102</td>
<td>51.3 (5.1)</td>
<td>1.3 (0.3)</td>
<td>$-31.4$ (0.3) z</td>
<td>$-4.2$ (1.4) z</td>
<td>10</td>
</tr>
<tr>
<td>MP</td>
<td>85, 89</td>
<td>57.1 (2.4)</td>
<td>1.6 (0.2)</td>
<td>$-30.2$ (1.0) y</td>
<td>$0.1$ (2.3) y</td>
<td>10</td>
</tr>
<tr>
<td>MP</td>
<td>74, 78</td>
<td>68.0 (4.1)</td>
<td>2.5 (0.3)</td>
<td>$-27.8$ (2.3) x</td>
<td>$2.9$ (4.7) y</td>
<td>7</td>
</tr>
<tr>
<td>YK</td>
<td>15</td>
<td>93.1 (4.1)</td>
<td>5.1 (0.8)</td>
<td>$-21.8$ (1.2) w</td>
<td>$10.3$ (1.7) x</td>
<td>10</td>
</tr>
</tbody>
</table>

**FIGURE 5.**—The $\delta^{13}C$ and $\delta^{34}S$ values of age-0 American shad sampled from the Mattaponi River on 30 June 2002 and the York River on 13 January 2003 (rkm 52 is the mouth of the Mattaponi River). Salinity was 17.4% (rkm 15) and 20.0% (rkm 9) at the river bottom on 13 January 2003.

**FIGURE 6.**—The $\delta^{13}C$ turnover of age-0 American shad captured at rkm 104–109, including the best fitting equation from Fry and Arnold’s (1982) turnover model (equation 1 in the text; solid line) and the model assuming growth turnover only ($c = -1.0$; dashed line). The values for the variables $R_f$, $R_i$, and $c$ were estimated by means of nonlinear regression.
δ¹³C value similar to the estimated $R_f$ (−31.8‰; Table 1), implying that age-0 fish were at isotopic equilibrium in this region. The average δ¹³C (SD) estimated from sampling at rkm 109 (27 May, 30 June, and 21 July) was −32.2‰ (0.8) for Eurytemora affinis (a calanoid copepod), −32.6‰ (1.2) for Bosmina freyi (a cladoceran), and −32.4‰ (1.7) for Chironomus spp. (Diptera larvae; Hoffman 2006). Moreover, movement from an upstream region was unlikely because this region is the farthest upriver within the nursery zone. While immigration from a downstream habitat could confound the analysis, the effect would be slight because there is a small isotopic gradient (~1‰) between fish at rkm 104–109 and those at rkm 96–100.

To simulate migration using the turnover model, each region was assigned an $R_f$ value using the δ¹³C or δ³⁴S distribution of fish captured within that region. The advantage of using the empirical probability distribution with the turnover model is that we do not need to make assumptions regarding the behavior of any individual fish. Rather, we can postulate that a fish could equilibrate at any of the isotopic values observed downstream and thereby obtain a biologically realistic range of $t_{eq}$ values. Migration, however, could bias the region’s mean δ¹³C or δ³⁴S, resulting in a lighter estimate than is representative of the region due to the presence of recent arrivals. The problem is not unique to this study but applies to any in which a local variable is measured from a potentially mobile population. In this study, the available data (particulate organic matter, zooplankton) provide independent support for our equilibrium values (Table 1).

The growth rate used in the model application, 0.05/d, is applicable to age-0 American shad of 40–80 mm TL but may be an overestimate for larger age-0 fish because growth during year 1 is asymptotic, slowing at around 80–100 mm TL (Crecco et al. 1983; Limburg 1996). The average size of estuarine age-0 shad used in this study was about 100 mm TL; therefore, $t_{eq}$ may be underestimated in the simulation of migration from the Mattaponi River to the York River. It could be underestimated by as much as 40 d if the growth rate declined to 0.02/d and metabolic turnover was relatively low (Figure 3). Some growth turnover, however, must have occurred between the summer and winter sampling because the average weight of age-0 fish increased by a factor of four over the period corresponding to the migration from the river (Table 1).

In general, the role of metabolism is difficult to assess in wild populations owing to the variation in individual growth, metabolism, and diet. As growth slows the contribution of maintenance metabolism to isotopic turnover increases, which is the premise behind turnover studies of adult animals. Although the average ± SD weights of the age-0 American shad captured in the upper York River estuary during November (7.1 ± 2.6 g) and January (8.6 ± 5.1 g) were similar, albeit highly variable, the δ¹³C of age-0 fish was more enriched in January (mean ± SE, −22.7 ± 0.4‰) than in November (−24.9 ± 0.5‰), implying that some metabolic turnover occurred during the winter months. Metabolic processes remain relatively unexplored with respect to isotopic discrimination. Different tissues are subject to different turnover rates (Tieszen et al. 1983); however, assessing the relative contribution by growth and metabolism to isotopic turnover, especially in field studies, remains extremely difficult. As others have argued (Gannes et al. 1997; Hobson 1999), more experimental work is needed. The contribution of metabolism could be indirectly measured in a field experiment in which direct estimates of growth, and possibly even the change in an individual’s isotopic composition, are obtained, perhaps by combining conventional tagging and stable isotope studies (if that is possible) or by means of cage experiments (e.g., Herzka et al. 2001).

### American Shad Habitat Use

American shad are the focus of conservation efforts along the Atlantic coast of North America owing to a widespread population decline caused by pollution, dam construction, and overfishing (ASMFC 1999). Young American shad are habitat generalists and use all available habitats within a river (Ross et al. 1997).

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**Table 3.—Comparison of equilibrium times ($t_{eq}$ [d]) for age-0 American shad simulated to migrate downstream in turnover model simulations using either δ¹³C or δ³⁴S, the corresponding mean stable isotope ratios at $t_{eq}$ ($R_f$), and the differences from their equilibrium values ($R_t$) at various river locations (rkm; see text); $n$ = the number of fish simulated.**

<table>
<thead>
<tr>
<th>Stable isotope ratio</th>
<th>Variable</th>
<th>104 to 96–102</th>
<th>96–102 to 85–89</th>
<th>85–89 to 74–78</th>
<th>74–78 to 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ¹³C $t_{eq}$ (range)</td>
<td>31 (15–38)</td>
<td>28 (19–37)</td>
<td>53 (48–60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ³⁴S $t_{eq}$ (range)</td>
<td>26 (16–33)</td>
<td>26 (17–33)</td>
<td>46 (32–66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_f$ $R_t$ – $R_f$</td>
<td>−3.1, −0.1</td>
<td>−2.9, −0.1</td>
<td>−2.9, −0.1</td>
<td>−2.9, −0.1</td>
<td></td>
</tr>
<tr>
<td>$R_t$ $R_f$ – $R_t$</td>
<td>−4.4, −0.2</td>
<td>−4.4, −0.2</td>
<td>−4.4, −0.2</td>
<td>−4.4, −0.2</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
In this study, the distinct $\delta^{13}C$ and $\delta^{34}S$ between regions within the nursery zone, combined with the turnover model results, indicate that the fish remain in small-scale habitats (5–10 km) for a month or longer. This suggests that habitat restoration efforts on the spatial scale of 5–10 km (i.e., a river reach) would be well matched to the scale of habitat use by American shad in the York River estuary.

In contrast, Hudson River juvenile American shad actively migrate downstream and enter brackish water as early as June (Limburg 1996). Most of the age-0 American shad captured in the upper York River estuary had an isotopic composition similar to the expected $\delta^{13}C$ of an estuary ($\delta^{13}C = -20$ to $-25\%$; Figures 4, 5), indicating that they were at or approaching isotopic equilibrium with the new habitat. With either $\delta^{13}C$ or $\delta^{34}S$ data, the turnover model indicates that American shad captured in the upper York River estuary in early January 2003 would have had to enter this brackish habitat no later than early December or late November 2002. This result is consistent with analyses of trawl data from the York River system that suggest that age-0 American shad remain within freshwater habitats until November before migrating out during the winter months (Hoffman and Olney 2005; Hoffman et al., in press). It is likely, therefore, that juveniles slowly exit the upper and middle reaches of the tidal freshwater portion over the summer and early fall, accumulating in the lower freshwater and oligohaline portions of the estuary before their oceanic migration. In contrast to the Hudson River, the York River is a warm, short, coastal-plain tributary; further research may reveal patterns in fish movement and habitat use among Atlantic coast tributaries in relation to their physical characteristics. These findings are relevant to the population dynamics of American shad. A residence time of weeks to months is sufficient for habitat differences to influence population demographics because larval and juvenile American shad have high mortality and growth rates (Crecco et al. 1983; Houde 1997).

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Appendix: Model Code for MATLAB 7.0.1

This appendix presents a stable isotope turnover rate model to estimate the time span required for two populations (initial and final) with different stable isotope means and standard deviations to become statistically similar. The variable D returns the matrix of stable isotope ratios given the mean ratio of the final population (mu) and its standard deviation (SD), the mean ratio of the initial population (mu2) and its standard deviation (sd2), the combined growth and metabolic rates of the initial population (r), the number of individuals in the population (n), and the time length of the run in days (t). The variable result is the output at each day of the Kolmogorov–Smirnov (K–S) goodness-of-fit test comparing the population at time t with the final population.

```matlab
function [D,result] = turnover(mu,SD,mu2,sd2,r,n,t)
    F = mu + SD * randn(1,n);
    I = mu2 + sd2 * randn(1,n);
    s = 0:1:t;
    j = 0; u = zeros(length(s),n);
    v = zeros(length(s),1);
    for b = 0:1:t
        E = F + (I - F) * exp(-r*b);
        [H,P] = kstest2(F,E);
        j = j + 1;
        u(j,:) = E;
        v(j) = P;
    end;
    D = [s' u];
    result = [s' v];
    plot(s',v')
xlabel('Time (d)')
ylabel('K-S P-value')
end
```