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 (δ^{18}O and

7 ^{87}Sr:^{86}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 δ^{18}O and ^{87}Sr:^{86}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 δ^{18}O ratios showing substantial inter-annual shifts while ^{87}Sr:^{86}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.

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

20 variability.

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1. Introduction

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

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

47 1997). For instance, otolith $^{87}Sr:^{86}Sr$ 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 $^{87}Sr:^{86}Sr$

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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 $^{87}Sr:^{86}Sr$, $\delta^{18}O$,

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 $\delta^{18}O$ and $^{87}Sr:^{86}Sr$,

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.

4

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 $\delta^{18}O$ 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 m•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 x 200 μm 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}Sr:^{86}Sr ratios sampled from an otolith CRM ($n = 74$) and solutions of

123   SRM987 ($n = 41$) were 0.70916 (± 0.00002) and 0.71025 (± 0.00002), respectively.

124   These data compare favourably to the accepted marine ^{87}Sr:^{86}Sr value (0.70918;

125   Ingram and Sloan, 1992) and the certified ^{87}Sr:^{86}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 x 400 μm raster with a

128   75 μm 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 ± 0.07‰ (Ostermann and Curry, 2000).

135   Samples from 2000-2002 were additionally analysed for Mn:Ca, Mg:Ca and

136   δ^{13}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}Sr:^{86}Sr, and δ^{18}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}Sr:^{86}Sr in the Mattaponi River showed statistically

169 insignificant inter-annual variation (Table 1). Surprisingly, ^{87}Sr:^{86}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}Sr:^{86}Sr ratios accounted

172 for only 5-19% of the total variability within a river. In contrast, δ^{18}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 δ^{18}O ratios were

183 excluded from the multivariate signature. In contrast, excluding ^{87}Sr:^{86}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}Sr:^{86}Sr, δ^{18}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 $\delta^{18}O$. Because

200   otolith $\delta^{18}O$ 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 $\delta^{18}O$ values. A wide variety of environmental

203   forces can drive temporal shifts in riverine $\delta^{18}O$ 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 $\delta^{18}O$ otolith signatures in the absence of detailed water

212   samples constraining variability in ambient waters. Regardless of the cause, $\delta^{18}O$

213   ratios varied enough to cause significant biases in estimates of natal origin if fish were

214   classified using inappropriate baseline maps. Indeed, $\delta^{18}O$ 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 $\delta^{18}O$ must be careful to use

219   temporally appropriate baseline maps when classifying migrants of unknown origins.

220   Otolith ratios of $^{87}Sr{:}^{86}Sr$ directly reflect ambient freshwater composition and

221   are not trophically fractionated (Capo et al., 1998; Kennedy et al., 2000). In general,

222   freshwater ^{87}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}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}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}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}Sr:^{86}Sr of otoliths. Yet, although inter-

231   annual differences in otolith ^{87}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}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}Sr:^{86}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 *Oncorhyncus*

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}Sr:^{86}Sr,

271   δ^{18}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 $\delta^{18}O$ is more likely to result in classification errors than a more stable signature like

275 $^{87}Sr:^{86}Sr$, 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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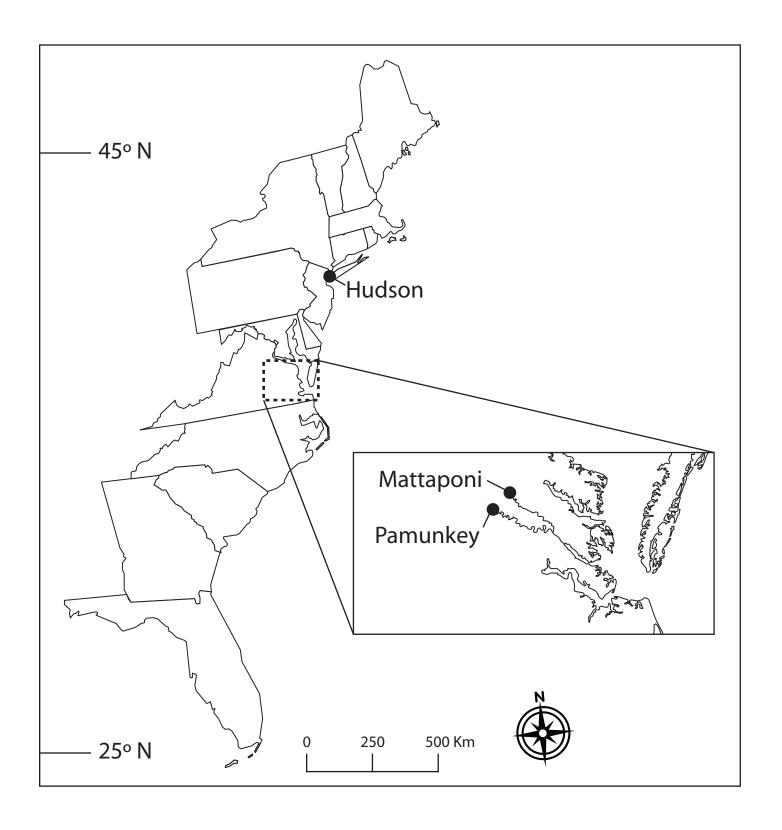323   Fisheries Society, 136(2): 469-476.

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420

421 **Figure captions**

422

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) $\delta^{18}O$, (b) $^{87}Sr{:}^{86}Sr$, (c) Sr:Ca,

427 and (d) Ba:Ca ratios for the Hudson, Mattaponi, and Pamunkey rivers across years.

428

45° N

Hudson

Mattaponi

Pamunkey

25° N

0   250   500 Km

N

(a)

δ¹⁸O (VPDB)

(b)

⁸⁷Sr:⁸⁶Sr

(c)

Sr:Ca (mmol•mol⁻¹)

(d)

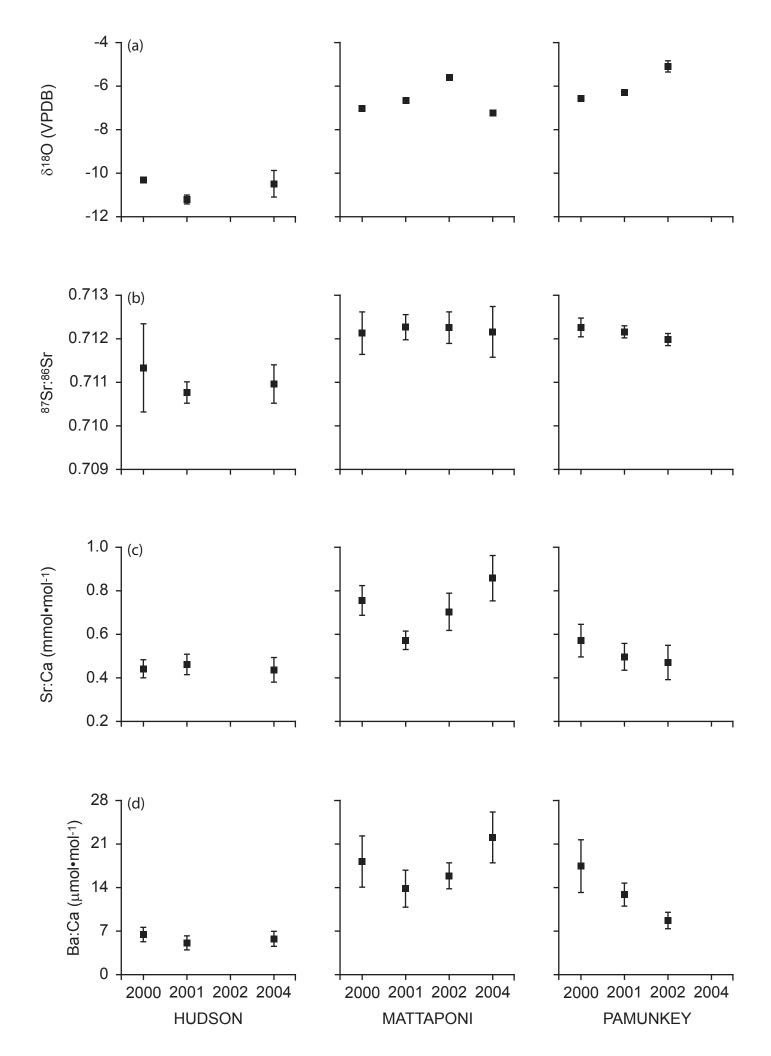Ba:Ca (μmol•mol⁻¹)

HUDSON   MATTAPONI   PAMUNKEY

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 ($‰\omega^2$) are given as the percentage of the total variance for each ANOVA.

	df	$\delta^{18}O$			$^{87}Sr:^{86}Sr$			Sr:Ca			Ba:Ca		
		MS	F	$‰\omega^2$	MS	F	$‰\omega^2$	MS	F	$‰\omega^2$	MS	F	$‰\omega^2$
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 $\delta^{18}O$, $^{87}Sr{:}^{86}Sr$, Sr:Ca, and Ba:Ca

435   ratios. Following columns show QDFA results excluding each chemical ratio in turn.

		Excluding			
Hudson	All	$\delta^{18}O$	$^{87}Sr{:}^{86}Sr$	Sr:Ca	Ba:Ca
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