

Inter-annual variability in isotope and elemental ratios recorded in otoliths of an anadromous fish

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1 **Abstract**

2 Isotope ratios and elemental concentrations in otoliths are often used as natural tags to
3 reconstruct migratory movements and connectivity patterns in marine and
4 anadromous fishes. Although differences in otolith geochemistry have been
5 documented among geographically separated populations, inter-annual variation
6 within locations is less frequently examined. We compared otolith isotope ($\delta^{18}\text{O}$ and
7 $^{87}\text{Sr}:^{86}\text{Sr}$) and elemental ratios (Sr:Ca and Ba:Ca) from several annual cohorts of
8 juvenile American shad (*Alosa sapidissima*) in three rivers. These four geochemical
9 signatures distinguished among river-specific populations of this species at both large
10 and small geographic scales, with $\delta^{18}\text{O}$ and $^{87}\text{Sr}:^{86}\text{Sr}$ generating the majority of
11 multivariate variation. We found significant variation among years for all variables in
12 two to three rivers. However, the magnitude of variability differed among ratios, with
13 $\delta^{18}\text{O}$ ratios showing substantial inter-annual shifts while $^{87}\text{Sr}:^{86}\text{Sr}$ ratios were
14 relatively stable across years. Sr:Ca and Ba:Ca ratios also varied among years. These
15 results imply that investigators using environmentally labile signatures must quantify
16 geochemical signatures for each cohort of interest in order to confidently identify
17 origins of migrants.

18

19 Keywords: otolith chemistry; strontium isotopes; oxygen isotopes; inter-annual
20 variability.

21

22 **1. Introduction**

23 Geochemical signatures recorded in calcified tissues of fishes have the potential
24 to resolve outstanding questions about dispersal and migration dynamics of a wide
25 variety of species. Isotope and elemental ratios of aragonitic otoliths, or ear stones,
26 have proved particularly useful for identifying rates of natal homing (Thorrold et al.,
27 2001), dispersal (Thorrold et al., 2002), thermal histories (Valle and Herzka, 2008),
28 and movements across salinity gradients (Milton and Chenery, 2005). Because
29 otoliths are metabolically inert, accrete discrete layers incrementally, and incorporate
30 some isotopes and elements in proportion to their ambient abundance, they can serve
31 as useful natural tags that reflect the environmental history of a fish (Campana, 1999).
32 When natal geochemical signatures are unique and distinct at appropriate
33 geographical scales, they can then be used to identify origins of individuals at
34 subsequent life history stages. Thus, the first step in many investigations using
35 otoliths as natural tags is to create a baseline map of elemental and isotope signatures
36 from potential source regions. Yet while much attention has been paid to
37 geographical scales of variability for the ratios of interest, temporal stability is less
38 frequently investigated. Understanding temporal variability in both isotope and
39 elemental signatures is essential to determine whether classifications of unknown
40 individuals can only be made using baseline data from the same cohort, or if previous
41 baselines can be applied.

42 For the previous two decades, investigations into the natural tag properties of
43 otoliths have focused on the relative abundances of elements such as Sr and Ba,
44 typically expressed relative to Ca (Campana, 1999). However, geographical
45 variability in isotope ratios have emerged as powerful natural tags, particularly for
46 species that inhabit fresh water at some stage of their life history (Kennedy et al.,

47 1997). For instance, otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios directly reflect dissolved ambient ratios,
48 which in freshwater habitats depend on the geological composition of the drainage
49 basin (Palmer and Edmond, 1992). Because juvenile anadromous fishes reside in
50 discrete freshwater habitats that drain heterogeneous lithologies, otolith $^{87}\text{Sr}:^{86}\text{Sr}$
51 ratios have recently been used to discriminate origins of anadromous fishes at
52 remarkably fine geographical scales (Barnett-Johnson et al., 2008; Kennedy et al.,
53 2002). Similarly, otolith $\delta^{18}\text{O}$ ratios are deposited in isotopic equilibrium with
54 ambient water values (Høie et al., 2003; Thorrold et al., 1997). As a result, latitudinal
55 and orographic patterns in surface water $\delta^{18}\text{O}$ ratios are recorded in otoliths across
56 large geographic scales (Walther et al., 2008). The addition of these two isotope
57 ratios to the suite of geochemical signatures routinely analysed has increased
58 estimates of classification accuracy beyond that generally achievable based only on
59 elemental ratios.

60 In a test of the combined power of isotope and elemental ratios to discriminate
61 among source populations, Walther and Thorrold (in press) reported $^{87}\text{Sr}:^{86}\text{Sr}$, $\delta^{18}\text{O}$,
62 Sr:Ca, and Ba:Ca ratios in the otoliths of juvenile American shad (*Alosa sapidissima*)
63 from 20 rivers between Florida and Québec along the east coast of North America.
64 This combination of only four elemental and isotopic signatures yielded highly
65 distinct river-specific signatures; mean classification accuracies were 93%.
66 Moreover, signature separation was driven primarily by $\delta^{18}\text{O}$ and $^{87}\text{Sr}:^{86}\text{Sr}$,
67 highlighting the utility of these isotopes in discriminating among these rivers.
68 Although this prior work comprehensively addresses the spatial variability in these
69 chemical tracers, inter-annual variability has not been thoroughly investigated for
70 these systems. Here, we expand on our previous work to examine inter-annual
71 variability in otolith signatures for the Hudson, the Mattaponi and Pamunkey rivers.

72 These data are used to discuss potential errors that could arise if migrants are not
73 classified using baseline signatures from the appropriate cohort.

74

75 **2. Materials and Methods**

76 Juvenile American shad were collected in freshwater or upper estuarine
77 habitats from the Hudson, Mattaponi, and Pamunkey rivers (Figure 1) prior to their
78 emigration to the ocean. Collections were timed to occur during the late summer
79 months when juveniles were at their highest abundances in each river. Push nets and
80 beach seines were used to obtain representative samples and specimens were
81 subsequently returned to the lab and frozen whole. Sagittal otoliths were dissected
82 from each fish, cleaned of adhering tissue, and stored dry. Samples were unavailable
83 for analysis in 2003 for all rivers and in 2002 and 2004 for the Hudson and Pamunkey
84 rivers, respectively, due to recruitment failure in those systems or incomplete
85 collections. Analyses were performed on all available samples from these three
86 rivers. Prior to analysis, both sagittal otoliths from each fish were mounted on
87 petrographic slides with cyanoacrylic glue and ground to the midplane on 30 and 3
88 μm lapping film. One otolith from each pair was randomly chosen for Sr:Ca, Ba:Ca
89 and ^{87}Sr : ^{86}Sr analyses. To remove surface contaminants, this otolith was sonicated for
90 2 minutes and triple-rinsed in ultrapure water in a class 100 clean room. The
91 remaining otolith was used for $\delta^{18}\text{O}$ analyses.

92 The first otolith from each fish was used for analyses of Sr:Ca and Ba:Ca
93 ratios using a Thermo Finnigan Element 2 single collector inductively coupled plasma
94 mass spectrometer (ICP-MS) coupled to a 213 nm laser ablation system. A 200 x 200
95 μm raster was ablated adjacent to the core and extending toward the posterior lobe.
96 This raster ablated material laid down over approximately two to three months of the

97 juvenile freshwater residency period. Elemental ratios were quantified by monitoring
98 ^{48}Ca , ^{86}Sr , and ^{138}Ba using methods following those of Rosenthal et al. (1999) as
99 modified by Walther et al. (2008). Briefly, a He gas stream carried ablated material to
100 the ICP-MS where it was mixed with an Ar sample gas and a wet aerosol (2% HNO_3)
101 supplied by a self-aspirating ($20 \text{ m}\cdot\text{min}^{-1}$) PFA nebuliser in the concentric region of
102 the quartz dual inlet spray chamber. Instrument blanks of 2% HNO_3 and two certified
103 reference materials (CRM; Sturgeon et al., 2005; Yoshinaga et al., 2000) were run at
104 the beginning and end of each block of ten otoliths and used to correct for background
105 intensities and instrument mass bias. External precision (relative standard deviation)
106 of the technique, calculated by treating one of the CRMs as an unknown for 2000,
107 2001, and 2002 samples was 0.3% for Sr:Ca and 0.6% for Ba:Ca (CRM $n = 92$).
108 Otoliths collected in 2004 were analysed separately, and relative standard deviations
109 were 0.3% for Sr:Ca and 1% for Ba:Ca (CRM $n = 134$).

110 After elemental ratio analyses, the same otolith was used for ^{87}Sr : ^{86}Sr analyses
111 using a Thermo Finnigan Neptune multiple collector ICP-MS coupled to a 213 nm
112 laser ablation system. A $250 \times 200 \mu\text{m}$ raster was ablated adjacent to previous raster
113 and covering the same time period analysed for elemental ratios. A suite of isotopes,
114 including ^{84}Sr , ^{86}Sr , ^{87}Sr , ^{88}Sr , ^{83}Kr , and ^{85}Rb were monitored. Contributions of ^{87}Rb
115 to ^{87}Sr and ^{86}Kr to ^{86}Sr intensities were removed by applying mass bias corrections
116 described by Jackson and Hart (2006) as modified by Walther et al. (2008). All data
117 were normalized to a SRM987 ^{87}Sr : ^{86}Sr value of 0.71024 based on mean ^{87}Sr : ^{86}Sr
118 values measured in SRM987 for a given analysis day. For otoliths collected in 2000,
119 2001, and 2002, mean (± 1 SD) values of ^{87}Sr : ^{86}Sr ratios sampled from an otolith
120 CRM ($n = 38$) and solutions of SRM987 ($n = 40$) were 0.70915 (± 0.00002) and
121 0.71025 (± 0.00002), respectively. For otoliths collected in 2004, mean (± 1 SD)

122 values of $^{87}\text{Sr}:^{86}\text{Sr}$ ratios sampled from an otolith CRM ($n = 74$) and solutions of
123 SRM987 ($n = 41$) were $0.70916 (\pm 0.00002)$ and $0.71025 (\pm 0.00002)$, respectively.
124 These data compare favourably to the accepted marine $^{87}\text{Sr}:^{86}\text{Sr}$ value (0.70918 ;
125 Ingram and Sloan, 1992) and the certified $^{87}\text{Sr}:^{86}\text{Sr}$ value of SRM 987 (0.71024).

126 Oxygen isotope ratios were obtained from the second otolith of each fish using
127 isotope ratio monitoring mass spectrometry (irm-MS). A $400 \times 400 \mu\text{m}$ raster with a
128 $75 \mu\text{m}$ depth was removed from an area adjacent to the nucleus and extending toward
129 the posterior lobe. The powder from the milled region was placed in glass
130 scintillation vials and analysed on a Thermo Finnigan MAT 252 equipped with a Kiel
131 III carbonate device following methods outlined by Ostermann and Curry (2000).
132 Isotopic values are reported relative to Vienna Pee Dee belemnite (VPDB) in standard
133 δ notation. The precision estimate for the mass spectrometer based on long-term
134 monitoring of the NBS19 standards was $\pm 0.07\text{‰}$ (Ostermann and Curry, 2000).

135 Samples from 2000-2002 were additionally analysed for Mn:Ca, Mg:Ca and
136 $\delta^{13}\text{C}$ ratios (Walther et al., 2008). However, it was determined that the addition of
137 these ratios did not improve classification accuracies obtained using just Sr:Ca,
138 Ba:Ca, $^{87}\text{Sr}:^{86}\text{Sr}$, and $\delta^{18}\text{O}$ ratios (Walther and Thorrold, in press). Thus, analyses of
139 the additional ratios were not performed for 2004 samples and are excluded from this
140 investigation.

141 Variable numbers of juveniles were collected and analysed each year. For
142 instance, 50-59 individuals were analysed in 2004 compared to 18-28 individuals for
143 earlier year classes. In order to compare approximately equal sample sizes across
144 years for each river, we randomly selected a subset of individuals from the larger
145 sample sizes to achieve a balanced design. Numbers of individuals included in
146 analyses were between 27-28 for the Hudson River, 24-28 for the Mattaponi River,

147 and 18-19 for the Pamunkey River. The randomized subsampling procedure did not
148 significantly alter the means or standard deviations of isotope or elemental ratios.

149 One-way analyses of variance (ANOVAs) were performed on mean isotope
150 and elemental ratios within a river with year as a random factor. Variance
151 components for each ANOVA were also calculated to assess the percentage of total
152 variance explained by yearly variation in each ratio for that river. Variances were not
153 homogeneous across years for some ratios, and thus an $\ln(x+1)$ transformation was
154 applied to the data and the ANOVAs were recalculated. This transformation did not
155 alter the significance of any ANOVA, and therefore only the results for the
156 untransformed data are presented.

157 We used discriminant function analysis (DFA) to assess inter-annual
158 variability of multivariate signatures for different combinations of the four chemical
159 ratios. Quadratic DFAs were first calculated for each river using all four ratios, with
160 otoliths grouped by year. Higher misclassification rates indicated more homogeneous
161 multivariate signatures across years. We then recalculated the QDFA for each river,
162 sequentially excluding each ratio in turn to determine the effect on a single ratio on
163 inter-annual misclassification rates.

164

165 **3. Results**

166 Isotope and elemental ratios varied among years within each of the three rivers
167 (Figure 2). Most signatures were significantly different among years, and only Sr:Ca
168 in the Hudson River and $^{87}\text{Sr}:^{86}\text{Sr}$ in the Mattaponi River showed statistically
169 insignificant inter-annual variation (Table 1). Surprisingly, $^{87}\text{Sr}:^{86}\text{Sr}$ ratios were
170 significantly different among years in both the Hudson River and Pamunkey River.
171 Variance components, however, showed that variability in $^{87}\text{Sr}:^{86}\text{Sr}$ ratios accounted

172 for only 5-19% of the total variability within a river. In contrast, $\delta^{18}\text{O}$ accounted for
173 large proportions of the total variance (34-85%). Mean Sr:Ca and Ba:Ca ratios were
174 significantly different in two and three of the rivers, respectively, and accounted for
175 varying amounts of the total variance (14-38% for Sr:Ca and 11-45% for Ba:Ca).

176 The relative importance of each signature in homogenizing multivariate
177 signatures among years was shown by misclassification rates of quadratic DFAs
178 (Table 2). When all four signatures were included, misclassification rates were
179 generally low, averaging 28% for the Hudson River, 8% for the Mattaponi River, and
180 9% for the Pamunkey River. These low misclassification rates indicated the
181 multivariate signatures did not overlap substantially among years for a given river.
182 However, misclassification rates rose to 22-39% on average when $\delta^{18}\text{O}$ ratios were
183 excluded from the multivariate signature. In contrast, excluding $^{87}\text{Sr}:^{86}\text{Sr}$ ratios did
184 not significantly alter misclassification rates. Similarly, the exclusion of Ba:Ca led to
185 higher misclassification rates while the exclusion of Sr:Ca did not have a large effect.

186

187 **4. Discussion**

188 Temporal variability in chemical signatures can pose significant problems for
189 researchers who use them to identify natal origins of mobile organisms. If
190 geographical maps of isotope or elemental ratios are assumed to be stable when in fact
191 they shift over time, spatial and temporal differences may be confounded (Gillanders,
192 2002). As a result, estimates of source origins could be significantly biased if
193 temporally inappropriate baseline signatures are used to classify migrants. Here, we
194 report statistically significant inter-annual variability in mean $^{87}\text{Sr}:^{86}\text{Sr}$, $\delta^{18}\text{O}$, Sr:Ca
195 and Ba:Ca ratios recorded in otoliths of an anadromous fish during the freshwater
196 residency period. Because these combined signatures constitute the baseline map

197 identifying source rivers for this highly migratory fish, care must therefore be taken to
198 match cohorts to the appropriate annual map to identify fish of unknown origins.

199 Of the four ratios we examined here, the most variable was $\delta^{18}\text{O}$. Because
200 otolith $\delta^{18}\text{O}$ ratios is incorporated in isotopic equilibrium with ambient waters (Høie
201 et al., 2003; Thorrold et al., 1997), this variability likely reflected substantial inter-
202 annual shifts in ambient freshwater $\delta^{18}\text{O}$ values. A wide variety of environmental
203 forces can drive temporal shifts in riverine $\delta^{18}\text{O}$ values, including precipitation
204 amount, temperature, evaporation intensities, groundwater contribution, and storm
205 events (Kendall and Coplen, 2001). Indeed, the years sampled in this study covered
206 divergent climatic conditions. The Mattaponi and Pamunkey rivers experienced
207 severe drought conditions between 2000 and 2002, while river flows in 2004 were
208 above average (USGS, 2005). For the Hudson River, 2000 and 2004 were relatively
209 wet years with above average flows while 2001 was a drought year (USGS, 2004).
210 However, we have a limited ability to retrospectively determine mechanisms
211 generating variability in $\delta^{18}\text{O}$ otolith signatures in the absence of detailed water
212 samples constraining variability in ambient waters. Regardless of the cause, $\delta^{18}\text{O}$
213 ratios varied enough to cause significant biases in estimates of natal origin if fish were
214 classified using inappropriate baseline maps. Indeed, $\delta^{18}\text{O}$ shifted up to 1.5‰ among
215 years in the Mattaponi and Pamunkey rivers. Shifts of this magnitude would be
216 equivalent to erroneously classifying a Chesapeake Bay fish as coming from either
217 Georgia or Delaware, depending on the direction of the shift. Clearly, researchers
218 who use environmentally labile signatures such as $\delta^{18}\text{O}$ must be careful to use
219 temporally appropriate baseline maps when classifying migrants of unknown origins.

220 Otolith ratios of $^{87}\text{Sr}:^{86}\text{Sr}$ directly reflect ambient freshwater composition and
221 are not trophically fractionated (Capo et al., 1998; Kennedy et al., 2000). In general,

222 freshwater $^{87}\text{Sr}:$ ^{86}Sr ratios are assumed to be temporally stable since they reflect the
223 combined geological composition of the drainage basin (Palmer and Edmond, 1992),
224 To date, otolith $^{87}\text{Sr}:$ ^{86}Sr ratios have been reported as temporally stable for splittail
225 *Pogonichthys macrolepidotus* (Feyrer et al., 2007), and Atlantic salmon *Salmo salar*
226 (Kennedy et al., 2000). We found that mean otolith $^{87}\text{Sr}:$ ^{86}Sr ratios were significantly
227 different among years in the Hudson and Pamunkey rivers. The reason for this
228 variability is unknown, although increased discharge rates can potentially alter
229 $^{87}\text{Sr}:$ ^{86}Sr ratios (Åberg et al., 1989). Also, inter-annual shifts in spatial patterns of
230 habitat use within a river could alter the $^{87}\text{Sr}:$ ^{86}Sr of otoliths. Yet, although inter-
231 annual differences in otolith $^{87}\text{Sr}:$ ^{86}Sr ratios were statistically significant, overall the
232 variability was relatively small as measured by both variance components and the
233 contribution to misclassification rates. In addition, the magnitude of inter-annual
234 variation is much less than average geographical variation reported by Walther and
235 Thorrold (in press). Pair-wise differences in $^{87}\text{Sr}:$ ^{86}Sr ratios between years were
236 0.0002 on average, an order of magnitude less than average pair-wise differences
237 between rivers (Walther and Thorrold, in press). Further, for the 20 rivers examined
238 by Walther and Thorrold (in press), only 3% of the pair-wise geographic differences
239 between rivers were less than the average inter-annual pair-wise difference. The
240 reason for the higher variance in Hudson river strontium isotope ratios in 2000 is
241 unknown, although it likely reflects the inclusion of fish from isotopically distinct
242 tributaries that were not present in subsequent collections. This indicates the need to
243 obtain sufficient sample sizes to accurately characterize the spread of values
244 encountered in a particular watershed. However, this increased variance likely did not
245 bias the minimal effect of Sr isotope ratios on misclassification rates, since the other
246 year classes from the Hudson River also recorded similar ratios despite smaller

247 variances. Thus, while we observed statistically significant inter-annual variability in
248 $^{87}\text{Sr}:^{86}\text{Sr}$ ratios for two rivers, this variability is minor compared to geographic
249 variability and unlikely to bias classification estimates.

250 Several studies have reported significant temporal variability in otolith Sr:Ca
251 and Ba:Ca ratios for a variety of species (reviewed by Gillanders, 2002). The
252 majority of these studies focus on estuarine or marine species, with many reporting
253 significant variability in Sr and Ba otolith signatures at time scales ranging from
254 seasonal to inter-annual (Bergenius et al., 2005; Elsdon and Gillanders, 2006;
255 Gillanders, 2002; Hamer et al., 2003; Patterson and Kingsford, 2005; Patterson et al.,
256 2004; Patterson et al., 2008; Rooker et al., 2003). Temporal variation in freshwater
257 systems is less frequently reported and not always significant. Feyrer et al. (2007)
258 reported significant differences in otolith Sr:Ca and Ba:Ca across two year classes of
259 splittail *Pogonichthys macrolepidotus*. In contrast, Wells et al (2003) and Munro et
260 al. (2005) report inter-annual stability in Sr:Ca ratios of cutthroat trout *Oncorhynchus*
261 *clarki lewisi* and lake trout *Salvelinus namaycush*, respectively. Using the same
262 species reported here, Thorrold et al. (1998) report significant seasonal variability in
263 Sr and Ba for the Connecticut, Delaware, and Hudson rivers, although the variability
264 was not enough to significantly bias accurate classifications of known-origin fish.
265 Because otolith Sr:Ca and Ba:Ca reflect ambient water composition, as modified by
266 temperature (Bath et al., 2000; Walther and Thorrold, 2006), the variability we
267 detected in American shad otoliths likely resulted from forces that altered ambient
268 composition, such as fluctuations in flow rates or tidally-driven resuspension (Jarvie
269 et al., 2000).

270 In conclusion, we observed statistically significant differences in $^{87}\text{Sr}:^{86}\text{Sr}$,
271 $\delta^{18}\text{O}$, Sr:Ca, and Ba:Ca ratios among years for three rivers. This variability limits the

272 ability of researchers to use a database of juvenile signatures collected in one year to
273 classify fish born in other years. Although inter-annual variability in a ratio such as
274 $\delta^{18}\text{O}$ is more likely to result in classification errors than a more stable signature like
275 $^{87}\text{Sr}:^{86}\text{Sr}$, it would be prudent to match cohorts whenever possible, regardless of the
276 signature used. However, this is not always possible due to a lack of available
277 juvenile otoliths from the cohort of interest. An alternative would be to restrict the
278 database to more temporally stable signatures and pool juvenile otoliths from several
279 years. This approach, taken by Walther et al. (2008), accounts for the range of values
280 likely to be found in the cohort of interest, although it has the potential to decrease
281 overall classification accuracies. Also, this approach assumes that the range of values
282 of the pooled signatures reflects the variability that occurred over longer time periods.
283 The benefit of including or excluding temporally variable chemical ratios will
284 ultimately depend on the system in question and to what extent those ratios
285 significantly improve natal classification accuracies. Extended time series of otolith
286 analyses from one location, ideally with accompanying water samples, would help
287 explore variability on these time scales. Clearly, this issue is of paramount concern to
288 those wishing to accurately identify origins of fish and temporal variability must be
289 accounted for in any well-designed study using otolith chemistry as a natural tag.

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420

421 **Figure captions**

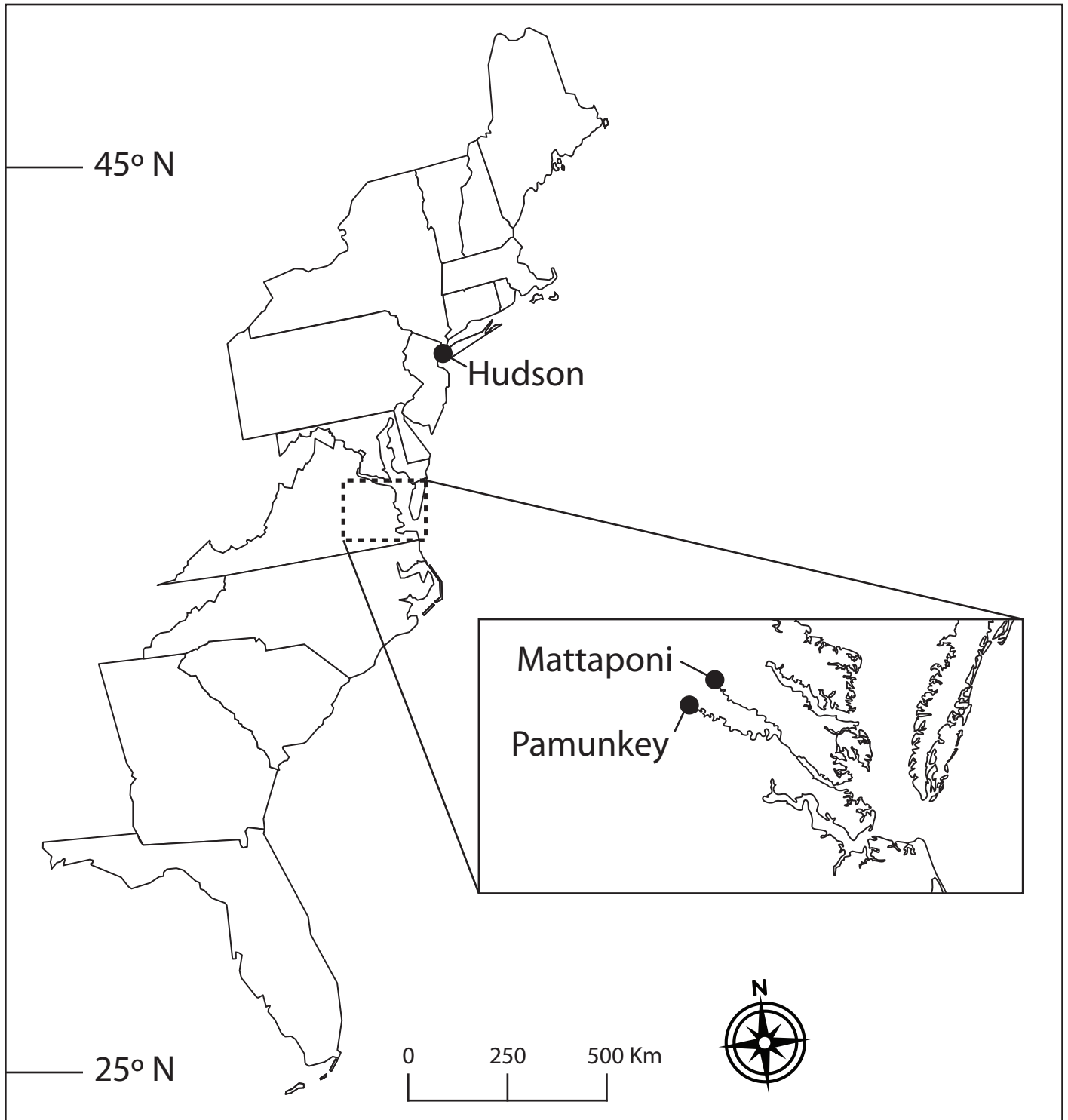
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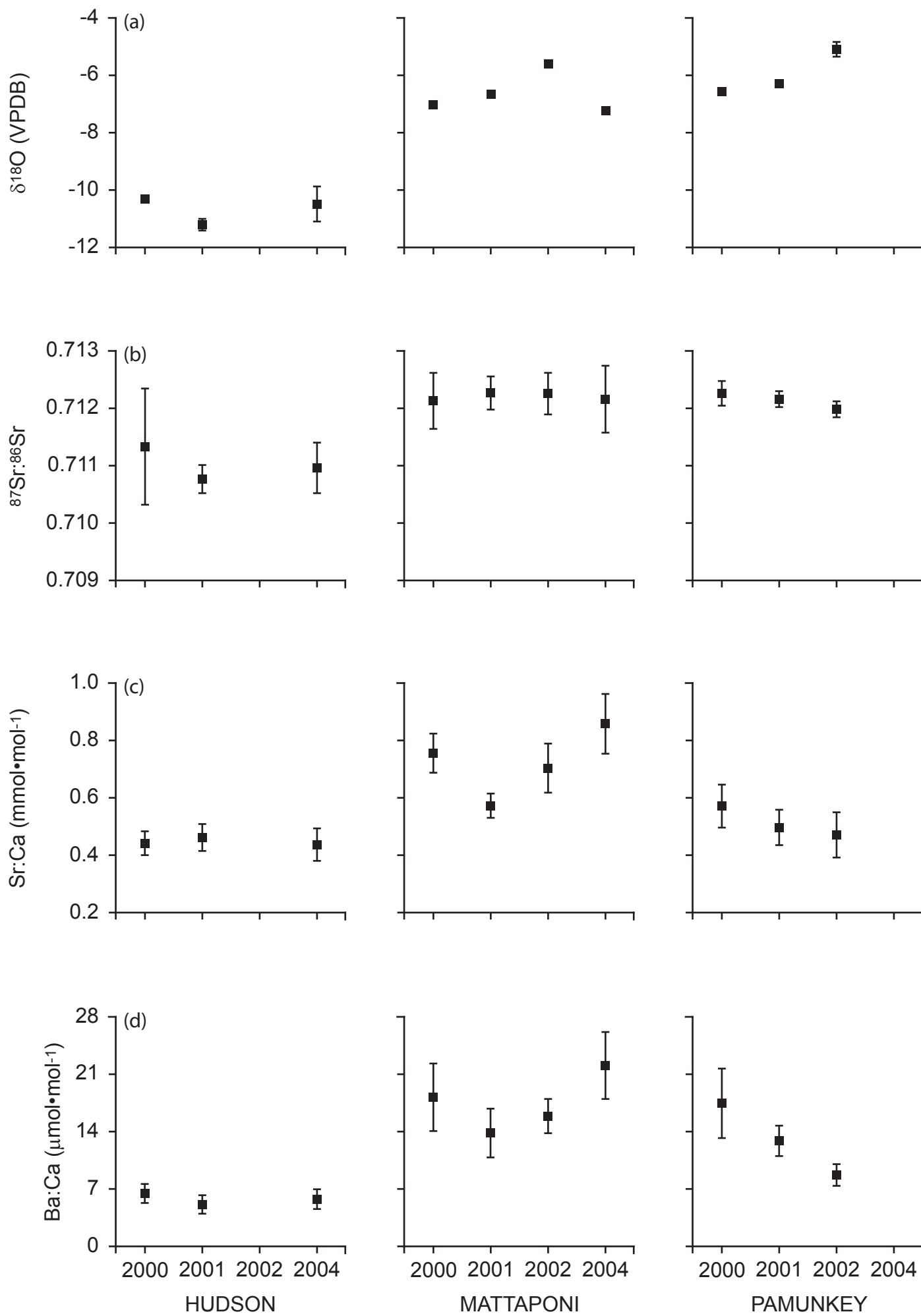
423 Figure 1. Map indicating locations of the Hudson, Mattaponi, and Pamunkey rivers
424 where juvenile American shad were collected..

425

426 Figure 2. Mean (± 1 standard deviation) values of (a) $\delta^{18}\text{O}$, (b) $^{87}\text{Sr}:^{86}\text{Sr}$, (c) Sr:Ca,
427 and (d) Ba:Ca ratios for the Hudson, Mattaponi, and Pamunkey rivers across years.

428





429 Table 1. Single factor ANOVA results for yearly variation in isotope and elemental ratios for each river. NS = not significant, *p < 0.01, **p <
 430 0.001. Variance components (% ω^2) are given as the percentage of the total variance for each ANOVA.

	df	$\delta^{18}\text{O}$			$^{87}\text{Sr}:^{86}\text{Sr}$			Sr:Ca			Ba:Ca		
		MS	F	% ω^2	MS	F	% ω^2	MS	F	% ω^2	MS	F	% ω^2
Hudson													
Year	2	6.21	43.16**	34	2.35×10^{-6}	5.50*	5	4.83×10^{-3}	2.16 ^{NS}	0	13.16	11.10**	11
Residual	79	0.14		66	4.27×10^{-7}		95	2.23×10^{-3}		100	1.19		89
Mattaponi													
Year	3	13.07	594.06**	85	1.37×10^{-7}	0.73 ^{NS}	0	0.37	63.03**	38	318.42	27.57**	21
Residual	99	0.02		15	1.87×10^{-7}		100	0.01		62	11.55		79
Pamunkey													
Year	2	11.45	241.33**	81	3.97×10^{-7}	14.37**	19	0.05	9.88**	14	353.80	46.28**	45
Residual	52	0.05		19	2.80×10^{-8}		81	0.01		86	7.64		55

431

432 Table 2. Percentages of misclassifications among years for a given river from
 433 quadratic discriminant function analyses (QDFA). The first column shows
 434 misclassification results for QDFAs using otolith $\delta^{18}\text{O}$, $^{87}\text{Sr}:^{86}\text{Sr}$, Sr:Ca, and Ba:Ca
 435 ratios. Following columns show QDFA results excluding each chemical ratio in turn.

		Excluding			
	All	$\delta^{18}\text{O}$	$^{87}\text{Sr}:^{86}\text{Sr}$	Sr:Ca	Ba:Ca
Hudson					
2000	26	41	15	22	30
2001	11	14	7	4	14
2004	48	63	44	52	48
Average	28	39	22	26	31
Mattaponi					
2000	15	52	7	7	26
2001	4	14	7	4	7
2002	0	57	0	0	0
2004	12	33	12	12	25
Average	8	39	7	6	15
Pamunkey					
2000	11	22	17	11	28
2001	11	28	11	11	17
2002	5	16	5	5	5
Average	9	22	11	9	17

436