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Evaluation of Otolith Microchemistry for Identifying Natal Origin of Anadromous River Herring in Connecticut

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Abstract

Over the past two decades, declines in the populations of river herring (alewife *Alosa pseudoharengus* and blueback herring *Alosa aestivalis*) have been documented across both species' ranges. Information concerning the natal origins of spawning adult fish could aid in their restoration but has been unattainable. We investigated the efficacy of otolith microchemistry for identifying the natal environment of alewife and blueback herring in Connecticut. In both 2008 and 2009, water and juvenile and adult fish were sampled from 10 sites across the state. The relationships between water and otolith Sr:Ca ratios differed for alewives and blueback herring, necessitating the development of separate classification models for assigning individuals to capture locations. Reclassification of age-0 alewives to their site of origin was variable (50–100%) but demonstrated that age-0 fish could be accurately reclassified to their natal environment at some sites. Discriminant function reclassification rates to site of capture for adult alewives varied greatly (10–85%); the similarity of water chemical signatures among many sites likely contributed to the relatively low rates of assignment to the majority of collection locations. Adult blueback herring reclassification rates were low (15–33%) for three of four collection sites, probably due to overlapping elemental signatures. Blueback herring collected at the fourth site had a distinct signature and a reclassification rate (81%) comparable to that of the highest alewife rate. Changes in Sr:Ca and Ba:Ca ratios across sectioned otoliths suggested a higher degree of movement across salinity boundaries during the first year of life than previously documented for anadromous alewives and blueback herring. Otolith microchemistry may be a useful tool with which to investigate alewife and blueback herring natal homing and life history, but its utility appears to be confined to particular sites with unique signatures or to broader regional assignments of natal origin.

Natal homing, or philopatry, is a life history characteristic shared by many species of fish. Evidence for spawning site fidelity has been documented across a diverse group of fishes, including many species of salmon (Quinn 1993; Quinn et al. 1999), weakfish *Cynoscion regalis* (Thorrold et al. 2001), bluefin tuna *Thunnus thynnus* (Rooker et al. 2008), Atlantic cod *Gadus morhua* (Svedang et al. 2007), Atlantic herring *Clupea harengus* (Brophy et al. 2006), and American shad *Alosa sapidissima* (Walther and Thorrold 2008; Walther et al. 2008). Natal fidelity has important ramifications for population connectivity, population persistence, and the potential management of fish stocks. Complete or nearly complete natal fidelity can isolate breeding populations as distinct genetic units, requiring that the breeding population at a site be managed as a distinct biological or population unit. Lower rates of natal fidelity are characteristic of loosely connected populations in which individuals primarily return to specific sites but some stray, making genetic contributions to other sites.

Alewives and blueback herring *Alosa aestivalis* are anadromous alosine species with populations along the coastline of Canada and the United States in the northwestern Atlantic Ocean. The two species are similar in appearance and are often collectively referred to as river herring. In response to recent declines in breeding populations across much of the fishes' range (NMFS 2007; Limburg and Waldman 2009), multiple states have now closed commercial and recreational river herring fisheries. The first moratorium was initiated by Connecticut in 2002, and since then Massachusetts, Rhode Island, and North Carolina have followed suit. In 2007 the National Oceanic and Atmospheric Administration declared alewives and blueback herring species of concern under the Endangered Species Act (NOAA 2007). River herring are jointly managed with a single "run" being the typical unit of focus. This management perspective relies on the untested assumption that river herring home to their natal rivers at high rates and that stock mixing is negligible.

Prior research has suggested that both species of river herring home to their natal rivers at a moderate level, but no precise estimates have been generated. Analyses of meristic characters (Messieh 1977), physical tagging of spawning adults (Jessop 1994), and a genetic analysis (Bentzen and Paterson 2005) have provided some evidence of philopatry. Additional evidence comes from olfactory choice experiments showing that adult river herring preferred water from their natal streams over alternative choices (Thunberg 1971). Research has shown that stock–recruitment relationships exist in some systems (Jessop 1990), and the stocking of nearly ripe adults reestablished remnant runs in Maine (Havey 1961), suggesting that at least some adults are returning to their natal sites.

The lack of precise estimates of homing and straying is in large part due to the fragility of juveniles, which makes physical tagging problematic. Adult tagging studies provide useful information but are hindered by the number of fish that need to be tagged to obtain precise return rate estimates, nonreporting of recovered tags (particularly in areas with closed fisheries), and

the apparently low survival of postspawn river herring (Davis and Schultz 2009). A more serious issue with this method is that tag recoveries do not represent natal homing (the natal origin of a fish tagged as an adult remains unknown) but rather spawning site fidelity. Genetic analyses designed to clarify population structure and gene flow are not well suited for estimating rates of homing and straying for several reasons. Some methods, such as those analyzing mitochondrial DNA haplotypes (e.g., Palkovacs et al. 2008) or meristic variants, do not yield sufficient differentiation among sites to inform estimates of among-site movement. Methods focusing on more variable parts of the genome, such as microsatellites and single-nucleotide polymorphisms, would in theory provide a more accurate estimate of a regionwide straying rate. However, restoration work by the Connecticut Department of Environmental Protection (CDEP) and U.S. Fish and Wildlife Service, wherein adult spawners are trapped and transported to other sites, has likely altered population genetic structure.

Otolith microchemistry is an approach that has been used to estimate philopatry in other species and could provide estimates of river herring natal fidelity. Over the past two decades, studies have consistently shown that certain elements are incorporated into fish otoliths in concentrations that reflect the chemical composition of the water that the fish inhabited (Farrell and Campana 1996; Thorrold et al. 1997; Bath et al. 2000; Milton and Chenery 2001; Elsdon and Gillanders 2002; Dorval et al. 2007). Once incorporated into the otolith, elements are metabolically inert (Campana and Thorrold 2001). In contrast to genetic or meristic based approaches, trap and transport activities should have a minimal effect on otolith chemistry. It is possible for the primordia of the otolith to record traces of the environment experienced (i.e., marine or freshwater) by the maternal parent (Zimmerman and Reeves 2002); however, in this study any maternal legacy reflected in the primordia of otoliths would be similar across drainages since spawning adults are all migrating from the coastal Atlantic Ocean.

Otolith microchemistry has been used in retrospective examinations of diadromous fish life history due to the ability of the technique to detect changes in salinity experienced by individuals, largely through changes in the Sr concentration (Limburg 1995; Secor et al. 1995; Limburg 1998; Secor and Rooker 2000; Zimmerman 2005; Diouf et al. 2006). The microchemical demarcation of egress to sea in a life history is essential because it allows for the elemental signature of freshwater residency (i.e., a natal site) to be isolated, which enables the characterization of chemical signatures for natal sites (Milton et al. 1997; Thorrold et al. 1998a, 1998b; Brazner et al. 2004; Hobbs et al. 2005; Tomás et al. 2005; Kellison and Taylor 2007; Whitledge et al. 2007; Walther et al. 2008). This approach has not been applied to alewives or blueback herring; the capability of otoliths to act as natural tags of natal origin and tracers of individual movements could be a valuable tool with which to estimate natal fidelity and stock compositions.

The goal of this study was to estimate natal fidelity in alewives and blueback herring using otolith microchemistry.
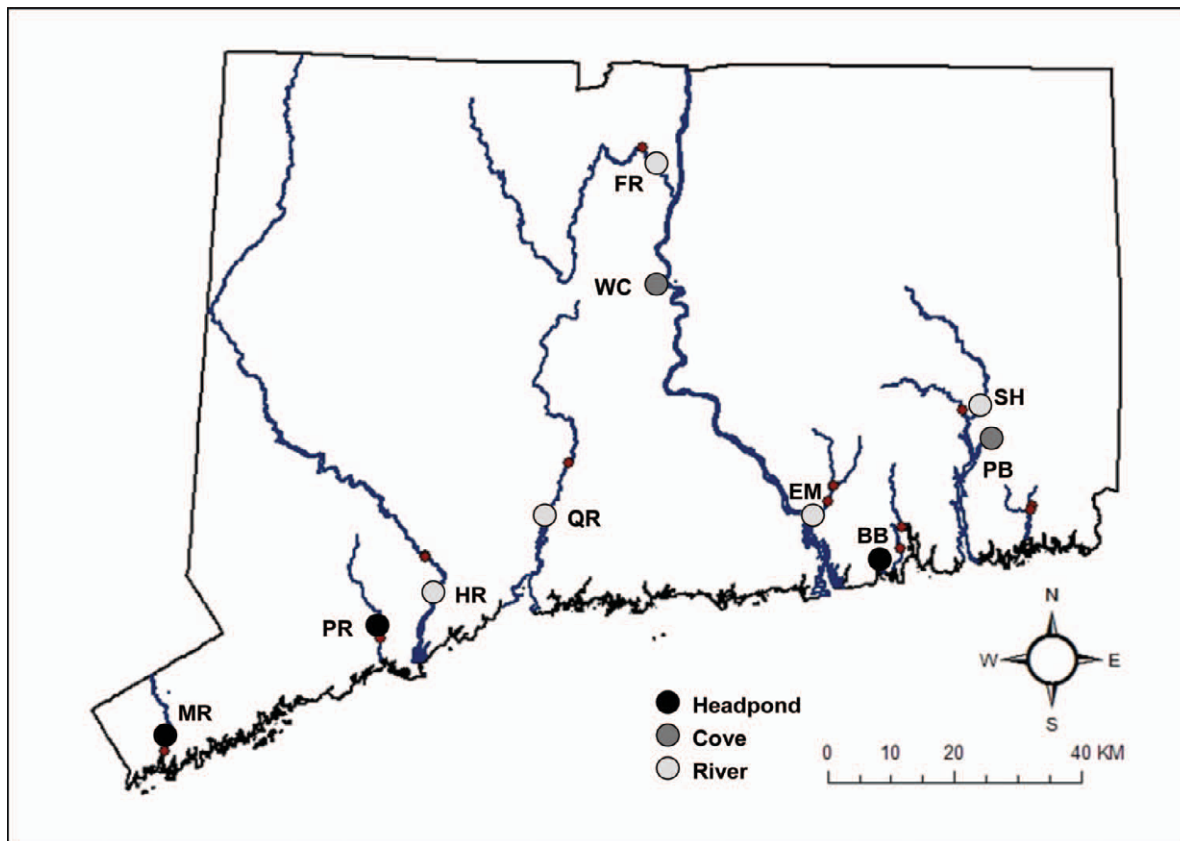
FIGURE 1. Map of the north shore of Long Island Sound showing the locations of the collection sites, with indications of habitat type. From west to east the sites are as follows: the Mianus River (MR), the Pequonnock River (PR), the Housatonic River (HR), the Quinnipiac River (QR), the Farmington River (FR), Wethersfield Cove (WC), the Eightmile River (EM), Bride Brook (BB), the Shetucket River (SR), and Poquetanuck Brook (PB). The red dots represent dams.

The first objective was to characterize the spatiotemporal variability in water chemistry. The second objective was to analyze the microchemical constituents of age-0 river herring otoliths to test whether local water chemistry affected otolith chemistry, determine whether there are differences in the incorporation of elements into otoliths between the two species, and attempt to correctly classify known individuals back to their site of collection. Finally, the chemical records from the cores of otoliths from adult fish collected during their spawning migration were used to assess the rate at which fish could be classified back to their collection site.

STUDY AREA

This study took place along the north shore of Long Island Sound, which has many rivers that support alewife and blueback herring runs. Long Island Sound is a 177 km long tidal estuary bounded by Connecticut and mainland New York to the north and west, and Long Island on the south. The eastern end is open to Block Island Sound and the Atlantic Ocean with the exception of a chain of small islands extending from the north fork of Long Island. All the rivers in this study are connected to Long Island Sound directly or through rivers to which they serve as tributaries.

Fish were collected from 10 sites in seven watersheds (Figure 1). The sites varied in size and distance from Long Island Sound. The 10 sites represent three different habitat types: riverine, coves of larger rivers, and headponds (natural and man-made lentic water bodies) connected to Long Island Sound by short stream segments.

METHODS

Water collection and analysis.—Water samples were collected from all 10 sites using a syringe filtration procedure (Shiller 2003) to characterize minor and trace element chemistry. The water sampling locations within sites represented nursery areas where age-0 fish were collected or observed by CDEP biologists in the past 5 years. Each site was sampled once in early summer and again in autumn in both 2008 and 2009 to capture the inter- and intraannual variability in elemental composition. Water samples were stored on ice in a cooler and transported to the Center for Environmental Science and Engineering at the University of Connecticut. Water samples were analyzed using inductively coupled mass spectrometry (ICPMS)

to quantify the concentrations of a suite of minor and trace elements, including Ca, Sr, Ba, Mg, K, Li, U, Mn, Fe, Ni, Cu, and Zn. These elements are known to occur in otoliths (Campana 1999) and have been used as tracers of natal habitats in previous studies (Campana et al. 2000; Thorrold et al. 2001; Brazner et al. 2004; Patterson et al. 2004; Forrester 2005; Miller et al. 2005; Whitledge et al. 2007) Elemental concentrations were originally calculated in units of µg/L. These values were subsequently normalized to Ca and are reported as molar element : Ca ratios (mmol/mol). The elemental ratios were averaged within each year for all sites.

River herring collection and identification.—Returning adult alewives and blueback herring were collected from March through June of 2008 and 2009. Five hundred forty-four alewives from 218 to 312 mm total length (TL) and 240 blueback herring from 210 to 307 mm TL were retained. To obtain a representative temporal sample of returning adults, fish were collected from each site every 8–12 d for a total of three to five sampling dates per site each year. To ensure complete representation of size- and age-class variation within runs, fish were classified by 5-mm size increments during each sampling event and one to two fish per size-class were retained for otolith extraction.

Adult fish were collected with dip nets, cast nets, backpack electrofishing, and nighttime boat electrofishing. The total length to the nearest mm and weight to the nearest 0.1 g of all retained fish were recorded. Sex was determined by expressing gametes from individual fish and gut cavity inspection for sexual organs. Species were identified by external coloration, morphology, and for those fish retained for otolith extraction, pigmentation of the peritoneal lining (Collette and Klein-MacPhee 2002). Fish were euthanatized by cold shock, sealed in individual bags and placed on ice. Fish were frozen whole until otoliths were removed several weeks subsequent to collection.

Age-0 fish were sampled during the latter portion of their freshwater residency. By this means, the amount of time they had spent in their natal environment was maximized, thereby increasing our ability to assess the intraannual variation in otolith chemistry during the period of juvenile freshwater residency. Five age-0 fish per site were collected from 6 of the 10 sites in September–October 2008 and from 8 of the 10 sites in September–October 2009. Age-0 fish were collected using the same gears used for collecting adults and were identified to species by head morphology and the color of the peritoneal lining (Walsh et al. 2005). Despite multiple efforts, no age-0 fish were collected from the Housatonic River or the Shetucket River in 2008 and from Poquetanuck Brook and the Farmington River in either year.

Otolith preparation and analysis.—Both sagittal otoliths were removed from each fish using nonmetallic forceps, thoroughly rinsed in reverse-osmosis de-ionized water to remove any organic materials, and stored in capped polyethylene vials. One otolith from each fish was chosen randomly for microchemical analysis. Adult otoliths were embedded in Epo-Fix

epoxy and sectioned in the transverse plane using an ISOMET low-speed saw (Buehler, Lake Bluff, Illinois) so that the section included the otolith core. Age-0 otoliths were placed in thermoplastic glue and ground in the sagittal plane. All otoliths were ground evenly with multiple grades of silicon carbide wet–dry sandpaper and polished with 3-µm lapping film to reveal growth increments and create a polished surface prior to ablation. Prepared otoliths were affixed to acid-washed glass slides using double-sided tape (3M, St. Paul, Minnesota), placed in polypropylene petri dishes, and ultrasonically cleaned for 5 min in ultrapure water (Fisher Scientific, Pittsburgh, Pennsylvania). Samples were then dried in a Class 100 laminar flow hood.

Adult otolith thin sections and juvenile otoliths ground in the sagittal plane were analyzed for ^{7}Li, ^{25}Mg, ^{39}K, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{62}Ni, ^{63}Cu, ^{65}Cu, ^{66}Zn, ^{67}Zn, ^{86}Sr, ^{88}Sr, ^{118}Sn, ^{120}Sn, ^{138}Ba, and ^{238}U using a Finnegan Thermo (ICPMS; Waltham, Massachusetts) Elemental X7 inductively coupled mass spectrometer coupled with a Quantronix (East Setauket, New York) Integra C femtosecond (fs) laser at the Great Lakes Institute for Environmental Research at the University of Windsor, Windsor, Ontario. Transects were ablated from the dorsal side of the otolith to the ventral edge passing through the core (beam diameter = 24 µm, laser pulse rate = 100 Hz, laser energy level = 0.026 mJ/pulse, wavelength = 785 nm, and dwell time = 253 ms). A National Institute of Standards and Technology standard (NIST 610) was ablated every 5–17 samples to adjust for possible instrument drift. Each sample analysis was preceded by a gas blank measurement. Isotopic counts were converted to elemental concentrations (µg/g) after correction for gas blank, matrix, and drift effects. Elemental concentrations in otoliths were normalized to Ca, which was considered an internal pseudostandard (Bickford and Hannigan 2005; Ludsin et al. 2006; Whitledge et al. 2007). All otolith microchemistry data are reported as element : Ca ratios (µmol/mol).

The element : Ca ratios were averaged over the entire juvenile freshwater residency period. In adult otoliths, the juvenile freshwater residency period was isolated by locating transitions in the Sr:Ca and Ba:Ca levels during the first year, indicating the onset of marine residency (Limburg 2001; Secor and Piccoli 1996). To determine the values of these ratios that are characteristic of the freshwater and marine residency periods during the first year of life, we calculated the 95th percentile value of Sr:Ca and the 5th percentile value of Ba:Ca from the age-0 fish that were known to inhabit freshwater (Lochet et al. 2008). These values provided benchmarks for freshwater habitation; transitions from "freshwater" Sr:Ca and Ba:Ca values to the elevated Sr:Ca and depressed Ba:Ca values that were observed prior to the first annulus in adult fish otoliths were assumed to be the result of emigration to saltwater; pronounced shifts were apparent in most adult otoliths. In cases where no transition was observed, the ablated otolith was examined under a compound microscope to discover whether the core had been removed during grinding or missed during ablation. If this was the case, the otolith and corresponding fish were removed from the study. If

the ablation passed through the intact core, then the otolith was retained in the study. Among the retained adult otoliths, 333 were alewives and 165 were blueback herring.

Data analysis.—Both univariate and multivariate approaches were used to assess differences in trace element concentrations among collection sites. All water and otolith concentrations had skewed distributions and were $\log_{10}$ transformed to correct for nonnormality. In most cases, both the water and otolith Sr:Ca and Ba:Ca values were still nonnormal after transformation. For all tests, a P-value of ≤ 0.05 was considered significant. To characterize the variability in mean water chemistry, two-way analyses of variance (ANOVA) with an interaction term combining site and year was used. Post hoc comparisons among sites of element : Ca ratios were made using Tukey's honestly significant difference (HSD) test. To assess the effect of species on differences in the age-0 element : Ca ratios, analysis of covariance (ANCOVA) tests were run with Tukey's adjustment; year-specific mean water element : Ca ratios from the collection sites were used as covariates in these analyses.

For an element : Ca ratio to aid in natal site identification, its value in the water must differ among sites and this variability must be reflected in the otoliths of the fish inhabiting those sites. Elements that did not meet both of these qualifications were removed from further consideration. Data from the two years of age-0 fish were pooled to meet sample size requirements, and differences in mean element : Ca among sites were identified using one-way ANOVA with Tukey's HSD test. Differences in the juvenile portion of adult otolith element : Ca ratios among sites were evaluated in identical fashion. The relationships between water chemistry at a site and year and individual otolith chemistry parameters were assessed by linear regression for each element.

To characterize the site-specific signatures, a number of multivariate techniques were used. Sites represented by inadequate sample sizes were removed prior to analysis (McGarigal et al. 2000). The minimum sample size was defined as

$$N > 3(P + M),$$

where N is the number of samples, P represents the variables in group 1 (i.e., the sites), and M represents the number of variables in group 2 (i.e., the elements). The sites were characterized in multivariate space using both multivariate analysis of variance (MANOVA) and canonical analysis of discrimination (using PROC CANDISC in program SAS 9.2; SAS, Inc., Cary, North Carolina). Pillai's trace statistic was used to assess significant differences in multivariate elemental signatures for the study sites. Classifications of individuals to collection sites were made through quadratic discriminant function analysis with a leave-one-out jackknife procedure (PROC DISCRIM; SAS 9.2). A classification was considered successful when an age-0 fish of known origin was classified to its site of origin rather than to an alternative collection site. We also analyzed the proportion of adult fish, which were all of unknown origin,

that were classified to their collection location based on their juvenile freshwater-period otolith chemical signatures. Differences in multivariate otolith chemical signatures among sites were visualized by plotting collection site group means with 95% confidence ellipses around the centroids in SYSTAT 13 (Systat Software, Inc., Chicago, Illinois).

Discriminatory performance was examined using the eigenvalue of each canonical variate describing the proportion of among-group variation. Performance was also evaluated using the squared canonical correlation (R_c^2), which represents the amount of dispersion explained in a canonical variate that is attributable to differences among the examined groups (McGarigal et al. 2000). The accuracy of the discriminant function analysis was also assessed using a randomization method (White and Ruttenberg 2007). A bootstrap procedure generated 5,000 randomizations of the otolith data to estimate the random chance of success and determine whether the mean rates of reclassification were significant.

Evidence for different movement patterns among individual fish during the juvenile freshwater residency period was examined by inspection of the trends along the laser ablation transect in otolith Sr:Ca and Ba:Ca values. Elemental concentrations were plotted beginning at the core and proceeding outward to the edge of the otolith. Analyses of movement patterns of age-0 fish examined data from the entire otolith, while analyses of adult fish only used data up the point of emigration to sea. Periods in which Sr:Ca values were sustained at near seawater levels (1,500–2,500 μmol/mol) only to revert to freshwater levels (200–1,000 μmol/mol) were interpreted as transitions among habitats, such as from a coastal pond to a downstream tidal estuary (Limburg 1995, 1998, 2001; Secor et al. 1995; Kennedy et al. 2002; Morris et al. 2003; Walther and Thorrold 2006). The values observed as indicators of environmental transitions in alewives were similar to those observed in prior studies of diadromous alosines (Limburg 1995; Secor et al. 2001; Lochet et al. 2008).

RESULTS

Water Chemistry

Element : Ca ratios for Mg, K, Mn, Fe, Zn, Sr, and Ba in water varied among sites. However only variability in Sr:Ca and Ba:Ca ratios produced corresponding differences in otolith chemistry among sites, so Mg, K, Mn, Fe, and Zn were not considered further. Water Sr:Ca and Ba:Ca values were not significantly different between the 2 years (two-way ANOVA; Sr:Ca: $F = 0.85$; df $= 1, 20$; $P = 0.37$; Ba:Ca: $F = 3.41$; df $= 1, 20$; $P = 0.08$). Water Sr:Ca varied significantly by site (two-way ANOVA; $F = 10.48$; df $= 9, 20$; $P < 0.0001$) in the same way each year (site $\times$ year interaction term: $F = 0.15$; df $= 9, 20$; $P = 0.9996$). Poquetanuck Brook had a relatively high Sr:Ca value, while the Quinnipiac and Housatonic rivers had relatively low Sr:Ca values (Figure 2a). Water Ba:Ca varied significantly by site (two-way ANOVA; $F = 6.74$; df $= 9, 20$; $P = 0.0002$) in
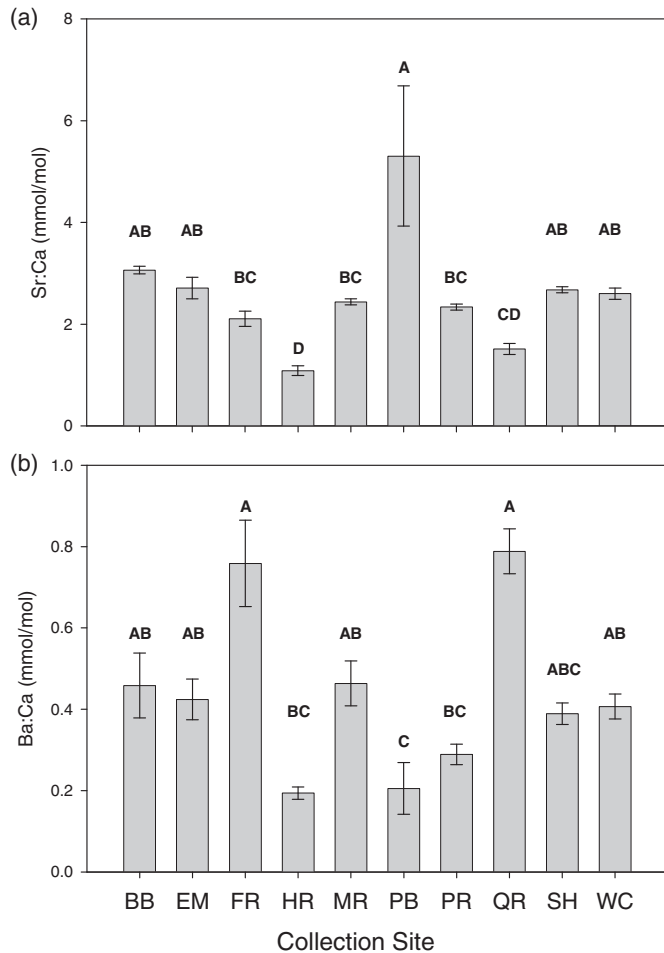
FIGURE 2. Mean **(a)** water Sr:Ca and **(b)** water Ba:Ca values at the 10 collection sites in 2008–2009. The error bars represent SEs. Means with the same letter are not significantly different (ANOVA with Tukey's HSD test on $\log_{10}$ transformed values; $P < 0.05$).

FIGURE 3. Linear regressions of **(a)** the otolith Sr:Ca values of individual age-0 alewives on the mean Sr:Ca values in the water at the collection sites and **(b)** the otolith Ba:Ca values of individual age-0 alewives on the mean Ba:Ca values in the water at the collection sites. All element : Ca ratios were based on the freshwater residency period of the fish and were $\log_{10}$ transformed.

the same way each year (site $\times$ year interaction term: $F = 0.45$; df $= 9, 20$; $P = 0.89$). The Farmington and Quinnipiac rivers had the highest mean Ba:Ca and the Housatonic River had the lowest (Figure 2b).

Age-0 Otolith Chemistry

The relationship between mean water Sr:Ca and otolith Sr:Ca values differed significantly between age-0 alewives and blueback herring (ANCOVA; $F = 51.70$; df $= 1, 64$; $P < 0.0001$). Blueback herring otoliths had lower Sr:Ca for a given water Sr:Ca than alewives (Figures 3a and 4a). The relationship between water and otolith Ba:Ca did not differ between species ($F = 0.23$; df $= 1, 64$; $P = 0.63$).

The Sr:Ca and Ba:Ca values in the water from natal sites were strongly correlated with the values in age-0 alewife otoliths (Sr:Ca: Figure 3a; $R^2 = 0.7070$, $P < 0.0001$; Ba:Ca: Figure 3b; $R^2 = 0.2172$, $P = 0.003$) and age-0 blueback herring otoliths (Sr:Ca: Figure 4a; $R^2 = 0.7560$, $P < 0.0001$; Ba:Ca:
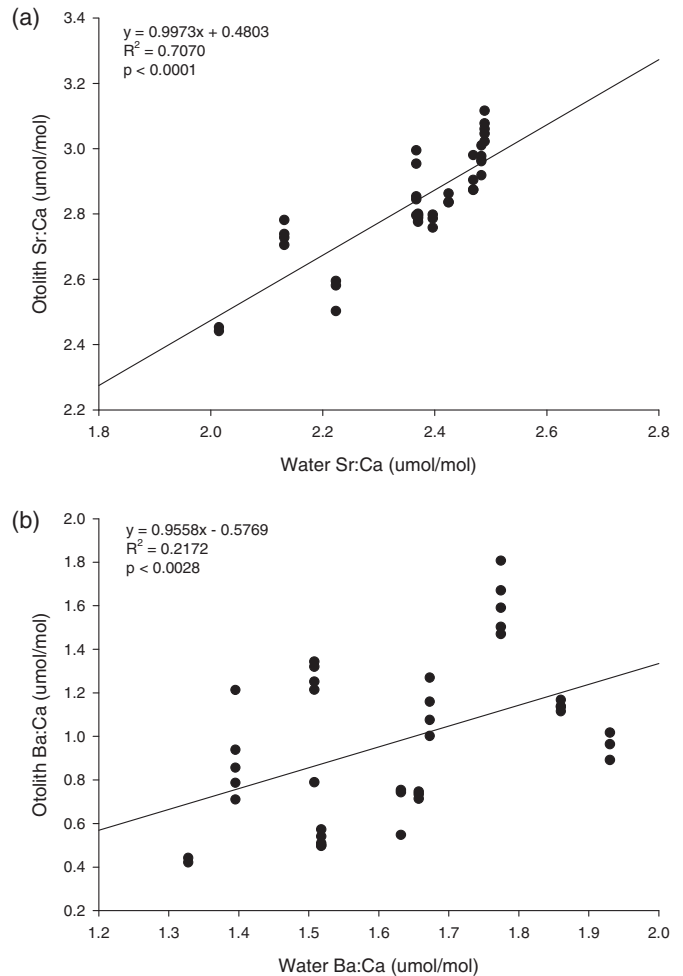
Figure 4b; $R^2 = 0.3070$; $P = 0.002$). The difference between species in the incorporation of Sr into the otoliths necessitated that the two species be analyzed separately in subsequent analyses.

The Ba:Ca and Sr:Ca values in age-0 alewife otoliths varied among sites and allowed for high reclassification rates at some sites. Age-0 fish collected from the Housatonic River, the Mianus River, and the Shetucket River were removed prior to multivariate analysis because of inadequate sample sizes at those sites. Fish from the four remaining alewife collection sites (Bride, Eightmile, Quinnipiac, and Pequonnock) had significantly different otolith chemistry signatures (Pillai's trace statistic; $F = 17.55$, df $= 6, 54$, $P < 0.0001$). Canonical scores suggested that most sites had distinctive chemical signatures; the Eightmile River was not distinct from other collection areas (Figure 5). The first canonical function (CAN 1) explained 77% of the total dispersion in the data, and 79% of the variation
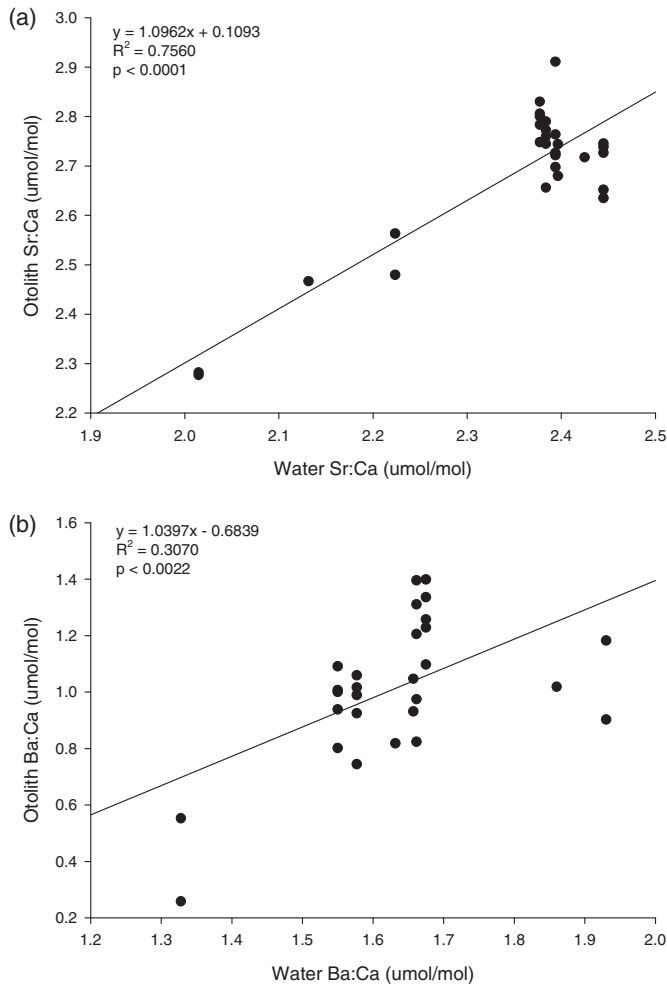
(a)

$y = 1.0962x + 0.1093$
$R^2 = 0.7560$
$p < 0.0001$

(b)

$y = 1.0397x - 0.6839$
$R^2 = 0.3070$
$p < 0.0022$

FIGURE 4. Linear regressions of **(a)** the otolith Sr:Ca values of individual age-0 blueback herring on the mean Sr:Ca values in the water at the collection sites and **(b)** the otolith Ba:Ca values of individual age-0 blueback herring on the mean Ba:Ca values in the water at the collection sites. See Figure 3 for additional information.

TABLE 1. Results of quadratic discriminant function analysis with the jack-knife procedure for age-0 alewives. The number of fish analyzed per site is the total for each row. Classification accuracy back to the site of collection was based on otolith Sr:Ca and Ba:Ca values.

	Assigned site				
Source site	Bride	Eightmile	Pequonnock	Quinnipiac	% Correct
Bride	9	1	0	0	90
Eightmile	1	2	0	1	50
Pequonnock	1	2	7	0	70
Quinnipiac	0	0	0	7	100

fication rate generated by the QDFA procedure was 78%, which was comparable to the mean reclassification rate generated by the randomization procedure (81%; $P = 0.0002$).

The Ba:Ca and Sr:Ca values in age-0 blueback herring otoliths varied little among sites, and reclassification rates were low. Juvenile blueback herring inhabit fewer aquatic systems in Connecticut and were collected from only six locations. Low sample sizes necessitated the omission of data from three rivers (the Housatonic, Quinnipiac, and Shetucket rivers) from the multivariate analyses. The MANOVA results indicated that there were no significant differences in age-0 blueback herring Sr:Ca and Ba:Ca values from the remaining sites (Eightmile, Wethersfield Cove, and Mianus; Pillai's trace statistic: $F = 2.17$, df = 4, 38, $P < 0.09$). The first canonical function explained 65% of the total dispersion in the data. However, differences in site means accounted for only 23% of the variation in CAN 1. Otolith Ba:Ca was correlated (correlation coefficient = 0.97, $P < 0.0001$) with CAN 1. The second canonical function was correlated with otolith Sr:Ca (correlation coefficient = 0.99, $P < 0.0001$), but differences among sites explained only 19% of the variation

in CAN 1 was attributed to differences among the sites. Both otolith Sr:Ca and Ba:Ca were correlated with CAN 1 (correlation coefficients: Sr:Ca = 0.97; Ba:Ca = 0.64; $P \leq 0.0001$). The second canonical function (CAN 2) explained the remainder of the dispersion in the data, and 53% of the variation in CAN 2 was attributed to differences among the sites. Only otolith Ba:Ca values were correlated with CAN 2 (correlation coefficient = 0.77, $P < 0.0001$). CAN 2 especially contributed to discrimination between Pequonnock and Quinnipiac (Figure 5). Quadratic discriminant function analysis (QDFA) was conducted using Sr:Ca and Ba:Ca since both elements contributed significantly to discriminatory power. Reclassification to site of capture was possible in most instances (Table 1); individuals were classified with 50–100% accuracy. All of the QDFA reclassification rates were higher than expected from chance alone (randomization null reclassification rate = 24%; SD = 0.10). The mean reclassi-
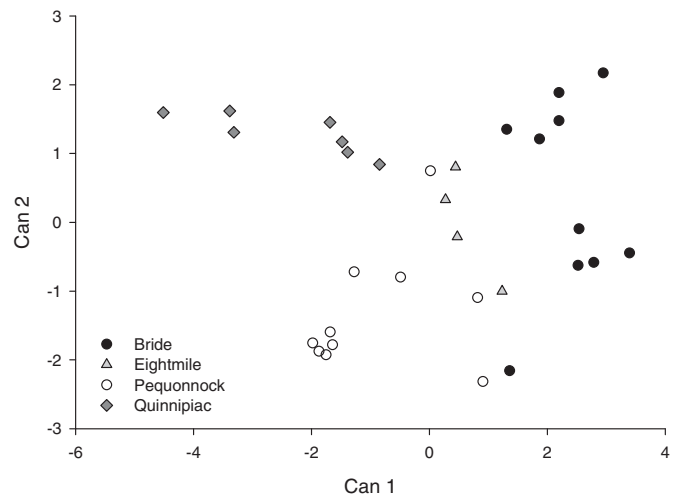

FIGURE 5. Plot of canonical variates 1 and 2 created through a quadratic discriminant function analysis including age-0 alewife Sr:Ca and Ba:Ca values.

in CAN 2. As a result, the QDFA jackknife reclassification accuracy for age-0 blueback herring to their site of collection was low (20–57%) and not reliable (randomization null reclassification rate = 33%; SD = 12%). All of the reclassification rates generated by the QDFA fell within the 95% confidence limits of the null reclassification estimate. The mean reclassification success rate generated by the randomization procedure (36%) was comparable to the mean rate of the QDFA (39%) but not significantly different from the null reclassification rate ($P = 0.46$).

Adult Otolith Chemistry

Returning adult alewives were collected from all 10 sites, although fewer than 10 fish were collected from Wethersfield Cove and the Farmington River and these sites were omitted from the multivariate analysis. Both otolith Sr:Ca and Ba:Ca during the juvenile freshwater residency period of adult alewives differed among collection sites (one-way ANOVA; Sr: $F = 41.73$, df = 7, 325, $P < 0.0001$; Ba: $F = 14.88$, df = 7, 325, $P < 0.0001$). Adult alewife otolith chemical signatures varied among sites (Pillai's trace statistic: $F = 27.06$; df = 14, 650; $P < 0.0001$). Some sites had distinctive signatures (Figure 6a). The 95% confidence ellipses of the group means suggest separation of signatures for Poquetanuck Brook, the Housatonic River, the Pequonnock River, and Bride Brook. The signals for the Quinnipiac, Mianus, Shetucket, and Eightmile rivers either overlapped or were relatively more dispersed. CAN 1 explained 76% of the total dispersion in the data. Differences among sites accounted for 50% of the total variation within CAN 1. Otolith Sr:Ca ratios were correlated with CAN 1 (correlation coefficient = 0.96; $P < 0.0001$). Among-site differences were responsible for 24% of the variation in CAN 2. Adult alewife otolith Ba:Ca levels were positively correlated with CAN 2 (correlation coefficient = 0.99; $P < 0.0001$), and Sr:Ca levels were negatively correlated with CAN 2 (correlation coefficient = –0.30; $P < 0.0001$). Reclassification of fish to their site of collection was variable; rates ranged between 10% and 85% (Table 2). Poquetanuck Brook, Bride Brook, and the Housatonic River, which had distinctive signatures, returned higher (64–85%) reclassification rates. Locations with overlapping and variable signatures (the Shetucket, Quinnipiac, and Eightmile rivers) had lower rates (10–20%) of reclassification. Adult alewife reclassifications were accurate; the estimated rate of successful reclassification due to chance alone was equivalent to the results for the three sites with the lowest reclassification rates (randomization null reclassification rate = 12%; SD = 2.5%). The overall mean QDFA reclassification rate (45%) compared favorably with the significant mean obtained by the randomization routine (mean reclassification rate = 40%; $P = 0.0002$).

Otolith chemistry differences among adult blueback herring were predominately restricted to one site. As expected, returning adult blueback herring were collected at fewer sites (five) than alewives. Only one individual was collected from the Shetucket River, so that the site was omitted from the multivariate analyses. Otolith Sr:Ca and Ba:Ca values during the juvenile
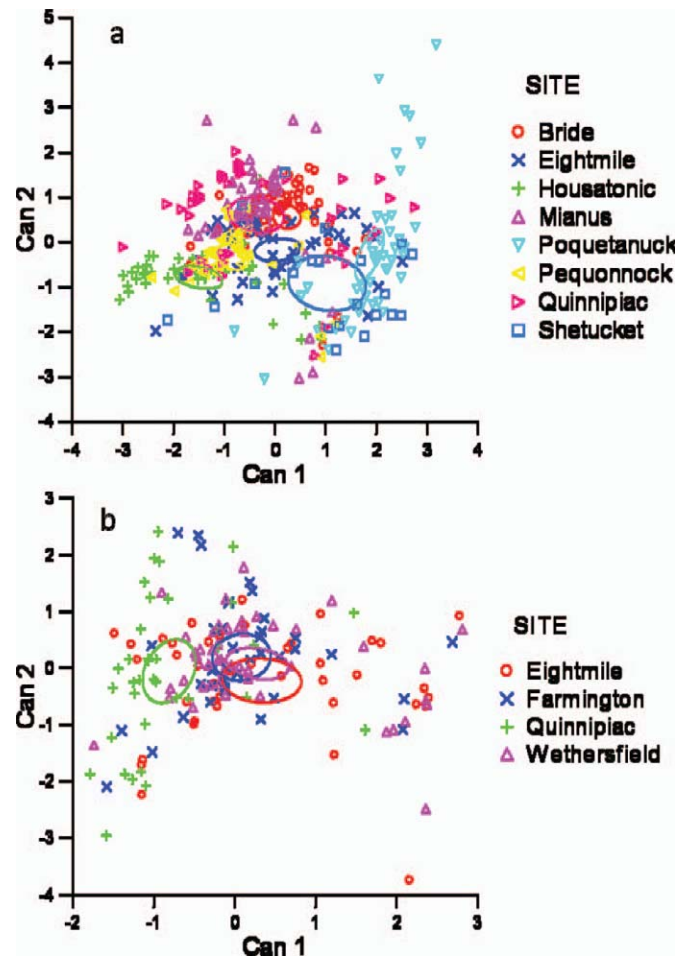

FIGURE 6. Plots of canonical variates 1 and 2 created through quadratic discriminant function analyses including Sr:Ca and Ba:Ca values from (**a**) adult alewife otoliths and (**b**) adult blueback herring otoliths. The points represent individuals collected from the indicated sites, and the ovals are 95% confidence ellipses around the group centroids generated from the samples.

freshwater residency period of adult blueback herring from the Quinnipiac River, Eightmile River, Farmington River, and Wethersfield Cove were significantly different among sites (one-way ANOVA; Sr: $F = 10.48$, df = 3, 160, $P < 0.0001$; Ba: $F = 3.10$, df = 3, 160, $P < 0.0285$). Multivariate otolith chemical signatures also differed among sites (Pillai's trace statistic: $F = 5.53$; df = 6, 320; $P < 0.0001$). Canonical plots suggested that the only distinct signature was that of the Quinnipiac River, which separated on CAN 1 (Figure 6b). CAN 1 explained 89% of the dispersion in the data, but only 17% of the variation in CAN 1 could be attributed to differences among sites. Adult blueback herring otolith Sr:Ca was positively correlated with CAN 1 (correlation coefficient = 0.99; $P < 0.0001$), and otolith Ba:Ca was negatively correlated (correlation coefficient = –0.48; $P < 0.0001$). CAN 2 made a negligible contribution to the discrimination among sites. Reclassification of individuals back to their site of capture was low for the three Connecticut River tributary sites (the Farmington River, Wethersfield Cove, and the Eightmile River) and ranged between 15% and 33%.

TABLE 2. Results of quadratic discriminant function analysis with the jackknife procedure for adult alewives. Site codes are as follows: BB = Bride Brook, EM = the Eightmile River, HR = the Housatonic River, MR = the Mianus River, PB = Poquetanuck Brook, PR = the Pequonnock River, QR = the Quinnipiac River, and SH = the Shetucket River. See Table 1 for additional information.

Source site	Assigned site								% Correct
	BB	EM	HR	MR	PB	PR	QR	SH	
BB	32	2	1	5	5	5	0	0	64
EM	8	7	4	5	6	12	0	0	17
HR	0	0	26	1	1	8	0	4	65
MR	12	2	0	22	4	6	2	1	45
PB	0	1	1	0	41	0	0	5	85
PR	1	2	8	5	3	20	0	0	51
QR	4	2	3	12	8	6	9	0	20
SH	2	3	2	1	10	1	0	2	10

The Quinnipiac River, however, had a reclassification rate of 81% (Table 3). This rate was comparable to that of the most distinct adult alewife collection site, Poquetanuck Brook, which had a reclassification rate of 85%. Some of the adult blueback herring QDFA classifications were not better than chance (randomization null reclassification rate = 25%; SD = 4.2%). The reclassification rates for the three Connecticut River sites fell within the 95% confidence limits for this estimate. The mean reclassification rate obtained by QDFA (40%) was comparable to the significant mean estimated by the randomization procedure (mean reclassification rate = 35%, $P = 0.0068$).

Early Life History Movements

Otolith records of the early life history of individual fish were often suggestive of occupancy of a discrete habitat (i.e., minimal variation in Sr:Ca and Ba:Ca) until emigration from natal sites, but a subset of fish did not conform to this pattern. Otolith chemistry from age-0 river herring collected in freshwater and from the portions of adult transects corresponding to marine residency (i.e., near the edge of the otolith) indicated that Sr:Ca values reflective of saltwater occupancy were above 2,000 µmol/mol while Sr:Ca values during freshwater residency were typically below 1,000 µmol/mol. The 95th percentile for otolith Sr:Ca generated from age-0 alewives was 1,195 µmol/mol, while the value for age-0 blueback herring was 676 µmol/mol. The Ba:Ca patterns were usually the inverse of those for Sr:Ca, but

sometimes Ba:Ca remained elevated even when Sr:Ca reached levels indicative of high-salinity environments. Ba:Ca ratios around 3 µmol/mol were typical of freshwater residency, while values below 2 µmol/mol were indicative of marine residency; the 5th percentile value for Ba:Ca generated from age-0 alewives was 2.76 µmol/mol and that for blueback herring was 3.57 µmol/mol.

Most fish displayed patterns that suggested direct emigration from freshwater natal habitats to the marine environment (Figure 7a, b). Records from age-0 fish sampled from headponds were assumed to reflect temporal rather than spatial variations in element : Ca ratios because once juveniles left these sites reentry into the nursery was impeded. Inspection of the individual otolith microchemistry transects of age-0 alewives and blueback herring from non–headpond locations sometimes revealed patterns that suggested spatial rather than temporal variation, although variation could have theoretically occurred from shifts in the position of the salt wedge at river sites closer to Long Island Sound. These patterns were typified by periods when the magnitude of Sr:Ca rapidly doubled or decreased by half. In many instances, Sr:Ca levels would approach or equal those typical of saltwater environments only to return to levels representative of freshwater (Figure 7c, d).

Otolith chemistry from the early life of returning adults also indicated that at age 0 some individuals made transitions between environments with different salinities prior to emigration

TABLE 3. Results of quadratic discriminant function analysis with the jackknife procedure for adult blueback herring. See Table 1 for additional information.

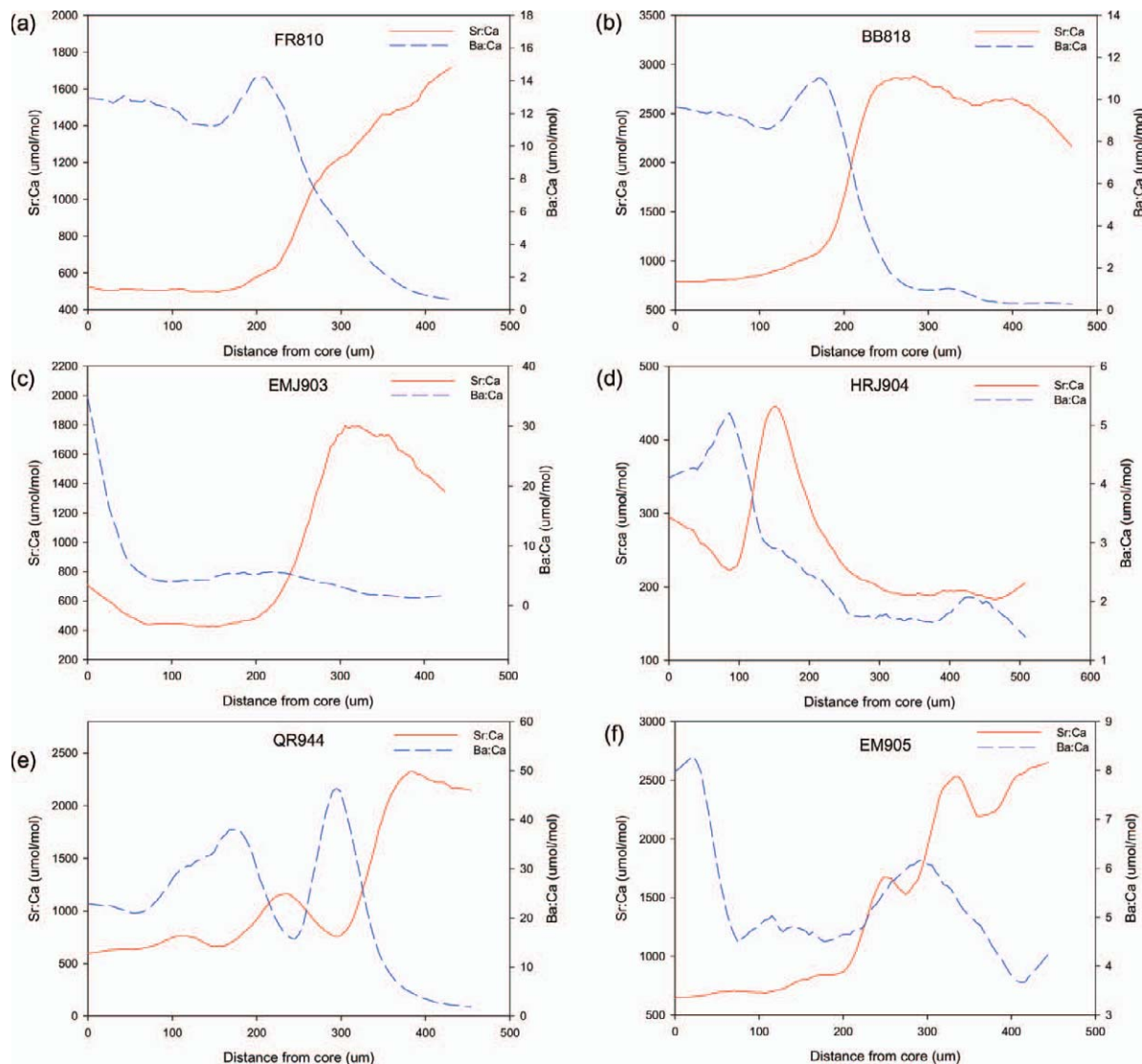| Source site	Assigned site				% Reclassified
	Eightmile	Farmington	Quinnipiac	Wethersfield	
Eightmile	6	9	13	13	15
Farmington	4	12	9	13	32
Quinnipiac	1	3	29	3	81
Wethersfield	8	17	8	16	33

FIGURE 7. Representative Sr:Ca and Ba:Ca transects of **(a)**–**(b)** adult fish that migrated directly from their natal sites to the ocean, **(c)**–**(d)** age-0 fish that migrated indirectly from their natal sites to the ocean, and **(e)**–**(f)** adult fish that migrated indirectly. All transects proceed from the core of the otolith toward the edge. Note that the scales vary for both the *y*- and *x*-axes; the transects for the age-0 fish are complete, but those for the adults are abbreviated at the point of migration to saltwater. The data were smoothed for presentational clarity.

to saltwater (Figure 7e, f), particularly in systems where movement between different habitats was unrestricted, such as tributaries of the Connecticut River and other riverine sites. Inspection of the early life history of all adult fish showed that 77 of the 491 fish examined (15.6%; 48 alewives and 29 blueback herring) moved multiple times between freshwater and marine habitats. The proportion of alewives and blueback herring in our samples displaying this alternative emigration pattern was similar to the overall proportion of the two species in our study.

DISCUSSION

There were significant differences in water chemistry among some study sites and in the otoliths of age-0 fish collected from these sites, but the differences were not sufficient for complete resolution among sites. Only two chemical markers (Sr:Ca and Ba:Ca) had significant differences among the water bodies sampled that were also reflected in the otoliths of age-0 fish. Most often Sr:Ca and Ba:Ca, along with stable isotopes, are the most effective habitat discriminators (Elsdon et al. 2008). The other markers that differed in the water samples (Mg:Ca, K:Ca, Mn:Ca, Fe:Ca, and Zn:Ca) can be effective but are inconsistent due to factors such as temporal variability and physiological regulation prior to or during incorporation into the otolith (Elsdon et al. 2008). Typically, reclassification rates of known-origin individuals to collection locations are greater than the rates seen in the age-0 and adult river herring in this study (Brazner

et al. 2004; Thorrold et al. 1998a; Walther and Thorrold 2008; Zeigler and Whitledge 2010), but similar rates have been reported in other areas (Proctor et al. 1995; Bronte et al. 1996; Gillanders et al. 2001). The difficulty observed in reclassifying individual fish back to their natal sites was probably linked to the relatively small spatial scale of our study and thus to minimal geologic heterogeneity. In contrast to sympatric anadromous species such as striped bass *Morone saxatilis* and American shad, which only ascend larger watersheds to reproduce, river herring (and in particular alewives) utilize watersheds of all sizes and often return to neighboring rivers. Unfortunately, classification resolution at this small spatial scale, which is important to understanding potential metapopulation dynamics, is precisely the information needed to inform river herring management and restoration activities.

The reclassification of age-0 alewives to their collection location was more successful than the reclassification of age-0 blueback herring. Initial concerns about the low within-group sample size (Williams and Titus 1988) were reduced by the significant results of the randomization procedure. Geologic similarity among the watersheds in Connecticut and the corresponding similarities in water Sr:Ca and Ba:Ca signatures likely account for many of the low reclassification rates for fish to their collection locations, particularly for age-0 blueback herring. Connecticut primarily consists of three geologic terranes: the Iapetos (Oceanic), Newark, and Avalonian: a fourth, the proto–North American terrane exists in the northwest corner of the state (Bell 1985) but is currently not available to river herring because of barriers to fish passage in the Housatonic River watershed. Multiple river herring runs occur in all three geologic terranes, with most of the coastal watersheds occurring in either the Iapetos or the Avalonian. The Newark terrane underlies the central valley through which the Connecticut River flows and includes the Wethersfield Cove and lower Farmington River study sites.

Another factor contributing to the low reclassification success of known-origin age-0 individuals to some sites was the apparent mobility of age-0 fish in nursery systems. Previous studies have documented age-1 river herring in estuaries and rivers that appeared to have never emigrated to the open ocean (Marcy 1969; Limburg 1998), but the general expectation of age-0 movement was that individuals remained in discrete nursery areas and then emigrated in large waves (Kissil 1974; Loesch 1987; Yako et al. 2002; Gahagan et al. 2010). The changes in Sr:Ca values along laser-ablated transects across the otoliths of the individual fish examined in this study suggested that age-0 fish did not always conform to the expected period of residence in a freshwater nursery area followed by a direct migration to saltwater. Several distinct patterns of Sr:Ca across the age-0 portion of adult otoliths were observed among individuals, suggesting that a variety of movement patterns were exhibited by individual river herring during their first year of life. Staircase increases in Sr:Ca (Figure 7c, d) suggested gradual downstream movement toward saltwater as fish developed. Some individuals (Figure 7b)

appear to have encountered higher salinity and subsequently moved back to freshwater. Another apparent pattern (Figure 7a) was movement from freshwater to an elevated-salinity environment followed by a gradual reduction of salinity, perhaps by movement to an estuary. The Sr:Ca ratios in otoliths appeared to reflect salinity transitions just as reported by others (Limburg 1995, 1998; Secor et al. 1995; Kennedy et al. 2002), and Ba:Ca ratios were also informative of salinity (Elsdon and Gillanders 2005) but may be complicated by more complex gradients in estuaries (Coffey et al. 1997; Shaw et al. 1998; Stecherlii and Kogut 1999). The otolith Ba:Ca ratios in our adult fish always decreased at the presumed entry into the marine environment but did not always appear to reflect environmental transitions as well as Sr:Ca ratios during the period of freshwater residency (Figure 7a, c).

The rate and variety of movements by age-0 river herring have not been documented previously, and this made the collection and designation of known-location fish and the establishment of otolith chemistry signatures for natal sites more complicated than anticipated (but see Limburg 1998). In some sites no age-0 fish were collected, even though adults were known to have been present and spawning. This was especially true for blueback herring, which were more frequently collected from lotic systems in which a high degree of mobility was possible. High mobility at early life stages can hinder designating natal signatures in the otolith cores of adult fish. In a management context, the possibility that in larger rivers stock mixing begins to occur prior to age-0 migration may preclude plans to manage at the tributary or stream run scale.

Reclassification was most successful for age-0 alewives at sites that were not tributaries of larger systems (i.e., Bride Brook, the Pequonnock River, and the Quinnipiac River). In contrast to blueback herring, alewives typically spawn in lentic environments such as coastal and river headwater ponds and impoundments (Loesch 1987; Collette and Klein-MacPhee 2002). In these circumstances, collecting known-origin age-0 fish was straightforward and the variability in the chemical signatures of these sites likely represented temporal rather than spatial variability, since impoundments blocked saltwater intrusion into the nursery area and prevented the reentry of fish that departed headponds. The defined space of these nursery environments also appeared to produce more cohesive otolith chemistry signatures than larger open systems. This was supported by the low reclassification rate of fish from the Eightmile River, which is connected to the tidal lower Connecticut River. The successful reclassification of some of the age-0 alewives demonstrated that despite issues with the mobility of individuals and relatively homogeneous water chemistry between sites, otolith chemistry could be used to discern the origins of river herring at Bride Brook, the Pequonnock River, and the Quinnipiac River.

For adult alewives, all three sites with poor reclassification success were riverine and the otolith chemical signatures were widely dispersed in multivariate space. A high degree of variability in the otolith signatures from a site could be caused by a

high rate of straying to that site, but it could also be caused by misclassification to a natal site as a result of homogeneous water chemistry among sites. Further, the mobility of fish in the earliest life stages can make retrieval of a distinct natal chemical signature at the otolith core difficult. Adult alewife otolith chemistry signatures from three collection locations (Poquetanuck Brook, Bride Brook, and the Housatonic River) were distinct and exhibited minimal dispersion among individuals within sites in multivariate space, which resulted in higher jackknife reclassification rates. Our results, however, do not allow us to evaluate the generality of homing and straying estimates among sites or to establish estimates of population connectivity.

The reclassification rates at three of the four blueback herring sites were not higher than random. The Farmington River, Wethersfield Cove, and the Eightmile River are all tributaries of the Connecticut River, and it is possible that in conjunction with similar water chemistry there may be higher straying among these three sites. Blueback herring are batch spawners (Richkus and Dinardo 1984), and the incidence of repeated spawning events appears to increase moving northwards in the species' range (Greene et al. 2009). It is possible that fish collected at locations upriver in the Connecticut River watershed had already spawned once, perhaps at their natal site downstream, and had moved elsewhere for a second spawning event. It appears that otolith microchemistry will not provide the resolution needed to address questions such as the possibility of within-watershed movements between batch spawning events at these locations.

There were two sites with distinct water chemistry that may have produced distinct signatures in the otolith cores of the adult fish captured there. Poquetanuck Brook was likely chemically distinct from the other sites due to high salinity, which created higher water Sr:Ca and lower Ba:Ca values that appeared to be corroborated in the juvenile-period otolith chemistry of most of the alewives captured there. Poquetanuck Brook grades into Poquetanuck Cove, which is a shallow (depth < 2 m in most areas) tidal cove connected to the lower Thames River. The cove is very well mixed by tidal actions and has elevated levels of salinity regardless of tide level (Loesch and Lund 1977). Returning adults collected from Poquetanuck Brook often had core Sr:Ca values higher (>3,000 μmol/mol) than those portions of the otolith corresponding to marine residency. These Sr:Ca values exceeded the mean levels in fish collected from other sites (Gahagan 2010). The distinct chemical signal provided a reclassification rate (85%) that may accurately reflect alewife natal fidelity, although it is possible that fish originated from unsampled sites with similar chemical signals. The Quinnipiac River signature was not as unique as that of Poquetanuck Brook but stood out from those of other sites where spawning blueback herring were collected and produced an estimated 81% reclassification rate. The reduced number of sites (and runs) of blueback herring increased the ability to separate the Quinnipiac River in multivariate space, as sites with chemical signatures inseparable from that of the Quinnipiac River only supported

alewife runs during the 2 years of sampling. Fewer numbers of blueback herring runs also likely lowers the chance of fish being misclassified to the Quinnipiac River, as there is less chance of fish originating from a chemically similar site. This study examined a number of sites, but the presence of many spawning sites, particularly for alewives, makes complete coverage a near impossibility. The prospect of future recoveries of previously extirpated runs will decrease the possibility of sampling all the existent runs even further.

The two reclassification rates produced from chemically distinct sites (Poquetanuck Brook and the Quinnipiac River) were similar (85% for adult alewives and 81% for adult blueback herring) but only represent two sites and a single datum for each species. The high reclassification rates of adult fish to these two sites suggest that individuals that were correctly classified as having been captured from these sites had a common environmental history over their lifetimes. The distinct water Sr:Ca and Ba:Ca signatures observed at Poquetanuck Brook and the Quinnipiac River, respectively, are also consistent with the high otolith core Sr:Ca and Ba:Ca values in adult fish returning to these sites, suggesting that some degree of natal homing to these sites is occurring. It is possible that interannual variability in water chemistry prior to 2008 and 2009 at our collection sites or fish from unsampled sites with equivalent signatures led to straying individuals (fish that are not returning to their natal environment) contributing to the sets of fish that were correctly classified as having been captured at these particular sites. However, we observed stability in water chemistry within and among collection sites during our study and sampled most of the major river herring runs in Connecticut, which leads us to believe that the most plausible explanation for the high reclassification rates at these two sites is natal site fidelity. If this supposition is correct, it is interesting to note that these rates are within the range of natal and spawning site fidelity estimates derived from other methods. Jessop (1994) measured spawning site fidelity by tagging spawning adults in the St. John River, New Brunswick, and recapturing individuals that survived to spawn in subsequent years. He observed that adult river herring returned not only to the rivers in which they were previously captured but to specific areas within those systems at rates of 63–97%. Messieh (1977) employed a discriminant function analysis of the meristic characters of alewives in the St. John River and produced a wide range of homing estimates, 20.8–82.6%, across sites (mean = 45.82; SE = 8.55). Preliminary genetic analysis of tributary runs in the St. Croix River, Maine and New Brunswick, indicated that alewives are capable of fine-scale philopatry (Bentzen and Paterson 2005), but a genetic study in eastern Connecticut concluded that there was homogenizing gene flow among anadromous runs and that it did not diminish across the spatial scale (80 km) of the study (Palkovacs et al. 2008). Philopatry estimates in the low 80% range indicate an inherent proclivity to spawn at natal locations, but the rates are less than estimates for many salmon species (Quinn 1993; Quinn et al. 1999) and

another alosine, the American shad (Hendricks et al. 2002; Walther et al. 2008).

It is important to note that the reclassification rates presented here should not be interpreted as true estimates of natal homing. In this study, no age-0 fish were available or collected prior to our sampling to generate an "atlas" of known chemical signatures from these sites with which to create statistical training sets to discriminate the origins of adult year-classes returning to spawn in 2008 and 2009. Without an atlas of chemical signatures, it was impossible to know whether fish classified as strays were indeed spawned at another location or represented variability in site chemistry across years (although chemistry was stable during the 2 years of this study). This research demonstrated that age-0 and adult river herring can be reclassified to the site of capture at sites that are geochemically distinct. However, the observed homogeneity of water chemistry in closely spaced watersheds and possible difficulties in sampling "known" age-0 fish due to early-life-history movements from nursery areas suggest that it may be difficult to use geochemical methods to resolve natal origins among closely spaced river herring runs. Despite these issues the technique may be useful for estimating homing to particular sites with unique chemical signatures, such as Poquetanuck Brook, provided that this unique chemical signature persists across years. Further discrimination may be possible in future studies if isotopic markers (e.g., δ^{18}O, δ^{13}C, and ^{87}Sr/^{86}Sr) are also employed. Future research into anadromous river herring philopatry may be more effectively accomplished through alternative techniques, including otolith microstructure research. Advancements in genetic analysis, new physical tagging methods that may work on fragile river herring juveniles, chemical batch marking, or a combination of several techniques might be better suited to explore homing in alewives and blueback herring.

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