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ARTICLE

Can different combinations of natural tags identify river herring natal origin at different levels of stock structure?

Sara M. Turner, Karin E. Limburg, and Eric P. Palkovacs

Abstract: We compared the accuracy of different combinations of natural markers, specifically otolith elemental (Sr:Ca and Ba:Ca) and isotopic ratios ($^{87:86}$Sr) with and without genetic stock constraints to evaluate their ability to distinguish among anadromous river herring (i.e., alewife, *Alosa pseudoharengus*, and blueback herring, *Alosa aestivalis*) populations from their US ranges. Model accuracy increased when constrained to a regional level by genetic stocks. Both species were misclassified to sites up to 1000 km from their collection location when only otolith chemistry was used. The inclusion of genetic constraints improved reclassification rates, and the longer time scale of genetic variation makes this method less sensitive to interannual variation. We recommend the combined approach of otolith chemistry and genetics as a means to trace river herring in marine bycatch back to river of origin.

Résumé : Nous avons comparé l'exactitude de différentes combinaisons de marqueurs naturels, plus précisément des rapports élémentaires (Sr:Ca et Ba:Ca) et isotopiques ($^{87:86}$Sr) d'otolites avec et sans information relative au stock génétique, afin d'évaluer leur pertinence pour distinguer différentes populations d'aloses (gaspareau, *Alosa pseudoharengus*, et alose d'été, *Alosa aestivalis*) anadromes dans leurs aires de répartition aux États-Unis. L'exactitude du modèle augmentait quand de l'information sur le stock génétique permettait de contraindre le modèle au niveau régional. Les deux espèces étaient erronément affectées à des sites distants de jusqu'à 1000 km du lieu de leur prélèvement quand seule la chimie des otolites était utilisée. L'inclusion de contraintes génétiques améliore les taux de reclassification, et l'échelle temporelle plus grande des variations génétiques rend ce modèle moins sensible aux variations interannuelles. Nous recommandons une approche combinée comprenant la chimie des otolites et la génétique pour retracer la rivière d'origine des aloses dans les prises accessoires en mer. [Traduit par la Rédaction]

Introduction

Alewife (*Alosa pseudoharengus*) and blueback herring (*Alosa aestivalis*) are two closely related species of iteroparous, anadromous fishes, collectively referred to as river herring (Fay et al. 1983). Both species spawn in natal rivers (63%–97% homing estimates; Jessop 1994) in spring, and juveniles emigrate to sea during late summer – early fall (Fay et al. 1983). Most populations have been reduced to a fraction of their historic abundances from a combination of overfishing and freshwater habitat loss and degradation (Belding 1920; Limburg and Waldman 2009; Hall et al. 2012). These species are an important food source for a wide range of fauna, including marine mammals, aquatic birds, and piscivorous fishes including many commercially important species such as striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), and Atlantic cod (*Gadus morhua*; Fay et al. 1983; Hall et al. 2012).

Freshwater behaviors have been well-documented, particularly migratory cues of juveniles and adults, because these migrations can easily be observed and monitored in many rivers (Kosa and Mather 2001; Davis and Schultz 2009; Gahagan et al. 2010). Restoring freshwater access and water quality are important, but as river herring spend the majority of their life at sea, marine stages must be protected as well (Limburg and Waldman 2009). Marine migrations are poorly understood; thus, if natural markers differ among populations, they may provide a unique opportunity for gaining insight to marine migrations and migratory behaviors (Elsdon et al. 2008).

"Natural tags": otoliths and genetics

Fish otoliths, or ear stones that form part of the hearing and balance system, are chronometric and grow in proportion to the fish (Campana 1999). Otoliths are composed of aragonitic calcium carbonate, become chemically inert upon formation, and take up some elements and isotopes in proportion to their ambient availability (Campana 1999). Elemental and isotopic markers are often used in combinations, referred to as signatures, and are frequently used to distinguish among populations or elucidate past habitat use (Campana 1999; Elsdon et al. 2008). Commonly used markers, such as Sr, Ba, $^{87:86}$Sr, and δ^{18}O, are influenced by the bedrock age and weathering, hydrology, water temperature, and salinity. To use signatures to distinguish among stocks, they must differ among groups; if the aim of a study is to identify the stock to which fish of unknown origin belong, then the signatures of all stocks must be known (Elsdon et al. 2008). Additionally, interannual variation can have substantial effects on signatures (especially δ^{18}O), and thus to determine the origin of unknown individuals, corresponding water chemistry or otolith chemistry of all potential cohorts must be known (Walther and Thorrold 2009).

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S.M. Turner* **and K.E. Limburg.** State University of New York College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210, USA.
E.P. Palkovacs. University of California – Santa Cruz, 100 Shaffer Rd., Santa Cruz, CA 95060, USA.
Corresponding author: Sara Turner (e-mail: sara.turner@noaa.gov).
*Present address: Integrated Statistics, Northeast Fisheries Science Center (NEFSC) Narragansett Laboratory, 28 Tarzwell Drive, Narragansett, RI 02882, USA.

Table 1. Sample collection locations and details.

River	State	Latitude (°N, mouth)	Latitude (°N, headwaters)	Species	YOY or adult	*n* (genetics)	*n* (otoliths)	*n* (both)
East Machias	ME	44.72	44.72	A	Adult	59	20	16
				B	Adult	58	20	16
St. George	ME	44.00	44.25	A	Adult	65	20	16
				B	Adult	50	20	18
Cocheco	NH	43.19	43.21	A	Adult	31	20	19
Oyster	NH	43.12	43.13	B	Adult	26	20	20
Lamprey	NH	43.07	43.09	A	Adult	47	20	19
Exeter	NH	43.05	42.98	B	Adult	41	20	18
Merrimack	MA	42.81	43.99	B	Adult	0	20	0
Mystic	MA	42.39	42.44	A	Adult	69	20	20
				B	Adult	69	20	20
Town Brook	MA	41.96	41.94	A	Adult	49	20	20
Nemasket	MA	41.94	41.78	A	Adult	0	20	0
Monument	MA	41.77	41.83	A	Adult	46	20	20
				B	Adult	51	20	20
Gilbert Stuart	RI	41.45	41.52	A	Adult	44	20	20
				B	Adult	38	20	20
Bride Brook	CT	41.30	41.33	A	Adult	27	18	18
Quinnipiac	CT	41.29	41.45	A	Adult	35	20	20
Hudson	NY	40.71	43.30	A	Both	61	20	4
				B	Both	79	20	20
Delaware	NJ	39.11	42.08	A	YOY	47	20	19
				B	YOY	49	20	20
Head of Chesapeake Bay	MD	39.11	39.11	A	YOY	0	20	0
				B	YOY	0	20	0
Nanticoke	DE	38.34	38.68	A	Adult	39	20	18
				B	Adult	24	19	18
Rappahannock	VA	37.49	38.41	A	YOY	62	20	19
				B	YOY	58	20	14
James	VA	36.98	37.50	B	YOY	98	20	16
Chowan	NC	36.04	37.10	A	Adult	54	20	9
				B	Adult	72	20	20
Alligator	NC	35.93	35.60	A	Adult	49	20	20
Santee-Cooper	SC	32.78	33.47	B	Adult	62	20	18
Savannah	GA	32.05	34.66	B	YOY	52	20	15
Altamaha	GA	31.31	31.96	B	YOY	53	20	19
St. John's	FL	30.41	28.71	B	Both	37	20	6

Note: A, alewife; B, blueback herring; YOY, young-of-year.

Genetic markers constitute another "natural tag" commonly used for stock identification and can provide information about connectivity and stock structure (Waples and Naish 2009). Genetically distinct stocks can be spawning populations if there is little gene flow among stocks (<10%) or can include multiple spawning groups if individuals are exchanged among spawning populations at a moderate rate (Waples and Naish 2009). Fisheries management typically focuses on spawning populations, but where population mixing occurs, groups of populations that comprise genetically distinguishable units, often referred to as evolutionarily significant units (ESUs) or distinct population segments (DPSs), deserve management consideration as well (Waples and Naish 2009). Genetic markers are also useful for distinguishing morphologically similar species and identifying hybrid individuals (Hasselman et al. 2014) and retain stable signatures over longer time scales (generations versus annual variation in otolith markers). Combinations of genetics and otolith chemistry may provide complementary information on the movements and connectivity of different stocks (Bradbury et al. 2008) or may be used either hierarchically or simultaneously for stock discrimination and determination of mixed stock composition (Barnett-Johnson et al. 2010; Smith and Campana 2010).

Natural tags provide an opportunity to gain information about unknown marine schooling and population mixing of river herring. In earlier work we found good separation among river herring populations at different spatial scales, with accuracy dependent on the markers used (Turner and Limburg 2014). Here, we predict that different combinations of natural tags will have different levels of accuracy for reclassifying fish of known origin and that misclassifications will be higher among more proximate sites. We test this prediction by using different combinations of natural tags for discriminating among river herring populations throughout the US range. Specifically, we will compare the percentages of fish reclassified with the population from which they were sampled using two methods:

1. otolith chemistry (Sr:Ca, Ba:Ca, and $^{87:86}$Sr); and
2. a hierarchical assignment method using genetic stocks to constrain possible assignments to a broader region and otolith chemistry to identify the natal watershed within that region.

Methods

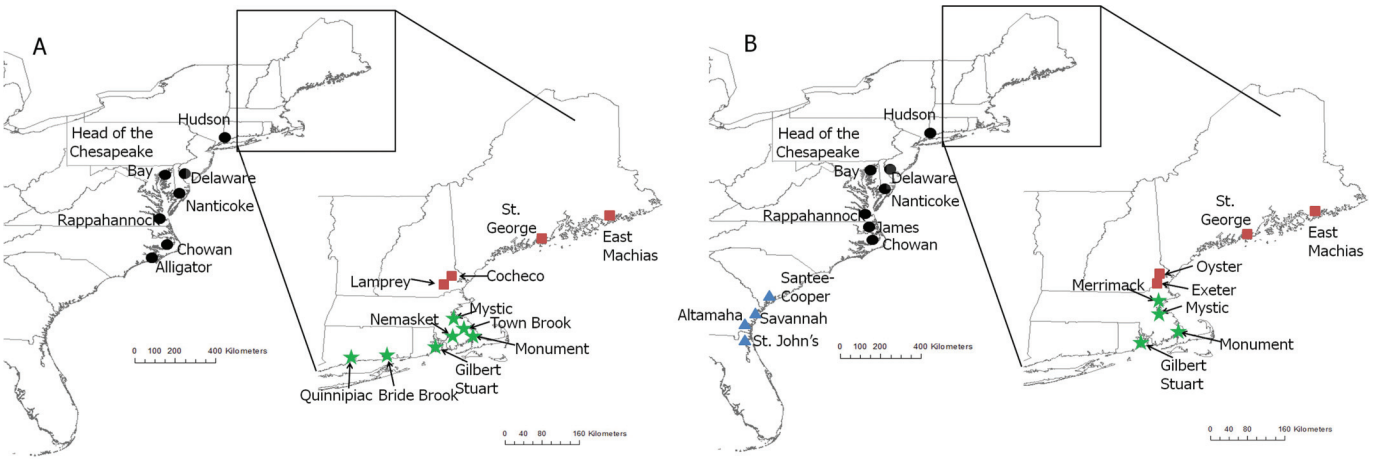
Study sites

River herring populations occupy many coastal watersheds from Florida to maritime Canada; populations from a total of 26 watersheds from Maine to Florida were included in this study, with alewife sampled at 18 sites and blueback herring at 19 sites (Table 1; Figs. 1A and 1B). More samples were collected from northern sites because more populations are monitored by governmental and university programs in the northeastern than the southeastern US.

Sample collections

Adult river herring were collected during spring spawning migrations at most sites, while young-of-year (YOY) were collected

Fig. 1. Map of the study sites for (A) alewife and (B) blueback herring, with different symbols indicating genetically distinct stocks.



during juvenile monitoring programs at others; the type of sample collected (YOY versus adult; Table 1) depended on the agency monitoring program focus. Whereas spawning adults may be strays that reared in another river, most fish included in this study had otolith signatures very similar to other individuals from the river in which they were captured. Fish being used for both genetics and otoliths were preserved immediately (either frozen or stored in ethanol) for later processing; we aimed for 20 fish per species per location for otolith chemistry (total $N = 734$) and 50 per species per location for genetics and attempted to maximize the number of samples included in both analyses (Table 1). Genetic samples were genotyped at 15 microsatellite loci (*Aa046*, *Aa070*, *Aa074*, *Aa081*, *Aa082*, *Aa091*, *Aa093*, *Ap010*, *Ap033*, *Ap037*, *Ap038*, *Ap047*, *Ap058*, *Ap070*, *Ap071*; genetic analyses are described in detail in Palkovacs et al. 2014).

Both sagittal otoliths were removed and ultrasonicated in 70% bleach and ultrapure water for 5 min each, then air-dried and stored in clean microcentrifuge tubes. For adult fish, both otoliths were embedded in epoxy resin (EpoFix); one otolith was randomly selected and sectioned in the transverse plane using a low-speed Isomet saw. Sections were polished to the core using 30 to 1 μm grit lapping film. Polished otolith sections were glued (Crystal Bond) to petrographic slides for laser analyses. One otolith from each YOY fish was selected at random, glued (Loctite) to a 1 cm coverslip square, and polished to the core in the sagittal plane.

Otoliths were analyzed for concentrations of ^{43}Ca, ^{88}Sr, and ^{138}Ba via laser ablation inductively coupled plasma mass spectrometry (LA ICPMS; with a PerkinElmer DRC-e mass spectrometer coupled to a New Wave 193 nm Nd-YAG laser) at the College of Environmental Science and Forestry using a straight-line transect (from the dorsal to ventral tip for adult transverse sections and from the core to the posterior edge for YOY otoliths), with a spot size of 35 μm at a speed of 3 μm·s^{-1} (power = 70%, frequency = 10 Hz). External precisions were monitored using NIST 610 glass and were 13.1% for ^{43}Ca, 13.0% for ^{88}Sr, and 13.5% for ^{138}Ba; raw counts for samples were converted to concentrations using a known concentration, compressed otolith pellet (Limburg et al. 2011). The same otoliths were also analyzed for $^{87:86}$Sr isotopic ratios using a Neptune multicollector LA ICPMS with a New Wave excimer 193 nm laser ablation unit using a 50 μm spot size at a speed of 3 μm·s^{-1} (power = 100%, frequency 5 Hz) at Woods Hole Oceanographic Institute. Laser precisions for $^{87:86}$Sr were monitored with the MACS3 carbonate standard (USGS), and corrected standard ratios deviated from published numbers by less than ±0.0008; all data were corrected offline for Kr and Rb interferences using the method described by Jackson and Hart (2006).

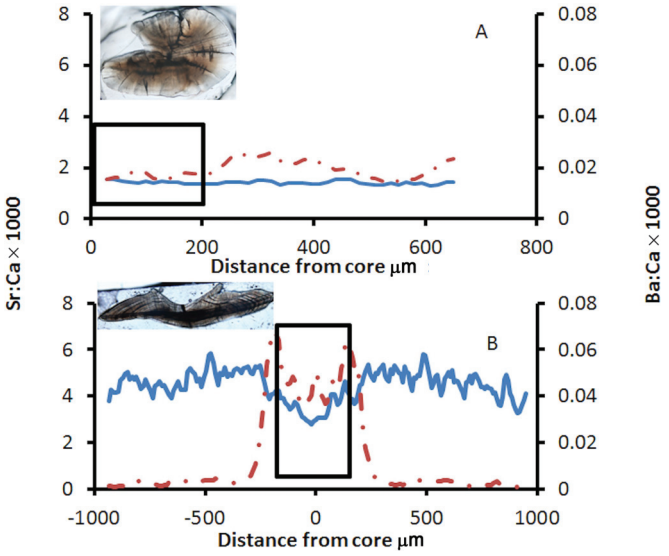
Statistical analyses

Natal signatures were contained in the portion of the transect encompassing the core (generally 100–300 μm) and were calculated by taking the mean within this portion of the transect (Fig. 2). As high variability has been observed in age and size at emigration, "natal" signatures were constrained by limiting the coefficients of variation at 30% for Sr:Ca and 50% for Ba:Ca. The $^{87:86}$Sr ratios were only analyzed for the center portion of the otolith and were calculated as the mean of the stable portion of the transect (to omit analysis of non-natal material, i.e., from seaward movements), and the coefficient of variation was limited at 0.1%.

Otolith signatures (Sr:Ca, Ba:Ca, and $^{87:86}$Sr) were tested for significant differences among populations using multivariate analysis of variance (MANOVA), and each ratio was tested individually for differences among groups with analysis of variance (ANOVA), and differences between individual populations were tested using Tukey's HSD. Locations where adult fish were sampled are expected to have higher within-group variation resulting from interannual differences in ambient ratios because samples are likely composed of multiple year classes. Differences in otolith signatures among populations were tested using quadratic discriminant function analysis (DFA). Tests were performed for each species separately, as previous river herring otolith chemistry work has found some differences in elemental uptake (Gahagan et al. 2012; Turner and Limburg 2014).

DFA was performed for each species separately using otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr, and rates of reclassification to collection site were quantified by using a jackknife leave-one-out procedure to test how well signatures could distinguish among populations throughout the sampling ranges. The percentages correctly assigned to their collection location were compared with constrained assignment models by using DFA to assign fish by otolith signatures within genetic stocks (composed of four to eight sampled populations) distinguished by Palkovacs et al. (2014). The same sites were included in both analyses (Figs. 1A and 1B); sites that were not included in genetic analyses were grouped with the stock in which the watershed was contained or to which it was most proximate. Sites with low numbers of samples analyzed for both markers were included in analyses; given the temporal stability of genetic markers, we assume no differences in genetic composition among these samples (samples for both analyses were collected during the same season). Three genetically distinct alewife stocks were detected, and analysis of molecular variance (AMOVA) showed variation among these groups was much higher than within (4.70%, $p < 0.001$ versus 1.30%, $p < 0.001$, respectively; Palkovacs et al. 2014). For blueback herring, four genetically dis-

Fig. 2. Otolith chemistry transects, illustrating the portion used for natal signatures (indicated by black rectangles) for (A) juvenile and (B) adult river herring. Sr:Ca is indicated with the solid line; Ba:Ca is represented with a dot-dashed line.



tinct stocks were found, with AMOVA showing higher among-group variation than within-group variation (2.45%, $p < 0.001$ versus 0.82%, $p < 0.001$, respectively; Palkovacs et al. 2014). The spatial extent of misclassifications was compared between models by plotting the percentages of misclassifications against the distance (km) between the collection site and the site to which fish were misclassified. The distance between sites was measured in Google Earth using a straight-line transect between river mouths, providing a minimum estimate of the distances between sites.

Results

Otolith chemistry discrimination

Otolith signatures differed significantly among groups for both species (MANOVA $p < 0.0001$), and individual ratios differed significantly among sites for both species as well (ANOVA $p < 0.0001$). Alewife Sr:Ca was most distinct for the Gilbert Stuart River, Rhode Island (RI), Nanticoke River, Delaware (DE), and Alligator River, North Carolina (NC), populations (Tukey's HSD, $p < 0.05$; Table 2). Town Brook, Massachusetts (MA), Monument River, MA, and Nanticoke River, DE, alewife populations were significantly different from most others based on Ba:Ca (Tukey's HSD, $p < 0.05$; Table 2). Strontium isotopes differed significantly among many populations, with Bride Brook, Connecticut (CT), the Quinnipiac River, CT, and the Rappahannock River, Virginia (VA), being some of the most distinct (Tukey's HSD, $p < 0.05$; Table 2). Blueback herring populations from the Nanticoke River, DE, and St. John's River, Florida (FL), had the most distinct Sr:Ca ratios (Tukey's HSD, $p < 0.05$; Table 2). Blueback herring Ba:Ca was most distinct in populations from the Monument River, MA, and the Nanticoke River, DE, and $^{87:86}$Sr was most distinct in the Gilbert Stuart River, RI, Rappahannock River, VA, and St. John's River, FL, populations (Tukey's HSD, $p < 0.05$; Table 2).

Overall assignments for each species across the sampling ranges using Sr:Ca, Ba:Ca, and $^{87:86}$Sr were 69.7% and 69.0% for alewife and blueback herring, respectively (Table 3). Reassignment rates were highest to the Cocheco River, New Hampshire (NH; 95%) for alewife and to East Machias, Maine (ME), Gilbert Stuart River, RI, and the St. John's River, FL (95%), for blueback herring (Appendix A). The lowest reassignment rates for both species were to the Head of the Chesapeake Bay, Maryland (MD; 10% for alewife, 16% for blueback herring; Appendix A). Misclassifications for both species using only the otolith markers occurred up to 1000 km away from the watershed in which individuals were captured (Fig. 3), but nevertheless such occurrences were low (<5% for the most part).

Hierarchical genetic–otolith discrimination

Constrained site discrimination using DFA of otolith signatures within genetic stocks resulted in overall reassignment rates of 81.5% for alewife and 79.8% for blueback herring (Table 3). Palkovacs et al. (2014) found three genetic stocks for alewife and four genetic stocks for blueback herring using Bayesian model-based clustering methods (Figs. 1A and 1B). Each of these genetic stocks contained four to eight populations that were analyzed for otolith signatures (Appendix A). Reclassification rates for alewife were highest (95%) for the Cocheco River, NH, the Lamprey River, NH, the Rappahannock River, VA, and the Alligator River, NC, and lowest for the Head of the Chesapeake Bay, MD (25%; Appendix A). Blueback herring reclassification rates were 100% for the Mystic River, MA, the Monument River, MA, the Santee-Cooper River, South Carolina (SC), the Altamaha River, Georgia (GA), and the St. John's River, FL, and lowest for the Head of the Chesapeake Bay, MD (47%; Appendix A). When genetic constraints were added to the classification model, alewife misclassifications were to sites up to 400 km away (i.e., the spatial extent of the genetic groups), but most were to locations within 100 km of the collection location (Fig. 3A). The majority of misclassifications of blueback herring were to sites within 200 km of the location at which the fish was captured (Fig. 3B).

Discussion

The effects of episodic bycatch in commercial marine fisheries is thought to cause substantial mortality to river herring populations, but the effect on river-specific populations is currently unknown. We tested the accuracy with which otolith chemical signatures could distinguish the natal origin of river herring to populations, both at the coast-wide scale and within genetic stocks. Other studies have found improved discriminatory power when combining genetic and otolith chemistry markers (e.g., Milton and Chenery 2001; Barnett-Johnson et al. 2010; Woods et al. 2010), and the use of multiple natural markers allowed Higgins et al. (2010) to pinpoint open-ocean capture locations of Atlantic cod.

Otolith chemistry discrimination

Pair-wise comparisons revealed that for alewives, sites with estuarine natal habitats were the most distinct, especially for Sr:Ca (Gilbert Stuart River, RI, Nanticoke River, DE, and Alligator River, NC) and to a lesser degree for Ba:Ca (Monument River, MA, and Nanticoke River, DE), while the most significant differences overall were for $^{87:86}$Sr. Similar trends were observed for blueback herring, with populations using estuaries the most distinct for Sr:Ca (Exeter River, NH, and Nanticoke River, DE), and again to a lesser degree for Ba:Ca (Nanticoke River, DE). Blueback herring from the St. John's River, FL, were significantly different from all other sites for both Sr:Ca and $^{87:86}$Sr because its bedrock is composed of Upper Eocene limestone (relatively young and depleted in ^{87}Sr; Mallinson et al. 1994); unlike all other sites, Sr:Ca was higher than marine ratios and $^{87:86}$Sr was below marine ratios, and this was reflected in their high reassignment rate. Walther et al. (2008) used otolith chemistry (Sr:Ca, Ba:Ca, $^{87:86}$Sr, and δ^{18}O) to distinguish among American shad (*Alosa sapidissima*) populations from throughout eastern North America and also noted that isotopic ratios were the strongest drivers of differences among populations.

The potential for use of $^{87:86}$Sr in otoliths to discriminate among habitats was first explored by Kennedy et al. (1997) in Connecticut River Atlantic salmon (*Salmo salar*) populations; they observed little discrimination in uptake and temporally stable ratios that

Table 2. Mean otolith ratios for each site for alewife (A) and blueback herring (B).

Site	Species	SrCa (SE)	BaCa (SE)	$^{87:86}$Sr (SE)
East Machias River, ME	A	1.48 (0.06)	0.016 (0.001)	0.7109 (2e^{-4})
	B	1.28 (0.04)	0.023 (0.001)	0.7128 (1e^{-4})
Saint George River, ME	A	1.80 (0.07)	0.011 (0.001)	0.7133 (3e^{-4})
	B	1.25 (0.06)	0.018 (0.002)	0.7152 (3e^{-4})
Cocheco River, NH	A	3.00 (0.09)	0.025 (0.002)	0.7158 (1e^{-4})
Oyster Riv er, NH	B	2.55 (0.36)	0.023 (0.003)	0.7121 (4e^{-4})
Lamprey River, NH	A	2.62 (0.12)	0.034 (0.002)	0.7125 (2e^{-4})
Exeter River, NH	B	3.14 (0.21)	0.005 (0.000)	0.7098 (7e^{-5})
Merrimack River, MA	B	2.75 (0.25)	0.021 (0.003)	0.7147 (5e^{-4})
Mystic River, MA	A	1.34 (0.04)	0.019 (0.002)	0.7109 (1e^{-4})
	B	0.96 (0.02)	0.026 (0.002)	0.7116 (1e^{-4})
Town Brook, MA	A	2.97 (0.07)	0.057 (0.002)	0.7107 (5e^{-5})
Nemasket River, MA	A	3.16 (0.17)	0.031 (0.003)	0.7114 (4e^{-4})
Monument River, MA	A	3.65 (0.13)	0.056 (0.004)	0.7103 (1e^{-4})
	B	2.44 (0.14)	0.127 (0.024)	0.7110 (3e^{-5})
Gilbert Stuart River, RI	A	6.29 (0.27)	0.005 (0.001)	0.7096 (3e^{-4})
	B	1.43 (0.06)	0.027 (0.002)	0.7178 (1e^{-4})
Bride Brook, CT	A	2.05 (0.07)	0.041 (0.003)	0.7170 (2e^{-4})
Quinnipiac River, CT	A	1.05 (0.03)	0.031 (0.002)	0.7141 (2e^{-4})
Hudson River, NY	A	1.46 (0.04)	0.017 (0.001)	0.7115 (2e^{-4})
	B	1.05 (0.07)	0.015 (0.001)	0.7107 (5e^{-4})
Delaware River, NJ	A	1.39 (0.05)	0.034 (0.003)	0.7122 (9e^{-5})
	B	1.07 (0.04)	0.035 (0.005)	0.7117 (2e^{-4})
Head of the Chesapeake Bay, MD	A	1.47 (0.10)	0.035 (0.003)	0.7123 (3e^{-4})
	B	1.07 (0.10)	0.051 (0.005)	0.7118 (4e^{-4})
Nanticoke River, DE	A	4.78 (0.29)	0.133 (0.010)	0.7105 (8e^{-5})
	B	3.74 (0.26)	0.173 (0.019)	0.7107 (1e^{-4})
Rappahannock River, VA	A	1.67 (0.09)	0.020 (0.001)	0.7157 (3e^{-4})
	B	1.14 (0.08)	0.096 (0.012)	0.7156 (4e^{-4})
James River, VA	B	0.98 (0.08)	0.053 (0.010)	0.7110 (7e^{-5})
Chowan River, NC	A	2.49 (0.22)	0.051 (0.004)	0.7099 (9e^{-5})
	B	1.70 (0.08)	0.066 (0.003)	0.7106 (5e^{-5})
Alligator River, NC	A	6.74 (0.35)	0.022 (0.003)	0.7095 (4e^{-5})
Santee-Cooper River, SC	B	2.07 (0.15)	0.069 (0.006)	0.7097 (3e^{-5})
Savannah River, GA	B	1.28 (0.06)	0.053 (0.004)	0.7109 (9e^{-5})
Altamaha River, GA	B	1.08 (0.04)	0.074 (0.004)	0.7119 (1e^{-4})
St. John's River, FL	B	5.71 (0.22)	0.016 (0.001)	0.7081 (1e^{-5})

Note: A, alewife; B, blueback herring.

Table 3. Overall jackknife reclassification rates from quadratic discriminant function analyses for 18 alewife and 19 blueback herring populations from throughout the eastern United States using otolith chemistry (Sr:Ca, Ba:Ca, and $^{87:86}$Sr) and otolith chemistry constrained by genetic stocks (from Palkovacs et al. 2014).

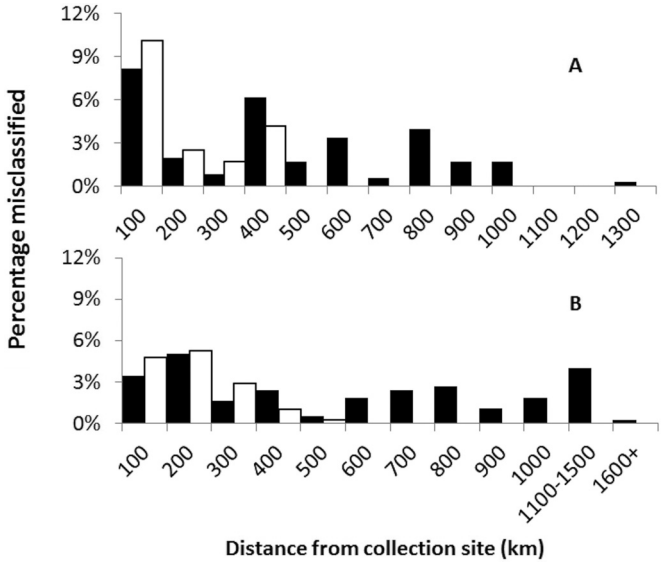
	Otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr	Genetically constrained otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr
Percentage of alewife correctly reclassified	69.7%	81.5%
Percentage of blueback herring correctly reclassified	69.0%	79.8%

varied at sub-basin spatial scales. Strontium isotopic ratios have been used relatively extensively to determine the natal origin of Pacific salmon populations; the highly variable bedrock geology within natal watersheds allows populations to be distinguished using only $^{87:86}$Sr (61%; Barnett-Johnson et al. 2010). Only a few studies have used $^{87:86}$Sr for discrimination of diadromous fishes in eastern North America despite its potential (Kennedy et al. 1997; Walther et al. 2008; Turner and Limburg 2014). At the continental scale, $^{87:86}$Sr may be confounded by the presence of similarly aged rocks at different locations or even similar formations. It may be the case that this marker is most effective at more local to intermediate scales (e.g., within river herring genetic stocks or <500 km) for use as an otolith discriminator.

Along a large geographic gradient, δ^{18}O has strong discriminatory power that is driven by a combination of meteoric and atmospheric processes (Kendall and Coplen 2001; Walther and Thorrold 2008; Turner and Limburg 2014). Classification rates in this study would likely be higher with inclusion of oxygen isotopes, because along the North American east coast δ^{18}O follows a latitudinal gradient, similar to the stock separation trend observed for North American alosine herrings (Walther and Thorrold 2008; Hasselman et al. 2013; Turner and Limburg 2014). We did not include this measurement here because of the difficulty in micromilling material cleanly from the cores of these small otoliths (Hoover and Jones 2013). However, in the case of river herring, we found that genetic classification can substitute to some extent for oxygen isotopes.

A major caveat to studies of migratory species that include adult specimens in baseline data is that homing to natal locations is not perfect. Homing rates for river herring have been widely estimated at 63%–97% via artificial tagging (Jessop 1994), but are likely at the higher end of that range, as homing rates for closely related

Fig. 3. Percentages of river herring misclassified plotted against the distance between the site at which the fish was collected and the site to which it was classified for (A) alewife and (B) blueback herring for different classification models. Solid bars = otolith chemistry model (Sr:Ca, Ba:Ca, and $^{87:86}$Sr); open bars = otolith chemistry using genetic groups as constraints.



American shad were 94% (Walther et al. 2008). Most adult fish included in otolith analyses for this study had similar ratios within sites, suggesting that most individuals were captured in their natal rivers, but no conclusions can be drawn here about homing or straying because corresponding water chemistry data for rearing periods for sampled year classes is lacking. Most samples included in this study were from larger watersheds; straying is likely higher among small, proximate coastal watersheds (Payne Wynne et al., in press). Turner and Limburg (2014) used juvenile otolith chemistry to classify the natal origin of spawning adults from corresponding year classes; reclassification rates were low with only moderate posterior probabilities, likely related to similarities in juvenile signatures from two sites.

Palkovacs et al. (2014) found significant differences in microsatellite markers among many populations for both species; nonsignificant differences were predominantly between geographically close populations, as would be expected because straying is generally assumed to be highest to nearby watersheds. Latitude was strongly correlated to genetic differentiation for both species (alewife R^2 = 0.85; blueback herring R^2 = 0.81); isolation by distance relationships were strong for both as well (alewife R^2 = 0.53; blueback herring R^2 = 0.50), with a steeper slope for the relationship for alewife, which infers lower straying rates (gene flow) among populations (Palkovacs et al. 2014).

Reclassifications to collection location for both species using otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr were generally high given the large number of sites, with the exception of the Head of the Chesapeake Bay. Alewife from the Head of the Chesapeake Bay were assigned to the Delaware River, NJ, at a relatively high rate (65%). Nonsignificant genetic differentiation was found among several rivers flowing into Chesapeake Bay and the Delaware River, NJ, for both alewife and blueback herring (Palkovacs et al. 2014). Together, these findings suggest a substantial amount of movement between the Delaware River and Chesapeake Bay, perhaps via the Chesapeake and Delaware Canal. Juveniles leaving the Delaware River, NJ, may access the upper Chesapeake Bay via the Chesapeake and Delaware Canal, and this may in part explain the misclassification. In the Delaware River, NJ, YOY fish were all captured from more than 128 km upstream from the mouth of the river and thus did not likely immigrate from another river system. While we lack corresponding water chemistry to support that Delaware River YOY alewife migrated to the Head of the Chesapeake Bay, upstream movements of that magnitude at small sizes are not likely. Assignments for blueback herring were not as striking, with assignments for Head of the Chesapeake Bay YOY fish classified to more than half of the sites in the model.

Misclassifications were not limited by geographic distance between sites; this is likely the effect of comparable geologic bedrocks in different areas. Alewife from East Machias, ME, the Mystic River, MA, and the Hudson River, NY, were assigned to each other at moderate rates (15%–30%), likely related to geological similarities causing similar signatures, rather than to straying, as the rivers are spatially isolated and these sites have few (if any) fish assigned to more proximal rivers. Trends in incorrect reassignments were not as striking for blueback herring; misclassified fish were not generally assigned to proximate populations, so this is likely the result of similar signatures (and therefore watershed geology) among groups and not to straying.

Hierarchical genetic–otolith discrimination

The hierarchical method for distinguishing among populations increased reclassifications to collection sites in 13 of 18 alewife populations and 15 of 19 blueback herring populations, while assignments for the rest of the populations remained the same. Alewife from the Head of the Chesapeake Bay assigned to the Delaware River, NJ, increased to 70%; a major portion of blueback herring from the Head of the Chesapeake Bay were assigned to the Delaware River with the hierarchical assignment as well (37%). This further supports our hypothesis that a major portion of YOY river herring in the Head of the Chesapeake Bay are migrants.

Reclassifications improved the most and were most accurate in stocks with fewer populations (northern New England for alewife and northern and southern New England and southern Atlantic stocks for blueback herring); assignments in these stocks with fewer populations sampled might decrease if more populations within these regions were sampled. Inclusion of genetics makes this model more robust by ensuring that even if interannual variability in otolith signatures causes a fish to be incorrectly assigned to a watershed, it is within the correct region (Walther and Thorrold 2009; Waples and Naish 2009). Using genetic groupings limited the geographic distances for misclassifications (400 km for alewife and 600 km for blueback herring), and while fish were misclassified to sites up to 400 km from their collection location, the majority of alewife were misclassified within 100 km and blueback herring to within 200 km. The distances between collection and misclassification sites observed for this coast-wide model were similar to previous work at a regional scale (Turner and Limburg 2014). Using genetic stocks for classifications also filters fish to genetically distinct groups (regions for alewife and blueback herring), thus reducing the number of groups being distinguished with otolith signatures (Barnett-Johnson et al. 2010).

Establishing combinations of natural tags with different levels of spatial discrimination and high discriminatory power is an important first step for answering basic questions about marine migratory behavior and identifying the natal origin of marine-captured individuals. Bycatch in marine fisheries is an important conservation concern (Cournane et al. 2013; Bethoney et al. 2014), and natural markers can provide answers to questions about the impacts of bycatch on different spawning stocks and populations. While sampling all extant river herring populations is impractical, statistical techniques can be applied with incomplete population baseline data to overcome issues related to missing baseline populations (Neubauer et al. 2013). Another remaining question is the temporal scales for baseline sample collection — the most accurate method is to collect and analyze otolith chemistry from YOY fish collected from all major populations on an annual basis, but in practice baseline sampling is limited by financial and hu-

man resources. Identifying the natal origin of fish accidentally caught in marine fisheries is critical to management; however, a more complete understanding of straying rates is required, which can stabilize the population dynamics of single runs (Schtickzelle and Quinn 2007) but at a potential cost to the overall stability within a region (Carlson and Satterthwaite 2011).

Several studies have tested different combinations of otolith chemistry for stock discrimination, with spatial scales varying from within an estuary to the entire species' range (e.g., Dorval et al. 2005; Walther and Thorrold 2008; Turner and Limburg 2014). Juvenile American shad from across their range (Florida to Quebec) had mean classification accuracies of 93% using a combination of trace metals (Sr and Ba) and isotopes ($^{87:86}$Sr and δ^{18}O; Walther and Thorrold 2008). In the present study, classifications within and among estuaries were somewhat lower, potentially related to the highly dynamic hydrology of estuarine environments as well as the potential for higher straying in river herring compared with American shad. Dorval et al. (2005) found significant differences among seagrass beds within the Chesapeake Bay and also among years; reclassifications based on Mn, Ba, La, Sr, δ^{13}C, and δ^{18}O were higher to sites near river mouths than to sites farther from rivers. Similarly, juvenile *Pelates sexlineatus* collected from multiple estuaries over 2 years were reclassified at moderate to excellent rates (50%–100%) using Sr, Ba, and Mn (Gillanders and Kingsford 2000). The results of these population discrimination studies as well as our work on river herring otolith chemistry to distinguish among populations at a range of spatial scales infer that populations can be consistently distinguished at relatively high rates when temporal variation is addressed; inclusion of stable isotopes also appears to increase discriminatory power.

Natural tags provided an accurate method of identifying the population to which river herring belong; different combinations of markers provided different levels of discriminatory power. Three otolith chemical ratios (Sr:Ca, Ba:Ca, and $^{87:86}$Sr) were able to distinguish among populations of each species from across their ranges with almost 70% accuracy. A hierarchical classification model, using genetics to distinguish the region from which a fish originates and then otolith chemistry to identify the population to which a fish belongs within that region, increased overall reclassification rates to approximately 80%. The addition of δ^{18}O and genetic-based stocks would likely have even higher reclassification rates, but high interannual variation of δ^{18}O makes their accurate use more complex than the other assignment methods (Walther and Thorrold 2009). While Sr:Ca and Ba:Ca (and to a lesser extent $^{87:86}$Sr) can vary interannually, the effects on population discrimination appear minor at this large spatial scale; variation in genetic markers occur over longer time scales. Thus, for determining the origin of unknown samples encompassing multiple year classes, a hierarchical genetic–otolith chemical assignment method is more forgiving if water or YOY otolith signatures for all corresponding year classes are incomplete or lacking.

For identifying the provenance of marine bycaught river herring, we recommend a combination of genetic and otolith methods. Because fish can be aged by otoliths and thus assigned to year classes, δ^{18}O could be used if river data are available in a region for specific years corresponding to those year classes. However, currently this is unlikely to be the case for many years and many rivers. Hence, combining genetic and other otolith markers can bring classification to a fairly high level of accuracy (80%) to natal river and higher if regional classifications are considered. This information will aid regional fishery management councils in recommending protective measures for the marine phase of river herring.

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Appendix A

Appendix tables appear on the following pages.

Table A1. Jackknife reclassification rates from quadratic discriminant function analysis for alewife by site using a combination of otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr.

	EMME	SGME	CONH	LANH	MYMA	TBMA	NEMA	MTMA	GSRI	BBCT	QRCT	HUNY	DLNJ	HBMD	NNDE	RAVA	CHNC	AGNC	% correct
EMME	**6**	1	0	0	4	0	0	0	0	0	0	6	1	1	0	0	1	0	30
SGME	2	**14**	0	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	70
CONH	0	0	**19**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95
LANH	1	0	0	**16**	0	0	3	0	0	0	0	0	0	0	0	0	0	0	80
MYMA	4	0	0	0	**9**	0	0	0	0	0	0	4	0	2	0	0	1	0	45
TBMA	0	0	0	0	0	**15**	0	3	0	0	0	0	0	0	0	0	2	0	75
NEMA	0	0	0	2	0	0	**12**	3	1	0	0	0	0	0	0	1	0	0	63
MTMA	0	0	0	0	0	3	2	**12**	0	0	0	0	0	0	2	0	1	0	60
GSRI	0	0	0	0	0	0	0	0	**17**	0	0	0	0	0	0	1	0	2	85
BBCT	0	0	0	0	0	0	0	0	0	**17**	0	0	0	1	0	0	0	0	94
QRCT	0	0	0	0	0	0	0	0	0	0	**17**	0	1	2	0	0	0	0	85
HUNY	3	0	0	0	5	0	0	0	0	0	1	**11**	0	0	0	0	0	0	55
DLNJ	0	0	0	0	1	0	0	0	0	0	0	1	**15**	3	0	0	0	0	75
HBMD	0	0	0	0	1	1	1	0	0	1	1	0	13	**2**	0	0	0	0	10
NNDE	0	0	0	0	0	1	0	2	0	0	0	0	0	0	**16**	0	0	1	80
RAVA	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	**17**	0	0	85
CHNC	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	**16**	0	80
AGNC	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	**18**	90

Note: EMME, East Machias, ME; SGME, St. George's River, ME; CONH, Cocheco River, NH; LANH, Lamprey River, NH; MYMA, Mystic River, MA; TBMA, Town Brook, MA; NEMA, Nemasket River, MA; MTMA, Monument River, MA; GSRI, Gilbert Stuart River, RI; BBCT, Bride's Brook, CT; QRCT, Quinnipiac River, CT; HUNY, Hudson River, NY; DLNJ, Delaware River, NJ; HBMD, Head of the Chesapeake Bay, MD; NNDE, Nanticoke River, DE; Rappahannock River, VA; CHNC, Chowan River, NC; AGNC, Alligator River, NC.

Table A2. Jackknife reclassification rates from quadratic discriminant function analysis for blueback herring by site using a combination of otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr.

	EMME	SGME	OYNH	EXNH	MRMA	MYMA	MTMA	GSRI	HUNY	DLNJ	HBMD	NNDE	RAVA	JAVA	CHNC	STSC	ALGA	SVGA	SJFL	% correct
EMME	**19**	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	95
SGME	1	**14**	0	0	3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	70
OYNH	1	2	**9**	2	2	1	0	0	2	0	0	0	0	0	0	1	0	0	0	45
EXNH	0	0	2	**18**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	90
MRMA	1	2	2	0	**13**	0	0	0	0	2	0	0	0	0	0	0	0	0	0	65
MYMA	1	0	0	0	0	**15**	0	0	0	2	0	0	0	0	0	0	0	2	0	75
MTMA	0	0	0	0	0	0	**15**	0	0	0	0	3	0	0	1	0	0	1	0	75
GSRI	0	0	0	0	1	0	0	**19**	0	0	0	0	0	0	0	0	0	0	0	95
HUNY	0	0	2	0	0	2	0	0	**12**	2	2	0	0	0	0	0	0	0	0	60
DLNJ	0	0	0	0	0	3	0	0	4	**7**	0	0	0	1	0	0	5	0	0	35
HBMD	2	0	2	0	0	1	0	0	0	2	**3**	0	1	1	0	1	4	2	0	16
NNDE	0	0	1	0	0	0	6	0	0	0	0	**11**	0	0	0	0	0	0	0	61
RAVA	0	0	1	0	0	0	0	0	0	1	0	0	**17**	1	0	0	0	0	0	85
JAVA	0	0	0	0	0	1	1	0	1	1	0	1	0	**11**	0	0	0	4	0	55
CHNC	0	0	0	0	0	0	0	0	0	0	0	0	0	1	**15**	1	0	3	0	75
STSC	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	**17**	0	0	0	85
ALGA	0	0	0	0	0	0	0	0	0	5	0	0	0	2	0	0	**13**	0	0	65
SVGA	0	0	0	0	0	0	0	0	1	0	0	0	0	2	2	1	1	**13**	0	65
SJFL	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**19**	95

Note: EMME, East Machias, ME; SGME, St. George's River, ME; OYNH, Oyster River, NH; EXNH, Exeter River, NH; MRMA, Merrimack River, MA; MYMA, Mystic River, MA; MTMA, Monument River, MA; GSRI, Gilbert Stuart River, RI; HUNY, Hudson River, NY; DLNJ, Delaware River, NJ; HBMD, Head of the Chesapeake Bay, MD; NNDE, Nanticoke River, DE; Rappahannock River, VA; JAVA, James River, VA; CHNC, Chowan River, NC; STSC, Santee-Cooper River, SC; ALGA, Altamaha River, GA; SVGA, Savannah River, GA; SJFL, St. John's River, FL.

Table A3. Jackknife reclassification rates from quadratic discriminant function analysis (DFA) for alewife using otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr after preliminary classification using genetic markers (genetic stocks are indicated; dashes indicate sites not included in DFA).

	Northern New England stock				Southern New England stock								Mid-Atlantic stock						
	EMME	SGME	CONH	LANH	MYMA	TBMA	NEMA	MTMA	GSRI	BBCT	QRCT	HUNY	DLNJ	HBMD	NNDE	RAVA	CHNC	AGNC	% correct
Northern New England stock																			
EMME	**18**	1	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	90
SGME	2	**18**	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	90
CONH	0	0	**19**	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	95
LANH	1	0	0	**19**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	95
Southern New England stock																			
MYMA	—	—	—	—	**15**	0	0	0	0	0	0	5	—	—	—	—	—	—	75
TBMA	—	—	—	—	0	**15**	0	5	0	0	0	0	—	—	—	—	—	—	75
NEMA	—	—	—	—	0	0	**15**	3	1	0	0	0	—	—	—	—	—	—	79
MTMA	—	—	—	—	0	3	2	**15**	0	0	0	0	—	—	—	—	—	—	75
GSRI	—	—	—	—	0	0	1	0	**18**	1	0	0	—	—	—	—	—	—	90
BBCT	—	—	—	—	0	0	1	0	0	**17**	0	0	—	—	—	—	—	—	94
QRCT	—	—	—	—	1	0	2	0	0	0	**17**	0	—	—	—	—	—	—	85
HUNY	—	—	—	—	7	0	0	0	0	0	1	**12**	—	—	—	—	—	—	60
Mid-Atlantic stock																			
DLNJ	—	—	—	—	—	—	—	—	—	—	—	—	**15**	5	0	0	0	0	75
HBMD	—	—	—	—	—	—	—	—	—	—	—	—	14	**5**	0	0	1	0	25
NNDE	—	—	—	—	—	—	—	—	—	—	—	—	0	0	**17**	0	2	1	85
RAVA	—	—	—	—	—	—	—	—	—	—	—	—	0	1	0	**19**	0	0	95
CHNC	—	—	—	—	—	—	—	—	—	—	—	—	0	0	2	0	**18**	0	90
AGNC	—	—	—	—	—	—	—	—	—	—	—	—	0	0	1	0	0	**19**	95

Note: EMME, East Machias, ME; SGME, St. George's River, ME; CONH, Cocheco River, NH; LANH, Lamprey River, NH; MYMA, Mystic River, MA; TBMA, Town Brook, MA; NEMA, Nemasket River, MA; MTMA, Monument River, MA; GSRI, Gilbert Stuart River, RI; BBCT, Bride's Brook, CT; QRCT, Quinnipiac River, CT; HUNY, Hudson River, NY; DLNJ, Delaware River, NJ; HBMD, Head of the Chesapeake Bay, MD; NNDE, Nanticoke River, DE; Rappahannock River, VA; CHNC, Chowan River, NC; AGNC, Alligator River, NC.

Table A4. Jackknife reclassification rates from quadratic discriminant function analysis (DFA) for blueback herring using otolith Sr:Ca, Ba:Ca, and $^{87:86}$Sr after preliminary classification using genetic markers (genetic stocks are indicated; dashes indicate sites not included in DFA).

	Northern New England stock				Southern New England stock				Mid-Atlantic stock							South Atlantic stock				
	EMME	SGME	OYNH	EXNH	MRMA	MYMA	MTMA	GSRI	HUNY	DLNJ	HBMD	NNDE	RAVA	JAVA	CHNC	STSC	ALGA	SVGA	SJFL	% correct
Northern New England stock																				
EMME	**19**	0	1	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	95
SGME	1	**15**	4	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	75
OYNH	2	3	**13**	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	65
EXNH	0	0	2	**18**	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	90
Southern New England stock																				
MRMA	—	—	—	—	**19**	1	0	0	—	—	—	—	—	—	—	—	—	—	—	95
MYMA	—	—	—	—	0	**20**	0	0	—	—	—	—	—	—	—	—	—	—	—	100
MTMA	—	—	—	—	0	0	**20**	0	—	—	—	—	—	—	—	—	—	—	—	100
GSRI	—	—	—	—	1	0	0	**19**	—	—	—	—	—	—	—	—	—	—	—	95
Mid-Atlantic stock																				
HUNY	—	—	—	—	—	—	—	—	**15**	2	3	0	0	0	0	—	—	—	—	75
DLNJ	—	—	—	—	—	—	—	—	4	**11**	3	0	0	2	0	—	—	—	—	55
HBMD	—	—	—	—	—	—	—	—	0	7	**9**	0	2	1	0	—	—	—	—	47
NNDE	—	—	—	—	—	—	—	—	0	0	0	**17**	0	0	1	—	—	—	—	94
RAVA	—	—	—	—	—	—	—	—	0	1	0	1	**17**	1	0	—	—	—	—	85
JAVA	—	—	—	—	—	—	—	—	1	1	1	2	0	**15**	0	—	—	—	—	75
CHNC	—	—	—	—	—	—	—	—	0	0	1	1	0	2	**16**	—	—	—	—	80
South Atlantic stock																				
STSC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**20**	0	0	0	100
ALGA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	**20**	0	0	100
SVGA	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	**18**	0	90
SJFL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0	**20**	100

Note: EMME, East Machias, ME; SGME, St. George's River, ME; OYNH, Oyster River, NH; EXNH, Exeter River, NH; MRMA, Merrimack River, MA; MYMA, Mystic River, MA; MTMA, Monument River, MA; GSRI, Gilbert Stuart River, RI; HUNY, Hudson River, NY; DLNJ, Delaware River, NJ; HBMD, Head of the Chesapeake Bay, MD; NNDE, Nanticoke River, DE; Rappahannock River, VA; JAVA, James River, VA; CHNC, Chowan River, NC; STSC, Santee-Cooper River, SC; ALGA, Altamaha River, GA; SVGA, Savannah River, GA; SJFL, St. John's River, FL.