Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*

Karin E. Limburg

Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545, USA

ABSTRACT: Sagittal otoliths of young-of-year American shad *Alosa sapidissima* from the Hudson River estuary, New York, USA, were transected with an X-ray-dispersive microprobe to examine temporal patterns of strontium, a micro-constituent found in otolith aragonite. Otoliths were assayed from fish reared on known diets (freshwater zooplankton, followed by artificial diet containing marine fishmeal) in fresh water. The switch from freshwater plankton to artificial diet resulted in a significant rise in Sr:Ca ratio in the otolith (mean increase 3.2-fold, $p < 0.001$) both for fish reared at 12.5°C and those reared at 22°C, although there was no significant difference in Sr:Ca increases between the 2 temperature treatments. In a field study, Sr:Ca values of otoliths from wild fish caught in the freshwater reaches of the Hudson were low (mean 0.79×10^{-3} Sr:Ca ± 0.32 SD, range 0.00 to 1.46×10^{-3}). Six fish captured in a single trawl in the lower estuary on 25 September 1990 had low Sr:Ca values on the inner parts of their otoliths (corresponding to younger age: mean Sr:Ca = 0.98×10^{-3} ± 0.38 SD), but the strontium content increased 3- to 5-fold (mean Sr:Ca = 3.62×10^{-3} ± 0.71 SD) on the outer parts, corresponding to dates when the fish were older. The change in strontium content is consistent with the movement of fish from freshwater to seawater, as seawater has a higher Sr concentration and ratio of Sr:Ca. The same change was seen in the otolith of a female adult shad and has been documented in other diadromous fishes. Three of these juveniles had such anomalous early growth patterns as to suggest their origin in a natal river other than the Hudson. The estimated seawater entry dates of these fish were as early as late June. This is the first demonstration of such early outmigration, and possibly vagrancy, in young-of-year of this species, and contributes to our understanding of American shad life history.

KEY WORDS: American shad · Otolith microchemistry · Strontium:calcium ratios · Anadromy · Migration

INTRODUCTION

Otoliths (earstones) of teleost fishes form a critical part of the acoustico-lateralis system (Moyle & Cech 1988). In most species, otoliths are composed of aragonite precipitated on a protein matrix (Degens et al. 1969) and appear to accrete new material throughout the life of the fish. Whereas coarse otolith macro-structures (annuli) have long been used to determine the age of fish ≥1 yr (e.g. Reibisch 1900, Dannevig 1933), much work in the past 20 yr has focused on microstructure (e.g. Campana & Neilson 1985, Brothers 1987). Micro-increments have been shown to accrete daily (Pannella 1971, Brothers et al. 1976), stimulating studies of age estimation in teleosts (Summerfelt & Hall 1987, Secor et al. 1994).

Chemical composition of otoliths has likewise interested investigators for some time (Dannevig 1956, Odum 1957b, Degens et al. 1969). Recent advances in X-ray spectroscopy have allowed fine-scale examination of trace inorganic constituents of otoliths (e.g. Radtke 1984, Gauldie et al. 1986, Kalish 1989, Gunn et al. 1992, Secor 1992). Strontium has been of particular interest because of its potential to reveal information about environments in which fish live. Strontium (Sr), like calcium (Ca) an alkaline earth element, has a similar crystal ionic radius (Ca = 0.99 Å, Sr = 1.13 Å; Atkins 1978), and can substitute into the aragonite crystal lattice as a trace constituent (Degens et al. 1969).

The incorporation of Sr vs Ca in inorganic aragonitic precipitations has been found to be inversely correlated with the temperature at which it was deposited (Kinsman & Holland 1969), and Radtke (1984, 1989) demonstrated a similar relationship in fish otoliths. However, even larger differences in Sr:Ca

exist when comparing freshwater to marine environments due to the relative scarcity of Sr in fresh water. Seawater strontium concentration is reported as relatively constant (ca 90 µM) throughout the world's oceans (Table 1). Freshwater strontium can vary considerably due to geology and weathering conditions (Odum 1957b, Durum & Haffty 1963, Skougstad & Horr 1963). In the contiguous United States, Sr:Ca levels are generally low in cooler, moister areas and higher in warm, arid ones (Table 1). Distributions of Sr:Ca in U.S. surface waters are typically lognormal: most surface water Sr:Ca ratios are less than 3.5 × 10^{-3} (Odum 1957a, Durum & Haffty 1963, Skougstad & Horr 1963).

Differences appear in relative Sr concentrations in otoliths from fish caught in different salinities, as well. For instance, Odum (1957b) noted nearly 5-fold greater Sr:Ca in marine (Long Island Sound and the Atlantic Ocean at North Carolina, USA) versus freshwater (Mill Pond, Connecticut) otoliths. More recently, microprobe analysis has shown that otoliths of diadromous fishes exhibit large Sr:Ca fluctuations as the freshwater-marine gradient is traversed (migrating eels in the St. Lawrence: Casselman 1982; Derwent (Tasmania) River anadromous salmonids: Kalish 1990; Chesapeake Bay (USA) striped bass: Secor 1992). Coutant & Chen (1993), studying strontium levels along transects of striped bass scales from the Roanoke River-Albemarle Sound (North Carolina) system, found patterns consistent with fish being spawned in higher salinity waters and then moving to fresh water.

I report here on 2 applications of the microprobe technique to examine the Sr:Ca ratios measured along transects on otoliths from juvenile American shad *Alosa sapidissima*, an anadromous herring common along the coastal western Atlantic. In the first application, otoliths were examined from fish that had been part of a growth experiment. These fish were reared entirely in fresh water from a single source, but their diet was switched 42 d post hatch from naturally occurring zooplankton to an artificial feed containing marine fishmeal, which had elevated strontium relative to the freshwater zooplankton. A set of observations on otoliths from 10 fish from this growth experiment, which lasted 12 wk, tests the hypothesis that diet alone can influence the incorporation of Sr into otoliths.

In a demographic study of the 1990 year-class from the Hudson River, New York, USA (Limburg 1994), several juveniles (7 out of 1504 examined) had total lengths ≥130 mm (Fig. 1). In addition, growth rate anomalies were observed in 3 other fish with back-calculated hatch dates in early May. The anomalous fish had otolith growth rate patterns that placed them in the 99 percentile for high growth in May.

Based on length at age or growth rate, these fish, all collected in the lower estuary between river kilometers (Km) 39 and 69, represented outliers in the population. In the second application, microprobe analysis was used to test the hypothesis that these individuals were not from the Hudson-spawned population, but rather were vagrants (sensu Sinclair 1988) from a different river system.

Table 1. Reported concentrations of strontium and calcium in marine and fresh water. Errors are standard deviations unless stated otherwise

Sr (µM)	Ca (mM)	Sr:Ca (× 10^{-3})	Source
Sea water			
92.4		9.23	Odum (1951)[a]
88.4 ± 4.4	10.28 ± 0.02	8.60	Culkin & Cox (1966)[b]
92.9 ± 4.4	10.29 ± 0.03	9.03	Riley & Tongudai (1967)[c]
88.3 ± 3.7			Bernat et al. (1972), Brass & Turekian (1972)[d]
		9.15	Broeker (1974)[e]
Fresh water			
0.99 ± 0.49	0.43 ± 0.14	2.45 ± 0.94	Odum (1957a)[f]
0.68 (range 0.07–9.15)			Durum & Haffty (1963)[g]
2.94		3.00	Skougstad & Horr (1963)[h]
Eastern seaboard (N = 10)		2.83 ± 1.15	
Great Lakes (N = 11)		2.24 ± 1.69	
Florida & Gulf states (N = 12)		4.56 ± 3.79	
Arid Southwest (N = 19)		6.36 ± 2.10	
West coast & Idaho (N = 9)		3.10 ± 1.53	
		1.72	Broeker (1974)[e]

[a] Atlantic Ocean (N = 160). Variation reported as 8.4 %

[b] Worldwide ocean sampling (N = 70). Values reported as Sr (ppm):chlorinity (‰) ratios. Converted by assuming 35‰ average salinity and using the regression (Riley & Chester 1971): Salinity ‰ = 0.03 + 1.8050 Cl ‰

[c] Worldwide ocean sampling (N = 80). Values reported as Sr (ppm):chlorinity (‰) ratios; converted as above

[d] Averages of data reported for Geosec stations in Atlantic, Pacific, and Mediterranean. Calcium not reported

[e] Global average

[f] Average of 6 U.S. eastern seaboard rivers: Housatonic (CT); Roundout Creek (NY); Hudson (NY); Delaware (PA); James (VA); Roanoke (NC)

[g] Median and range for large North American rivers. Neither the names nor the total number of rivers was reported

[h] Geometric means for U.S. rivers (N = 83). Sr:Ca ratios in different regions are broken out below
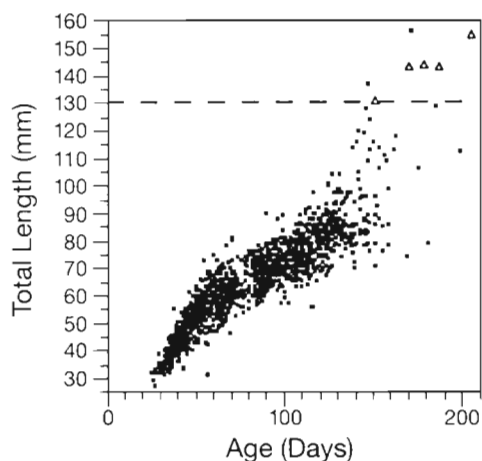
Fig. 1. *Alosa sapidissima*. Plot of total length at age of 1504 juvenile American shad collected in 1990. Dashed line indicates anomalously large fish (99.5 percentile); triangles mark fish analyzed in this study

MATERIALS AND METHODS

Study sites. The Hudson River is located in eastern New York State and drains some 34 680 km^2. It flows in a southerly direction for 507 km, draining into the New York Bight at New York Bay (Limburg et al. 1986). A dam located at Km 245 (Green Island) separates the upper, nontidal river from the lower, tidal portion. The lower Hudson, a partially stratified estuary (Abood 1974), receives salt water through the Verrazano Narrows in New York Bay. The extent of seawater intrusion varies with seasonal freshwater river flows (Abood 1974, Simpson et al. 1974). During summertime low freshwater flows, the salt front (0.5‰) is usually located ca 80 to 100 km up the estuary (Abood 1974). Vertical salinity stratification is usually weak at this time due to mixing (McCrone 1966, Busby & Darmer 1970, Abood 1974, Simpson et al. 1974) but surface-to-bottom gradients of 10‰ at Piermont (river Km 40) can occur (Simpson et al. 1974).

American shad use the tidal part of the river exclusively. Adult shad enter the Hudson in late March or early April, and spawn in fresh water from Km 145 to 245 (peak Km 182) for a prolonged period when temperatures occur between 14 and 20°C (Talbot 1954, Schmidt et al. 1988). Juveniles are found throughout the tidal riverine-estuarine system from July through November, but exit before the onset of winter.

Laboratory study. Laboratory studies were conducted at the National Fishery Research and Development Laboratory (NFRDL), United States Fish and Wildlife Service, located in Wellsboro, Pennsylvania. Experimental fish, of Hudson River origin, were reared from fertilized eggs (8 male and 15 female parents).

18 d old larvae were obtained from the Van Dyke Hatchery, Thompsontown, Pennsylvania, in June 1989 and transferred into an artificial rearing pond at NFRDL, and reared to approximately 50 mm total length (TL). After 24 d in the pond, the fish were given an open-formula feed, ASD-20 (Lemm et al. 1988). Two days later, approximately 1200 fish were collected from the pond and transported to the laboratory, using modifications of techniques described in Backman & Ross (1991). In the laboratory, fish were held in 500 l tanks for a 12 wk growth experiment. The water source for the pond and the lab were identical; both pond and tanks were fed continuously by fresh inflow. Fish were fed at 3 different ration levels on ASD-20 and held at 3 different temperatures. Full details of the growth experiment are described in Limburg (1994). For the present study, otoliths were selected from 5 fish reared in a 22°C, satiation ration treatment, and from 5 fish from a 12.5°C, satiation ration treatment, in order to test for temperature-mediated differences in Sr uptake.

Wild fish collections. Wild fish were collected during the summer and fall of 1990 at 1 upriver, 1 mid-river, and 2 downriver sites (Fig. 2, Table 2). Collections were made either with a 30.5 m bag seine (0.64 cm bar mesh), or with a 7.9 m headrope Carolina wing bottom trawl (3.8, 3.2, and 1.3 cm in the trawl body, cod end, and cod liner, respectively).

Fish for microchemical otolith analysis were selected at random from 4 upriver (Albany: Km 240) and 4 mid-river (Poughkeepsie: Km 120) individuals to provide data on the freshwater Sr:Ca baseline. Seven randomly selected fish were examined from the downriver beach seine site (Tappan Zee: Km 39 to 50). These fish had otolith microstructures which were typical for most juveniles collected throughout the Hudson River in 1990. Three fish with otolith microstructures that showed atypically high growth rates in May and 3 other fish (all 6 collected by bottom trawl in Haver-

Table 2. Description of sampling sites and gear used in 1990 Hudson River collections. Salinity regimes are as defined in Limburg et al. (1986)

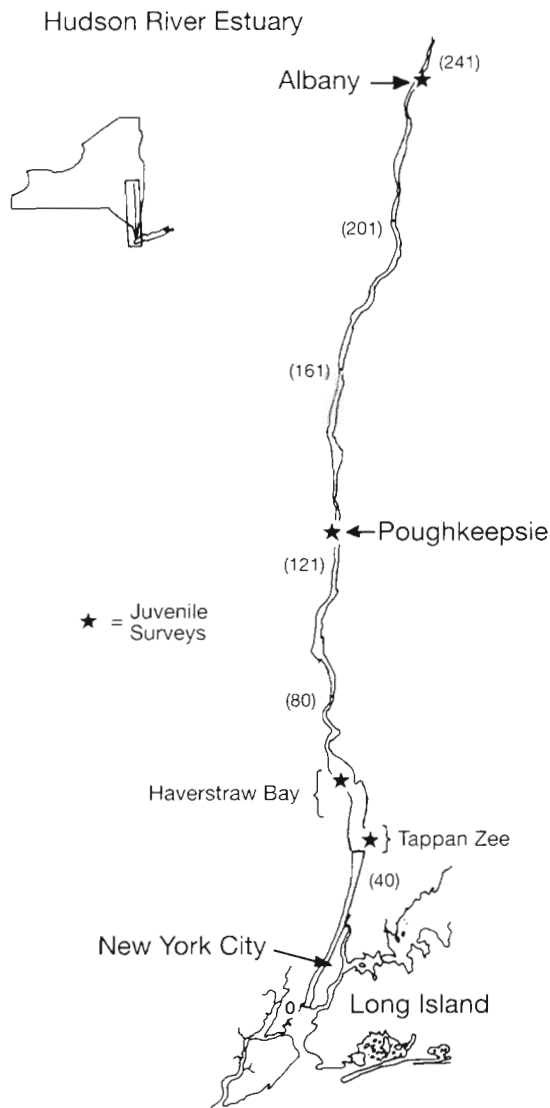| Site name	Distance from mouth (Km)	Salinity regime	Gear type
Albany	240	Freshwater (≤ 0.5‰)	Seine
Poughkeepsie	120	Freshwater (≤ 0.5‰)	Seine
Haverstraw Bay	39 to 69	Mesohaline (5 to 18‰)	Trawl
Tappan Zee	39 to 50	Mesohaline (5 to 18‰)	Seine

Hudson River Estuary

Fig. 2. Map of the tidal Hudson River showing sampling stations. Numbers in parentheses are river kilometers from the mouth

straw Bay on 25 September 1990) were also examined. Otoliths were also assayed from 5 young-of-year shad of unusually large size (> 130 mm TL), collected in the lower estuary in early November Finally, the sagittal otolith from a single adult female, caught at Kingston, New York (Km 145) during the 1991 spawning run, was probed.

Otolith preparation. Sagittal otoliths were extracted, cleaned with 10 % sodium hypochlorite (bleach), air-dried, and subsequently embedded in Spurr epoxy. The cured plastic stubs containing the otoliths were ground to roughly 0.5 mm thickness. Otoliths were then ground to approximately the mid-sagittal plane to expose the core, and polished with the following series of 3M lapping papers: 30, 15, 9, 5, and 3 μm. American

shad otoliths are relatively flat, so grinding to expose the core did not result in loss of outer-most increments. After examination of microstructures, the otoliths were re-polished, this time down to 0.5 μm. The otolith surface was cleaned with 95 % ethanol between polishings. Final cleaning was with ultrasonication, using isopropanol as the cleaning agent. Otolith stubs were then mounted on slides and coated with carbon (~ 250 Å thickness) by high-vacuum evaporation.

Otolith microstructure. Otoliths were examined with a Leitz Orthoplan compound microscope with video attachment. Daily increments have been validated for larval and juvenile American shad (Savoy & Crecco 1987, Limburg 1994). Increments were counted (2 to 3×) on the major posterior axis. Increment widths were measured by placing cash register tape on the video screen and marking increments on the tape. Distortion due to curvature of the screen was slight (< 5%). Increment marks were then digitized with a Graf/Bar GP-7 sonic digitizer (Science Accessories Corporation) and normalized to the length of the major posterior axis. Precision on counts was ± 3 d and accuracy on known-age juveniles was within 5% (Limburg 1994).

Microprobe analysis. Sr and Ca concentrations were quantified with a JEOL 733 wavelength dispersive electron microprobe at the Materials Science Center EM Facility, Cornell University, New York. Following recommendations by Gunn et al. (1992), long counting times along with a wide beam diameter of 20 μm were used to collect Sr counts; this reduces specimen damage and improves the quality of the data. Accelerating voltage was set at 20 kV and probe current at 20 nA. Sr was counted for 60 s and Ca for 20 s. Background was measured for 30 s on each side of the Sr peak and subtracted. Calcite was used for the calcium standard. The Sr standard was Corning Glass 'X' (distributed by the Microbeam Analysis Society), which has a low concentration of SrO (0.79% by weight) and is thus suitable for trace element analysis. Weight percents of SrO and CaO were computed from X-ray counts with the Bence-Albee correction factors (Albee & Ray 1970), and converted to atom percents of Sr and Ca, respectively. Precision of Ca and Sr (as measured on the standards) was 0.2 and 1.4%, respectively (Goldstein et al. 1981). The minimum detectable level of Sr was 290 ppm. Results are given as atoms Sr per 1000 atoms Ca.

Transects were made along the longest posterior axis of the otolith, as had been done for measuring growth. Between 9 and 20 equally spaced points were assayed, with interpoint distances ranging from 25 to 99 μm apart, depending on the size of the otolith and the number of data points read. After microprobe measurements, the distance of the first point (visible as a

faint mark on the carbon coating) to the otolith primordium was measured with an ocular micrometer.

Analysis of artificial diet. A sample of the diet fed to fish at NFRDL was archived and analyzed for Sr and Ca with inductively coupled argon plasma atomic emission spectrometry (ICP) at the Nutrient Analytical Laboratory, Food and Vegetable Science Department, Cornell University. 300 mg of sample was digested in 2.0 ml redistilled HNO_3 and 0.250 ml ultrapure $HClO_4$ at 180 to 200°C for several hours until near dryness, redissolved in 0.5 ml HCl and brought to 10.0 ml with distilled H_2O, and read on the ICP. Sample error was within 1%. Blanks (5% HCl) did not contain measureable amounts of either element.

RESULTS

Laboratory fish

Fish reared for laboratory experiments showed marked changes in Sr:Ca that corresponded to changes in diet, as seen in 2 typical Sr:Ca transects (Fig. 3). For all but 3 of the fish, initial values were very low (Table 3). Otoliths that were sampled directly on the primordium had low values, in contrast with anadromous salmonids (Kalish 1990). Otoliths of the remaining 3 fish had moderately high initial Sr:Ca values, followed by low values, then followed by high values again as the fish were brought into the laboratory, In many cases, the otolith was sampled during the pond period just when fish had been introduced to the artificial diet; this was reflected as an increase in Sr:Ca. Once in the laboratory, fed entirely on artificial diet, Sr:Ca values quickly reached a maximum value and then declined somewhat (Fig. 3). This may have been due to a slow-down in growth rate over time.
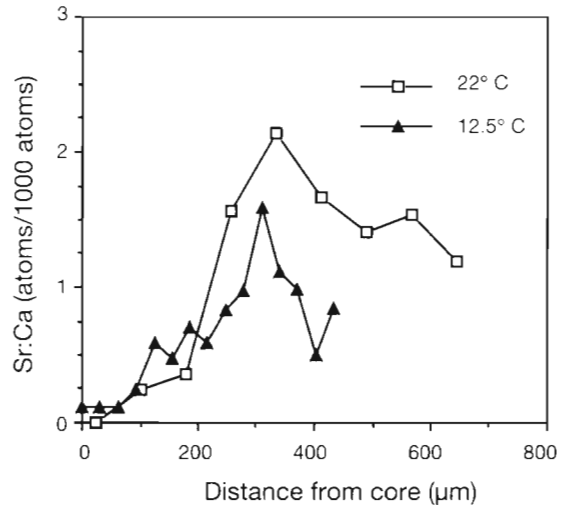

Fig. 3. *Alosa sapidissima*. Comparison of Sr:Ca transects from fish reared in the laboratory at 2 different temperatures. The peak Sr:Ca values occur just after the fish were moved into the laboratory. The data point just prior to the peak is from the rearing pond and reflects the introduction of ASD-20 diet into the pond

Repeated measures analyses of variance (ANOVA) revealed that diet was the main source of variation (p < 0.0001). Neither laboratory temperature treatment nor diet by temperature treatment had significant effects on otolith Sr:Ca, due to within-subject variability and small sample size (necessitated by cost constraints).

Sr in the artificial diet, determined by ICP analysis, was 158 ppm and Ca was 19 180 ppm, giving a Sr:Ca ratio of 3.77×10^{-3} (atom basis). The averaged peak value (reflecting maximum incorporation of Sr) of the pooled 22 and 12.5°C treatments is $1.77 \times 10^{-3} \pm 0.39 \times 10^{-3}$ (mean 2.10×10^{-3} at 22°C and 1.52×10^{-3} at 12.5°C). The average Sr:Ca for the hatchery/pond phase is 0.31×10^{-3} without the 3 fish with high initial values, and 0.54×10^{-3} with all fish. Using the latter number as the freshwater baseline, the artificial diet caused an increase in Sr:Ca of up to 3.3 times, averaged across all 10 fish. During uptake from the artificial diet, Sr was discriminated against by a factor of 3.06 [$= Sr:Ca_{ASD-20} \div (Sr:Ca_{Lab\,max.} - Sr:Ca_{Pond\,avg.})$].

Wild fish observations

Typical transects of individual fish assayed from each river region are shown in Fig. 4 and mean regional values from all fish in Fig. 5. Fish from freshwater reaches of the Hudson River had low Sr:Ca ratios (Fig. 4a, b). Values ranged

Table 3. Sr:Ca ratios ($\times 10^{-3}$) for 10 fish reared 18 d in a hatchery, 26 d in an artificial pond, and 144 d in the laboratory. Means for the hatchery/pond do not include data reflecting the introduction of artificial diet (ASD-20) into the pond

Fish ID	Lab. rearing temp. (°C)	Average Sr:Ca (± SE) Hatchery/pond	Lab	Max. Sr:Ca in the lab
C1–X7	22	0.20 (0.10)	1.59 (0.13)	2.14
C1–X2	22	0.21 (0.08)	1.46 (0.13)	2.17
C1–X11	22	1.23 (0.30)	1.46 (0.11)	2.17
C1–X8	22	0.99 (0.18)	1.32 (0.10)	2.01
C1–X4	22	0.31 (0.08)	1.15 (0.15)	2.02
A3–08	12.5	1.01 (0.31)	1.09 (0.16)	1.57
A3–07	12.5	0.31 (0.07)	0.99 (0.22)	1.82
A3–11	12.5	0.43 (0.09)	1.00 (0.15)	1.59
A3–20	12.5	0.43 (0.13)	0.98 (0.13)	1.45
A3–22	12.5	0.29 (0.08)	0.70 (0.13)	1.19

Table 4. Sr:Ca levels (atoms Sr/1000 atoms Ca) reported in freshwater fish otoliths

Species	Location	Mean	Range	Source
Lepomis gibbosus	Connecticut, USA	0.92	0.8–1.36	Odum (1957b)
Oncorhynchus mykiss	Tasmania	0.95	0.83–1.07	Kalish (1990)
Salmo trutta	Tasmania	0.8	0.4–1.1	Kalish (1990)[a]
Carassius aurata	Japan	0.4	0.2–0.6	Mugiya et al. (1991)
Morone saxatilis	S. Carolina, USA	0.59	0.19–1.00	Secor (1992)
Anguilla spp.	Canada and Ireland	1.4	1.0–1.9	Casselman (1982)[a]
Alosa sapidissima	Hudson River (Km 120 to 240)	0.79	0.57–1.13	This study

[a] Values reported from those parts of otoliths of anadromous fish that the authors state represent deposition in fresh water

from 0 (below Sr detection limit) to 1.46×10^{-3}. Mean values of transects from fish collected in Albany (upriver) were slightly higher than those collected in Poughkeepsie (Fig. 5), but not significantly so ($F_{1,6}$ =

2.757, p = 0.148). These results are consistent with data reported for fish from other freshwater environments (Table 4).

Otoliths from fish collected in near-shore habitats in the oligo- to mesohaline stretch of the estuary (Tappan Zee) showed more variability. One fish in particular, caught 14 August (Fig. 4c), had 3-fold variation in Sr:Ca. The pattern of Sr:Ca along that transect suggests an early experience with elevated Sr, then a decline, and finally a marked increase. Another fish, caught 18 October, had elevated Sr:Ca in the middle and the end of the transect. These excursions were higher than any values recorded in the freshwater reaches, and when the otoliths were re-assayed in the same regions, were repeatable.
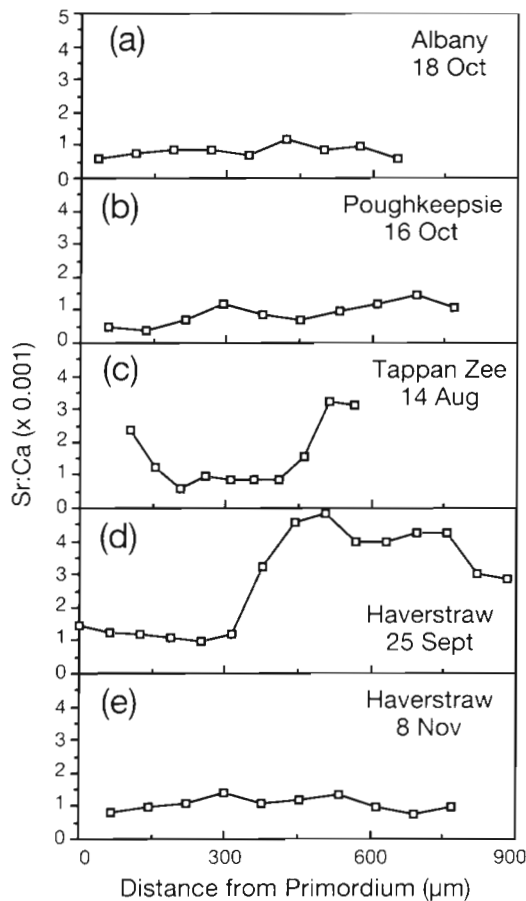

Fig. 4. *Alosa sapidissima*. Representative otolith Sr:Ca transects from individual fish collected in different regions of the estuary. (a) Upper estuary near Albany, New York (Km 240). (b) Mid-estuary near Poughkeepsie (Km 120). (c) Near-shore habitats in the lower Hudson River estuary, Tappan Zee area (Km 39 to 50). (d) Offshore habitats in the lower Hudson River estuary, Haverstraw Bay and Tappan Zee (Km 39 to 69). (e) Offshore in lower estuary
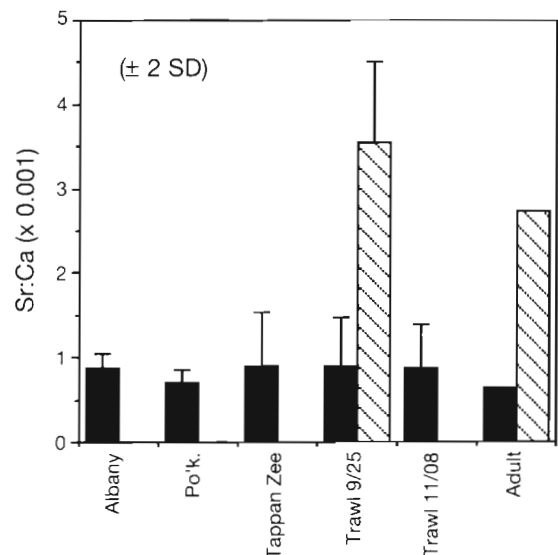

Fig. 5. *Alosa sapidissima*. Mean otolith Sr:Ca ratios from wild American shad collected in the Hudson River. Black bars indicate average whole-transect values for fish in Albany, Poughkeepsie, Tappan Zee, and the 8 November lower estuarine trawl, and only the presumed freshwater phase of 25 September lower estuarine trawl-collected and adult shad. Striped bars show mean transect values for the presumed seawater phase of the adult and the fish collected on 25 September
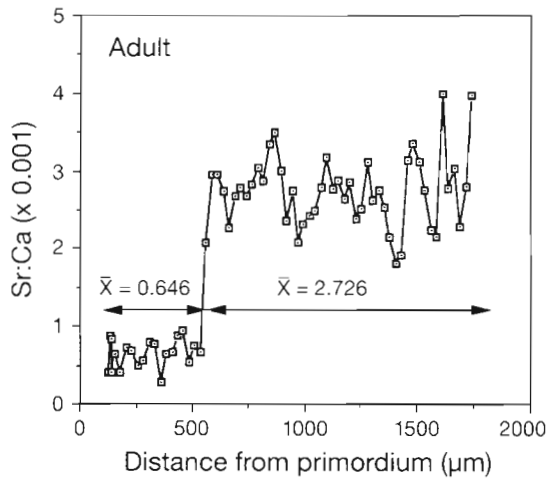
Fig. 6. *Alosa sapidissima*. Otolith Sr:Ca transect of female adult shad (age 4) caught near Km 145, May 1991

Sr:Ca transects of otoliths from young-of-year fishes that were suspected 'vagrants' (meaning they originated in a non-Hudson River population but entered the Hudson), collected by bottom trawl in the lower estuary (Haverstraw Bay), show different patterns (Fig. 4d). All 6 specimens examined from 25 Sep-

tember show a pattern of initially low Sr:Ca, followed by rapid increases, indicative of movement into a marine environment (Radtke et al. 1988). Mean increases (averaging the presumed 'freshwater' and 'marine' data) ranged from 250 to 620% in the 6 fish, and are in good agreement with the Sr:Ca record from the otolith of an adult shad (Fig. 6) and with adult anadromous striped bass (Secor 1992). In contrast, anomalously large specimens collected on 8 November (Figs. 4e & Fig. 5) show low Sr:Ca values consistent with data presented for freshwater resident fish.

Daily otolith increment widths and superimposed Sr:Ca transects for 1 of the Haverstraw Bay fish collected on 25 September (Fig. 7a), and for a fish collected in the same region in early November (Fig. 7b) show different patterns. The otolith growth tracks differ markedly in the timing of peak growth: the September-caught fish shows a peak in early June (Days 150 to 160), whereas the maxima in the November-caught fish center on Days 190 to 200 (mid-July). Growth rates in the first 30 d differ dramatically, despite proximity in back-calculated hatch dates: the November fish shows growth that agrees with the growth of larvae collected in late May and June 1990 (LMS 1992, Limburg 1994), and with water temperature data (LMS 1992). When compared statistically with the distribution of average May growth rates of fish hatched before 16 May, the probability that the fish shown in Fig. 7a belonged to the Hudson population is very low ($z = 4.280$; $p < 10^{-4}$).

The increase in Sr:Ca (Fig. 7a) appears to coincide with a decline in otolith growth rate. This may be fortuitous, as growth rate increases and declines are typical also of fish in freshwater habitats (Fig. 7b), without corresponding changes in Sr:Ca. Estimated 'sea entry dates' (dates when Sr:Ca rises on the otolith transect) for all fishes collected 25 September occur in June through mid August (Table 5).
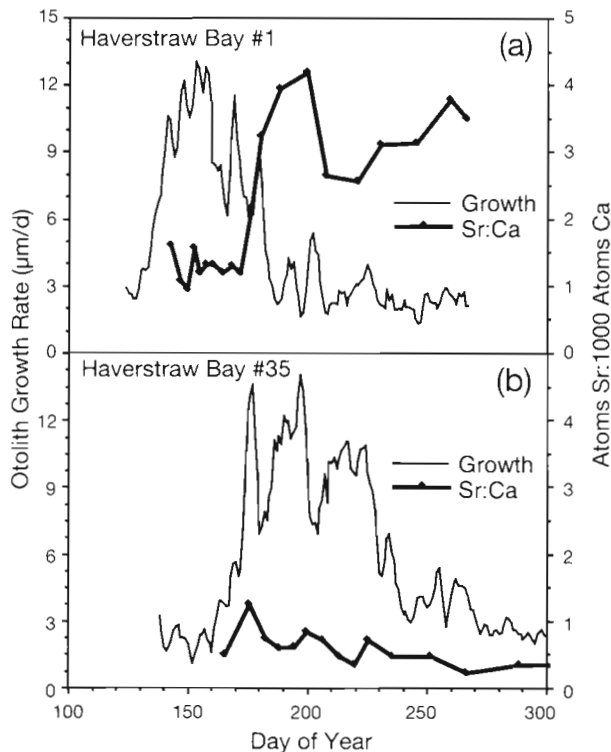

Fig. 7. *Alosa sapidissima*. Time-series plots of daily otolith growth rate and Sr:Ca for a migrant and a resident fish. (a) Fish caught in Haverstraw Bay trawl, 25 September 1990. (b) Fish caught in Haverstraw Bay trawl, 8 November 1990

Table 5. Total lengths (TL) at capture, estimated dates of entering seawater (defined as when Sr:Ca values increase above 2.5×10^{-3}), and estimated age of fishes when entering seawater for shad collected in the Hudson River, Km 39 to 69, 25 September 1990

ID	TL (mm)	Seawater entry Date	Age (d)
1	80	25 June	52
11	79	28 June	51
13	75	13 July	41
16	85	27 June	48
20	72	5 July	65
21	75	14 August	65

DISCUSSION

Sr uptake from food

Fish reared in fresh water had significantly higher otolith Sr:Ca ratios when switched from a diet of freshwater zooplankton to artificial food containing at least 50% marine herring and shrimp. The magnitude of Sr:Ca increase (average 3.3 times) was not quite as large as that reported by Mugiya et al. (1991). In that study, goldfish were exposed to 10 mg l^{-1} Sr in solution, and showed a >5-fold increase in otolith Sr:Ca over controls.

In the present study, within-subject variability was great enough to swamp the possible effects of temperature on Sr uptake by fish in the lab. However, fish reared at 22°C consistently had higher Sr:Ca values than fish reared at 12.5°C (Table 3), and a temperature effect might have been detectable with a larger sample size. Hoff & Fuiman (1993) found an increase in both Sr and Ca in red drum *Sciaenops ocellatus* otoliths with increasing temperature and age. In the fish I examined, the relationship appears more closely coupled with growth rate, since highest rates of Sr incorporation occurred shortly after the fish were moved into the laboratory when the otoliths were still growing rapidly, and declined as otolith growth rates decelerated, even as the temperature remained constant. On the other hand, the observed peaks in otolith Sr:Ca may also be associated with the stress of being moved from the pond into the laboratory; further work would be required to separate the effects of diet and stress.

Sr:Ca in wild fish otoliths

Otolith strontium concentration is affected by salinity more than it is affected by temperature (Secor et al. 1995). Although the degree to which otolith Sr varies along a seawater gradient has not been verified for American shad, the results in this study are consistent with previous findings (Casselman 1982, Radtke et al. 1988, Kalish 1989, 1990, Secor 1992).

Because a large part of the Hudson valley is composed of marine carbonates, it is of interest to know the Sr:Ca ratios of Hudson valley bedrock, and how bedrock influences strontium and calcium in river water. The lower Hudson drainage runs through limestone (Km 160 to 250), shales and slates (Km 100 to 160), and further south a mix of igneous, metamorphic, and sandstone rock (Johnsen 1966). Limestones have relatively low Sr:Ca ratios (average 0.81 ± 0.83 SD × 10^{-3}; Kulp et al. 1952). Odum (1957b) suggested that these low ratios were due not only to the predominance of calcite, the crystal lattice of which does not

incorporate Sr as readily as aragonite, but also because processes that cause calcite deposition may in turn release strontium into solution. Shales collected in the Hudson valley and adjoining Catskills were enriched in Sr compared to carbonates (range 1.55 to 12.62 × 10^{-3} Sr:Ca; Turekian & Kulp 1956). Adirondack rock (mainly Canadian Shield anorthosite), which underlies the uppermost Hudson drainage, is strontium enriched (average of 4 samples, 49.98 × 10^{-3} Sr:Ca, 10.09 × 10^{-3} SD; Turekian & Kulp 1956).

Relatively few data exist on Sr:Ca ratios in Hudson River waters. Fabricand et al. (1968) measured strontium, calcium, and salinity longitudinally on the Hudson from Km 56 to 240 in July 1965. A minimum of 1.62 × 10^{-3} Sr:Ca was found at Km 180 (North Germantown) and increased to 3.08 × 10^{-3} at Km 240. The highest ratio (7.32 × 10^{-3}) was found in bottom waters at Km 56 (Tappan Zee). In 1990, Sr:Ca values in the Hudson River measured at Green Island (Km 245) averaged 2.45 ± 0.14 × 10^{-3} (N = 4; Firda et al. 1991) in good agreement with Mohawk River data (2.66 × 10^{-3}) presented by Skougstad & Horr (1963). The Sr:Ca minimum in Hudson River water between Poughkeepsie and Germantown (Km 120 to 180) may reflect passage through low Sr:Ca drainages. Otoliths of shad collected in Poughkeepsie had slightly lower Sr:Ca ratios compared to Albany shad (Fig. 5).

Otolith Sr:Ca was generally low (< 2 × 10^{-3}) in wild Hudson River fish, with the notable exception of 6 fish collected by bottom trawl in the lower estuary. Since these fish all came from the same net haul, it is possible they were part of a school that had wandered in to the Hudson from saline waters surrounding New York Bay, i.e. the New York Bight or Long Island Sound. Odum (1957a) reported Sr:Ca values for Long Island Sound averaging 9.39 × 10^{-3} (± 1.10 × 10^{-3} SD) and 1 datum from the Hudson River at Poughkeepsie, New York of 2.65 × 10^{-3}. The 3.5-fold difference in seawater versus freshwater Hudson Sr:Ca is consistent with differences observed in the migrating fish.

Alternatively, these fish may have moved into higher salinity bottom waters near the estuary mouth. Mean bottom salinities in the lower estuary (Yonkers vicinity, Km 19 to 32) ranged from 3.3 to 11.4‰ during June-October (LMS 1992). A single datum exists from Fabricand et al. (1968) of 7.32 × 10^{-3} Sr:Ca at 10.2‰ salinity. This value is 4.5 times higher than the minimum reported in the same study. Such a difference is also consistent with differences seen in the shad otoliths, albeit from a single data point. Further collection of strontium data in the lower Hudson, in combination with controlled rearing of shad in different salinities, would help to clarify whether shad could pick up sufficient strontium in the lower estuary to show patterns as in Fig. 4d.

When the Sr:Ca data are aligned with otolith growth data to obtain estimates of calendar dates corresponding to the Sr:Ca samples, Sr:Ca levels rise in late June through mid August (Table 5), indicating early experience with elevated salinity. It is likely that at least 3 of the fish were of Hudson River origin, based upon otolith microstructure. The 3 remaining fish showed 4- to 5-fold higher growth rates in late May and early June than would be expected from otoliths of larvae collected during that period (Limburg 1994). Growth rates of larvae, which were collected throughout the tidal Hudson River, corresponded well with temperature records in May and June (LMS 1992). The entire Hudson was cool during May (14.3°C, Km 0 and 120), due in part to heavy rainfalls (Limburg 1994) and larval lengths remained around 10 to 12 mm (LMS 1992) with correspondingly low otolith increment widths (Limburg 1994). The larval and thermal data are at odds with the otolith growth tracks of the remaining 3 fish collected on 25 September. While it is possible these fish had discovered a completely different (most likely warmer) environment within the Hudson, it is also possible that they were hatched in another river system with a different thermal regime during May. The evidence from fish size distributions, otolith growth rates, and hydrographic data (Limburg 1994) points to the second possibility.

None of the other trawl-collected fish (8 November) showed elevated Sr:Ca ratios, nor were their otolith growth patterns atypical when compared to other fishes from the study. However, their large sizes were anomalous: whereas most fish were <100 mm TL and weighed <6 g, these fish were >130 mm TL (up to 155 mm) and weighed in excess of 12 g (up to 26 g). Such large fish may have been using a different habitat than fish in the near-shore areas; however, in general juvenile shad appear to use near-shore areas during the daytime and move off-shore at night (Limburg pers. obs.).

In addition, 2 or 3 otolith trajectories from fish caught by beach seine in near-shore habitats in the lower Hudson estuary showed moderate to marked increases in Sr:Ca at various points. These variations may have arisen when saline water masses moved into the estuary as summertime freshwater flows declined; however, one might expect to see similar variations on all the otoliths from fish caught in that area, if they were present there during these proposed seawater intrusions. Alternatively, individual fish may have made excursions into waters of increased salinity, and then moved to lower salinity areas. Such movements might be either longitudinal (downriver to saltier waters, then retreat upriver) or horizontal (movement into deeper, more saline channels and then back into fresher shallows).

These results have important implications for our understanding of American shad life history. The Sr:Ca data provide evidence of departure (Table 5) from the natal freshwater environment much earlier than has been previously reported (Walburg & Nichols 1969, Leggett & Whitney 1972, Leggett 1976, O'Leary & Kynard 1986), and at a much younger age (ca 6 to 9 wk old). Assuming the patterns of elevated Sr:Ca do reflect seawater entry, then these are the first data to confirm early (pre-autumn) out-migration of juvenile American shad from natal rivers, a phenomenon that has been hypothesized by Chittenden (1969) and Marcy (1976). Further, juvenile American shad may make brief forays back and forth between estuary and sea, or wander back into estuaries during the first year, phenomena which have not been reported at all.

To confirm adequately that the 3 'suspected vagrants' originated in another river system, temperature records from other spawning rivers would be needed, in addition to stock identification data, e.g. mitochondrial DNA or meristic information. If these are truly 'vagrants' from another population (Sinclair 1988), interesting questions could be posed. For instance, would these fish have eventually returned to their natal river to spawn, or alternatively would they have not shown natal river fidelity? Lack of fidelity to a natal river can have important consequences for the evolution of population structure.

The immediate fate of out-migrating young-of-year shad is largely unknown, although there is evidence that juveniles shoal just off the coast during the first winter (Milstein 1981). The relative success of early versus late out-migrants in terms of their contribution to spawning populations is also unknown. With the microprobe technique, it should be possible to examine the first year's growth in otoliths of returning adult shad, and to estimate fairly accurately the time of their departure from a natal freshwater environment. This information would shed light on successful migration strategies.

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