Resolving natal tags using otolith geochemistry in an estuarine fish, rainbow smelt *Osmerus mordax*

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ABSTRACT: Dispersal and connectivity are central to stability and persistence in natural populations. The use of otolith composition as geo-referenced tags may provide unparalleled resolution of spatial movements in marine and anadromous fish, although these geochemical signatures remain largely undescribed and the factors influencing otolith composition poorly understood. We examined spatial variation in the otolith geochemistry of juvenile rainbow smelt *Osmerus mordax* using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and isotope ratio monitoring mass spectrometry to evaluate its potential as a natural tag to resolve fine-scale geographic patterns and dispersal of estuarine early life history stages. Otolith element ratios (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) and isotope ratios (δ^{13}C, δ^{18}O, $^{87:86}$Sr) varied significantly among locations (n = 9) and provided 83% cross-validated accuracy using a quadratic discriminant function analysis. Assignments based only on the 3 isotope variables resulted in the highest rates of correct assignment (87%), largely driven by a significant increase (~11%) in correct assignments at nearby locations (<20 km distant). Five of the 7 elements examined were significantly correlated with each other, consistent with a common response to estuarine differences. We also observed a significant effect of habitat on assignment success, in that sites with partially restricted marine access (i.e. sand bars) assigned correctly at significantly higher rates. This study demonstrates that geochemical otolith signatures are intimately linked to estuarine structure, which in turn directly influences assignment power. It also demonstrates that assignment over fine spatial scales (<20 km) can be maximized by the examination of isotope signatures and sampling of specific habitat types.

KEY WORDS: Otolith chemistry · Rainbow smelt · Connectivity · Population structure · Elemental fingerprint

INTRODUCTION

Geographic translocation (i.e. dispersal) from birth to reproduction is a principal determinant of population structure, stability, and persistence (Hastings & Botsford 2006, Campbell Grant et al. 2010). Connectivity in marine species is often the culmination of passive and active processes during both the larval and adult stages (Bradbury & Snelgrove 2001, Pineda et al. 2007,

Botsford et al. 2009). The measurement of connectivity in species where lifetime dispersal may span large geographic distances and multiple life history stages has therefore proven challenging. Despite recent progress in measuring dispersal (e.g. Thorrold et al. 2001, Jones et al. 2005), the few marine and estuarine species for which estimates of dispersal exist are insufficient to infer general trends (Bradbury et al. 2008a). Nonetheless, studies increasingly suggest significant

interspecific and intraspecific variability in dispersal, and contradict previous assumptions of widespread, long-distance larval transport among marine taxa (Levin 2006). Clearly, a better understanding of connectivity and dispersal pathways in marine and estuarine organisms is necessary for improved sustainability and conservation of marine species and communities (Sale et al. 2005, Almany et al. 2009).

Several approaches to measuring movement patterns in marine and estuarine species exist (see reviews in Cadrin et al. 2005, DiBacco et al. 2006, Elsdon et al. 2008). Indirect molecular genetic approaches rely on population-scale differences in allele frequency and on models of population structure to estimate rates of average exchange among populations (e.g. Bradbury & Bentzen 2007). However, the application of population allele frequency estimates of connectivity to contemporary management remains tenuous, given the possibility of alternate demographic influences (e.g. bottlenecks and non-equilibrium conditions) and the large temporal scales over which they integrate. Direct measures of dispersal, by contrast, often rely on genetic (e.g. Jones et al. 2005, Saenz-Agudelo et al. 2009) or geochemical (e.g. Campana et al. 1999, Thorrold et al. 2001, Elsdon et al. 2008) tags. Over fine spatial scales of km to hundreds of km, movement is likely most frequent, and non-genetic tags such as otolith composition are often most informative (e.g. Thorrold et al. 2001, Fodrie & Herzka 2008). The utility of otolith composition as a geographic tag stems from the fact that otoliths are largely metabolically inert, are accreted continuously with age, and can reflect ambient hydrography (Campana & Thorrold 2001, Elsdon et al. 2008). Where differences in local hydrography (viz. temperature, salinity) exist, multi-chemical signatures of otolith composition may act as a natal tag, allowing assignment of individuals to discrete natal habitats (Elsdon et al. 2008, Fodrie & Levin 2008).

Rainbow smelt *Osmerus mordax* (Mitchill, 1814) is a small pelagic fish found in coastal and freshwater systems throughout northeastern North America (Nellbring 1989). Anadromous smelt spawn near the head of the tide in coastal rivers and streams during the spring, with peak spawning timing varying among sites from late April to June. Eggs hatch over a 1 mo period and larvae develop in downstream estuaries during the summer and early autumn (e.g. Ouellet & Dodson 1985, Bradbury et al. 2004). Smelt typically mature at 2 to 3 yr of age and may live from 4 to 5 yr (McKenzie 1964). Prior work suggests significant behavioural contributions to early life-history patterns in the form of synchronized hatch (Bradbury et al. 2004) and diel vertical migration (Laprise & Dodson 1989, Bradbury et al. 2006), which may result in larval

retention within single estuaries (Bradbury et al. 2006). Straying among estuaries during both early life and adult stages has nonetheless been reported even among locations displaying clear evidence of genetic isolation (Bradbury et al. 2008b,c). The persistence of isolation in the face of dispersal may reflect local adaptation and strong selection against straying. Given that smelt populations inhabit a diverse array of habitats ranging from open estuaries, to estuaries with obstructions (such as sand bars) that partially restrict sea access, to coastal ponds where sea access is virtually absent, there is considerable potential for distinct natal or nursery area signatures that may permit further insight into the contribution of various ecological and evolutionary forces influencing connectivity.

The overall goal of this work, carried out at several sites in southeastern Newfoundland, was to evaluate the use of otolith geochemistry as a natural tag for natal habitat in rainbow smelt and to investigate the factors influencing assignment success. Our objectives were: (1) to examine elemental and isotopic signatures (as well as associations among elements and isotopes) at the core of juvenile (~3 mo old) otoliths; (2) to evaluate the potential for assignment of juvenile individuals to habitats of origin based on these geochemical signatures, and to identify the most informative suite of geochemical variables for accurate, fine-scale spatial assignment; and (3) to evaluate the influence that distinct habitat features may have upon otolith composition and assignment success, specifically addressing the question of whether estuaries with limited exchange with the ocean provide greater classification accuracy. The aim was both to describe the geochemical signatures for natal assignment in rainbow smelt and to discover which tools and habitats are most promising for fine-scale assignment of individuals in estuarine and marine species.

MATERIALS AND METHODS

Sample collection. Sampling locations were spread over ~500 km of coastline in southeastern Newfoundland, Canada (Fig. 1). We sampled 9 known smelt spawning locations identified from previous coastal surveys (e.g. Methven et al. 1998) by deploying a 25 m seine from a small boat in shallow water (usually <10 m deep) which 2 people then hauled ashore. Each haul swept the bottom 2 m of the water column over an area of ~880 m^2 (16 m along the shore by 55 m from offshore to the beach). The seine retained fishes of >20 mm total length (TL) within the codend. Multiple tows were completed at each site in adjacent but non-overlapping locations in rapid succession until adequate numbers of fish (n = 50 juvenile smelt per site) were
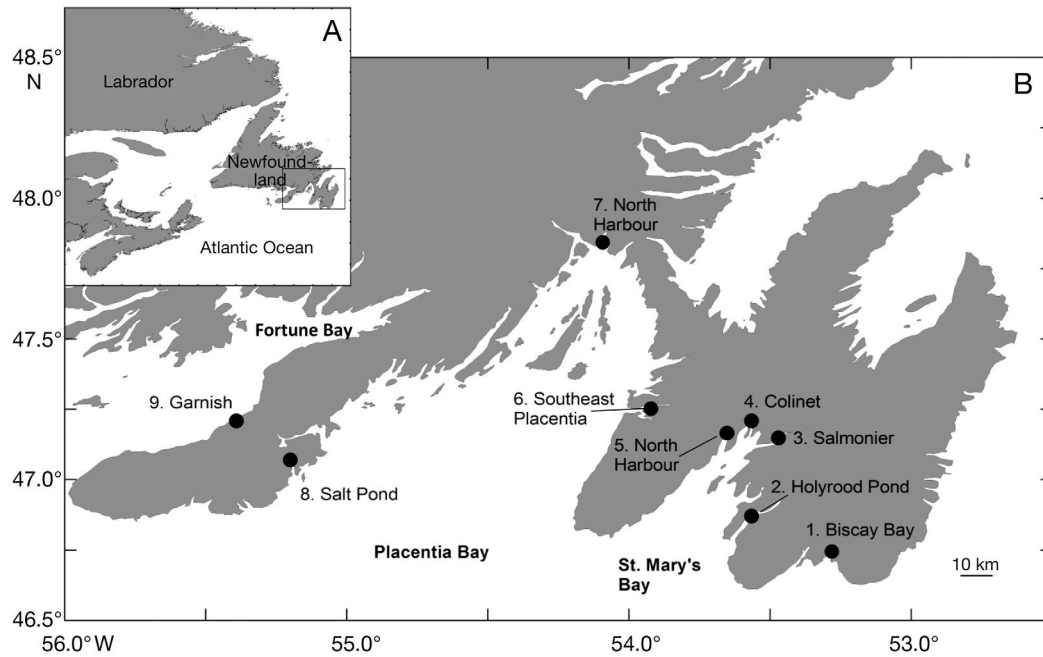
Fig. 1. *Osmerus mordax*. (a) Location of Newfoundland with respect to eastern Canada. Study area outlined at lower right. (b) Study area showing the 9 sampling locations

collected, measured for TL, and preserved in 95% ethanol (Hedges et al. 2004). All sites were sampled between October and November 2004.

Otolith preparation and geochemical analysis. We selected individuals measuring ≤80 mm TL for otolith extraction, assuming that those individuals had been recruited that year (McKenzie 1964; Table 1). The number of individuals for the elemental and Sr isotope ($^{87:86}$Sr) analysis ranged from 41 at Garnish to 20 at North Harbour (Placentia Bay); for all $\delta^{13}C$ and $\delta^{18}O$ analysis, sample sizes were 20 ind. per location (Table 1). Sampled individuals varied in average fork length from 46.1 mm at Biscay Bay to 61.3 mm at Holyrood Pond. Both sagittal otoliths were removed, cleaned with ultrapure water, air-dried, and stored in acid-washed glass vials until they were mounted on glass microscope slides and polished to the core using fine lapping film. Mounted otoliths were then cleaned again with a nylon brush, triple-rinsed in ultrapure water, and sonified for 2 min before being air-dried in a laminar-flow hood and transferred to clean Petri dishes. We measured elemental composition of the core of 1 of the 2 otoliths using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), which coupled an ArF excimer 193 nm laser to a Thermo Finnigan Element 2

ICP-MS for detection and measurement at the Woods Hole Oceanographic Institution Plasma Mass Spectrometry facility. We chose to quantify isotopes of 5 elements (^{25}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba), because they are commonly used in discrimination and assignment studies (Elsdon et al. 2008), preliminary analysis had indicated that these elements were consistently higher than background environmental levels. Assays consisted of a single 80 μm spot with a repetition rate of 5 Hz and dwell time of 60 s. A certified reference material (CRM) consisting of powdered otoliths (Yoshinaga et al. 2000), dissolved in 2% HNO_3 and diluted to a Ca concentration of 40 μg g^{-1}, was used to correct

Table 1. Sample locations, average total length (TL) and sample sizes (N) for geochemical analysis of rainbow smelt otoliths, coastal Newfoundland. See also Fig. 1. Location codes — SMB: St. Mary's Bay; PB: Placentia Bay; FB: Fortune Bay; B: barred estuary; O: open estuary

ID	Location Name (estuary type)	Code	TL (mm)	N element and $^{87/86}$ Sr	N $\delta^{18}O/$ $\delta^{13}C$
1	Biscay Bay, SMB (B)	BIS	46.1	34	20
2	Holyrood Pond, SMB (B)	HRP	61.3	30	20
3	Salmonier, SMB (O)	SAL	48.6	37	20
4	Colinet, SMB (O)	COL	53.9	34	20
5	North Harbour, SMB (O)	NSM	53.1	31	20
6	Southeast Placentia, PB (B)	SEP	55.7	33	20
7	North Harbour, PB (O)	NPB	47.2	20	20
8	Salt Pond, PB (B)	SLT	53.0	31	20
9	Garnish, FB (B)	GAR	62.8	41	20
	Total			291	180

for instrument bias and drift, following Thorrold & Swearer (2009). We estimated external precision periodically throughout the laser analyses by assaying a second otolith CRM (Sturgeon et al. 2005), also dissolved in HNO_3 and diluted to a Ca concentration of 40 µg g^{-1}. We estimated limits of detection (LOD) as 3× the mean intensities of the blanks (2% HNO_3), run every 10 assays, which produced LOD values of 0.28% for Ca, 1.3% for Mg, 2.16% for Mn, 0.05% for Sr, and 0.97% for Ba. Estimates of precision based on the relative SD values of the second CRM were 4.2% for Mg, 21.4% for Mn, 0.35% for Sr, and 0.87% for Ba.

Sr isotope ratio ($^{87:86}$Sr) was analyzed on the second otolith using a Thermo Finnigan Neptune MC-ICP-MS at the Woods Hole Oceanographic Institution Plasma Mass Spectrometry facility. We sampled an 80 µm spot on the core of the second otolith with a 105 s dwell time, and measured ^{82}Kr, ^{83}Kr, ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr, and ^{88}Sr during each ablation. Although multiple potential sources of interference on Sr isotopes exist in carbonates, previous work identified only Rb and Kr as significant challenges to estimation of $^{87:86}$Sr for this instrument (Barnett-Johnson et al. 2005, Jackson & Hart 2006). We therefore used a mass-bias correction with ^{85}Rb to remove the influence of ^{87}Rb on ^{87}Sr. To correct for Kr interference on δ^{86}Sr we followed Jackson & Hart (2006), as outlined in Walther et al. (2008). Finally, all data were normalized to the standard reference material (SRM) 987. Daily sampling of National Institute of Standards and Technology SRM 987 (N = 35) and the otolith CRM (N = 52) produced means ± SD of 0.71027 ± 0.00003 and 0.70917 ± 0.00003, respectively, which are comparable to the global marine $^{87:86}$Sr value of 0.70917 (Barnett-Johnson et al. 2005) and the certified SRM 987 value of 0.71024.

Otolith δ^{13}C and δ^{18}O signatures in one of the sagittal otoliths of each individual were also examined by milling otoliths with a MicroMill sampler (New Wave Research) or a handheld dental drill under a stereomicroscope until we obtained 50 to 200 µg of the remaining material around the core. Otoliths were decontaminated again prior to milling as described above. Analysis was conducted at the Bloomsbury Environmental Isotope facility at University College London on a ThermoFinnigan DeltaPLUS XP stable isotope mass spectrometer attached to a ThermoScientific Gas Bench II device. Standards and samples (>100 µg) were loaded into glass vials, methanol-rinsed and kept overnight in a 70°C oven. For analysis, each vial was acidified by manually injecting 100% H_3PO_4 (0.1 ml) via the screw-cap septum. Precision of all internal (BDH, IAEA, IFC) and external (NBS19) standards is ±0.03 for δ^{13}C and ±0.08 for δ^{18}O. All values are reported in the Vienna Pee Dee Bee (VPDB) notation, relative to NBS19.

Statistical analysis. Data for each elemental and isotope ratio were visually assessed for normality and log-transformed as necessary. We tested for geochemical differences among locations using both univariate ANOVA for each variable separately, and a multivariate analysis of variance (MANOVA) that simultaneously compared all ratios. For MANOVA we chose Pillai's trace as the test statistic because it is appropriate for small unequal samples. MANOVA was conducted at the scale of individual sample locations (Fig. 1), with sample locations 2, 3, 5, and 7 pooled based on the distribution of DFA assignments (see 'Results — Otolith geochemistry' below); and sample locations pooled within disjunct bays (Fig. 1). To identify consistent significant differences along locations and variables, we compared each location and chemical combination against pooled remaining samples or chemicals using ANOVA. Each population was compared with the total dataset, excluding itself. We evaluated correlations among chemical signatures using Pearson's correlation coefficient, and graphical analysis using hierarchical clustering of the correlation coefficient.

We examined the utility of geochemical signatures for spatial assignment at multiple geographic scales using a quadratic discriminant function analysis (QDFA) with cross-validation using the elemental and isotope data separately and for all variables combined. We chose QDFA over linear discriminant function analysis because it is free of assumptions regarding homogeneity of covariance matrices. Levels of correct assignment were compared among open and barred estuaries using ANOVA. Open and barred estuaries refer to estuaries with open access to the Atlantic Ocean or with a partial (usually >80%) obstruction.

RESULTS

Otolith geochemistry

Otolith geochemistry varied significantly among sample locations. Univariate analysis (ANOVA, Table 2, Figs. 2 & 3) showed significant differences among sample locations for all measured element and isotope ratios (Table 2). The r^2 values from the ANOVA exceeded 50% for 5 variables (see Table 2), of which δ^{13}C was the highest at 94.5%. Of the sampled locations, the Garnish sample deviated most from the pooled remaining samples with significant differences in all 7 variables examined (Table S1 in the supplement at www-int-res.com/articles/suppl/m433p195_supp.pdf), displaying the lowest values for Sr:Ca and all isotopes and the highest Ba:Ca values. Of the elements or isotopes examined here, δ^{13}C, δ^{18}O, and Mg:Ca differed significantly at the largest number (≥6)

Table 2. Univariate ANOVA examining element and isotopic ratios in juvenile rainbow smelt otolith cores at sample locations throughout southeastern Newfoundland (see Fig. 1). Combined data set had 180 individuals

	df	MS	F	p	r^2
Mg:Ca	8	0.145	7.63	<0.0001	23.3
Mn:Ca	8	0.196	4.21	<0.0001	12.7
Sr:Ca	8	0.000	60.4	<0.0001	60.4
Ba:Ca	8	0.000	55.1	<0.0001	70.8
$^{87:86}$Sr	8	0.000	78.2	<0.0001	77.6
δ^{13}C	8	338.5	381.5	<0.0001	94.5
δ^{18}O	8	115.9	28.4	<0.0001	55.2

of locations in comparison to pooled remaining samples (Table S1 in the supplement). Similarities were most apparent in Sr:Ca, δ^{18}O, and δ^{13}C for samples from the head of St. Mary's Bay. Several of the elements (e.g. Ba:Ca and Sr:Ca) were highly correlated (Pearson's correlation), and these elements were significantly associated with all isotope ratios (Table 3, Fig. S1 in the supplement). The nature of the correlations varied depending on the element or isotope considered (Table 3). Generally Mg:Ca, Sr:Ca, and the iso-

topes examined were all positively associated with each other, and negatively correlated with Ba:Ca and Mn:Ca. Multivariate analysis (MANOVA) showed significant variation at multiple spatial scales, where sample location (Pillai's Trace = 2.87, F = 14.3, p < 0.001), pooled (2, 3, 5, 7) sample locations (Pillai's Trace = 2.54, F = 24.3, p < 0.001), and bay (Pillai's Trace = 1.14, F = 31.8, p < 0.001) all explained significant components of the variance.

Classification success

The QDFA of the elemental data produced correct assignments ranging from 26 to 85% per location, averaging 63%. Overall Garnish, Holyrood Pond, and Salt Pond were most distinct using the QDFA (Fig. S2 in the supplement). Correct assignment was highest for Garnish River (85%, Table 4) and lowest for North Harbour (Placentia Bay) and the samples from the head of St. Mary's Bay (Table 4). The QDFA of the isotopic data correctly assigned individuals from 60 to 100% per location, with an average of 87%. Again, correct assignment was highest for Garnish River
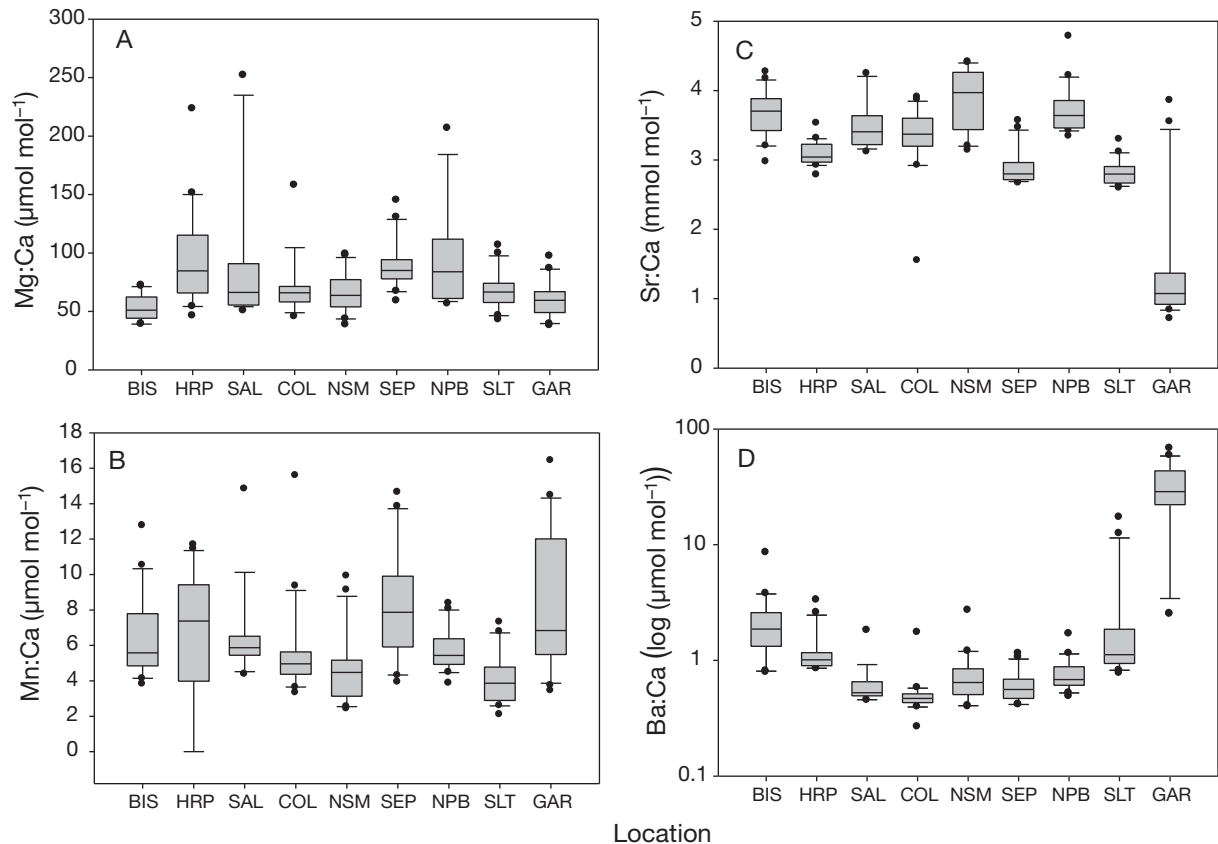

Fig. 2. Four otolith element ratios in rainbow smelt otoliths by sampling location. (A) Mg:Ca, (B) Mn:Ca, (C) Sr:Ca, (D) Ba:Ca. Horizontal line within box: median value; top and bottom edges of box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; filled circles: outliers beyond 95th percentile. Location codes in Table 1

(100%, Table 4). With all elemental and isotope ratios included, average correct assignment was 83%, ranging from 63 to 100%, with Garnish assigning 100% correctly (Table 4, Fig. 4, Fig. S3 in the supplement). The decline in assignment success with both types of data in comparison to the isotopes alone is largely associated with an average 10% drop in successful assignment at fine (<20 km) spatial scales, primarily among Colinet,

Table 3. Pearson's correlation coefficient among elements and isotopes examined (above diagonal) and p-values (below diagonal). Bonferonni adjusted $\alpha = 0.002$

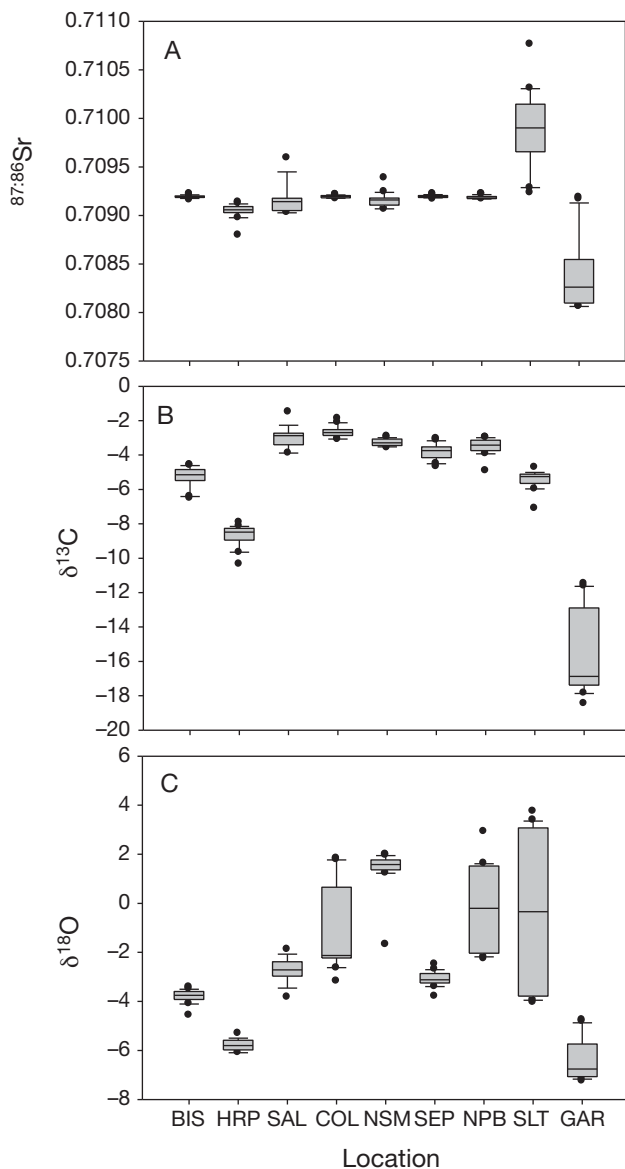|	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca	[87:86]Sr	δ^{13}C	δ^{18}O
Mg:Ca		0.077	0.106	−0.155	0.002	0.107	0.014
Mn:Ca	0.316		−0.327	0.181	−0.272	−0.255	−0.287
Sr:Ca	0.162	0.000		−0.762	0.375	0.710	0.448
Ba:Ca	0.040	0.016	0.000		−0.541	−0.741	−0.408
[87:86]Sr	0.983	0.000	0.000	0.000		0.625	0.448
δ^{13}C	0.159	0.001	0.000	0.000	0.000		0.578
δ^{18}O	0.852	0.000	0.000	0.000	0.000	0.000	

Fig. 3. Three otolith isotope ratios by sampling location. (A) [87:86]Sr, (B) δ^{18}O, (C) δ^{13}C. Horizontal line within box: median value; top and bottom edges of box: 25th and 75th percentiles; whiskers: 5th and 95th percentiles; filled circles: outliers beyond 95th percentile. Location codes see Table 1

Table 4. Percentage of rainbow smelt juveniles correctly assigned to sampling location using otolith composition, both elemental and stable isotope data, and a quadratic discriminant function analysis with cross-validation. For each location, highest self-assignment estimate is indicated in **bold**

ID	Location name	Elemental data only	Isotope only	All data
1	Biscay Bay, SMB	80	**95**	90
2	Holyrood Pond, SMB	65	**95**	**95**
3	Salmonier, SMB	78	**84**	78
4	Colinet, SMB	26	**80**	69
5	North Harbour, SMB	50	**85**	70
6	Southeast Placentia, PB	80	**90**	85
7	North Harbour, PB	37	60	**63**
8	Salt Pond, PB	65	**95**	**95**
9	Garnish, FB	85	**100**	**100**
	Average	**63**	87	83

Salmonier, and North Harbour (St. Mary's Bay; Table 4, Fig. 4). Locations characterized by limited exchange (e.g. partial sand bars versus open estuaries) differed significantly from those open to the coast for Mn:Ca, Sr:Ca, Ba:Ca, δ^{13}C, and δ^{18}O (Fig. 5). Habitat type also impacted assignment success, in that sites with partially restricted marine access (e.g. blocked by sand bars) assigned correctly at significantly higher rates than open locations (ANOVA, $F = 33.72$; $p < 0.001$; $r^2 = 80.36$). Pooling all open estuaries and all closed estuaries, a QDFA correctly assigned samples to habitat type with a total 84% accuracy, corresponding to 77% of closed estuary individuals and 91% of open estuary individuals, supporting significant differences among habitat type and greater homogeneity among individuals from open estuaries.

DISCUSSION

The persistence and stability of populations depends largely on patterns of connectivity and dispersal (Hastings & Botsford 2006, Botsford et al. 2009, Campbell Grant et al. 2010), yet for most marine and estuarine or-
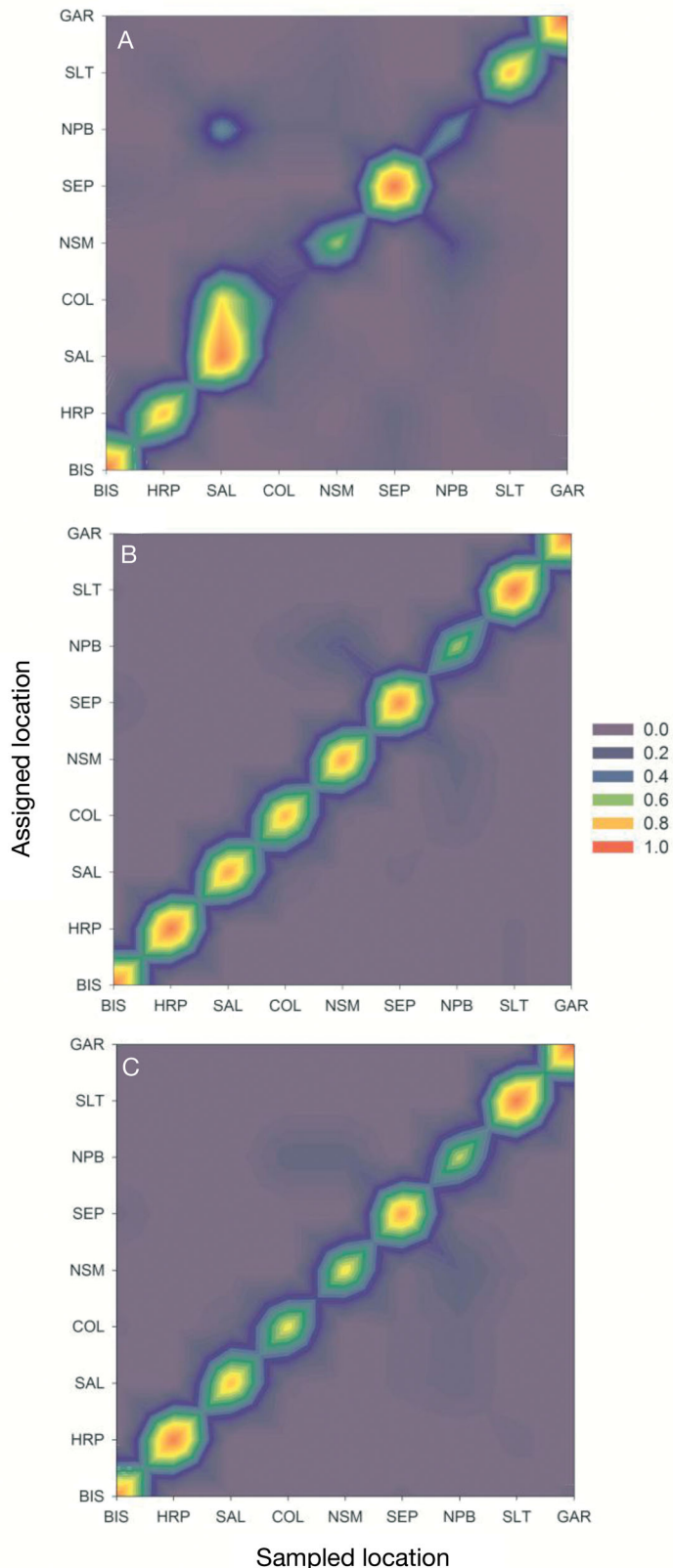
Fig. 4. Degree (proportion) of assignment success for juvenile smelt based on (A) elemental composition, (B) isotopic composition, and (C) combined data

ganisms the scales of dispersal remain unknown (Bradbury et al. 2008a). We evaluated the use of otolith geochemistry in coastal Newfoundland as a natural tag in juvenile rainbow smelt using a suite of elements and isotope ratios. We observed consistent spatial variation among individuals from estuaries and significant associations among several of the elements and isotopes examined, consistent with a common response to estuarine differences. Spatial variation in otolith composition permitted successful classification of individuals to estuary of capture with high (~87%) accuracy. Assignment accuracy was highest using isotope ratios and for sites with obstructions preventing open access to the sea, both of which relate to consistent stable differences in salinity among regions. These observations support a growing literature documenting significant spatial variation in otolith geochemistry of estuarine fishes (e.g. Thorrold et al. 2001, Fodrie & Levin 2008) and the potential for further insight into marine connectivity based on otolith composition. Geochemical signatures identified here, indicative of natal or nursery location, allow currently unparalleled resolution of dispersal patterns later in life and support further use in other marine species, particularly at fine spatial scales where movement among locations is common (e.g. Campana et al. 1999, Thorrold et al. 2001, Elsdon et al. 2008).

Numerous studies report spatial variation in otolith geochemistry in marine, estuarine, and anadromous species (Campana & Thorrold 2001, Gillanders et al. 2001, Elsdon et al. 2008). We observed significant spatial variation in otolith composition at large (100 km) and fine (5 to 10 km) spatial scales in rainbow smelt. Similar observations of fine-scale geochemical variation have been reported for other species and locations (e.g. Thorrold et al. 1998, Gillanders & Kingsford 2000, Clarke et al. 2009, Tanner et al. 2011) and, as frequently noted, much of the small-scale discrimination among the samples here is driven by variability in isotope ratios. Isotope ratios in particular accurately assigned the 3 locations at the head of St. Mary's Bay over fine (<20 km) spatial scales, where the 51% accurate assignment with elemental data increased to 83% for the isotope data. Of the geochemical variables among sites, we found that $\delta^{13}C$ values differed most. The proportion of metabolic carbon incorporated into otoliths has been shown to vary with diet, metabolic rate, and ontogeny (Solomon et al. 2006, Elsdon et al. 2010). Smelt across the study area varied widely in growth rate, age at maturity, and trophic strategy (e.g. planktivorous or piscivorous; Bradbury et al. 2008b, 2009b). A large variation in $\delta^{13}C$ is therefore perhaps not surprising.

In addition to dietary sources, spatial variation in salinity may explain a significant component of variation in the chemicals examined. Salinity differences may contribute to geochemical composition, and $\delta^{13}C$
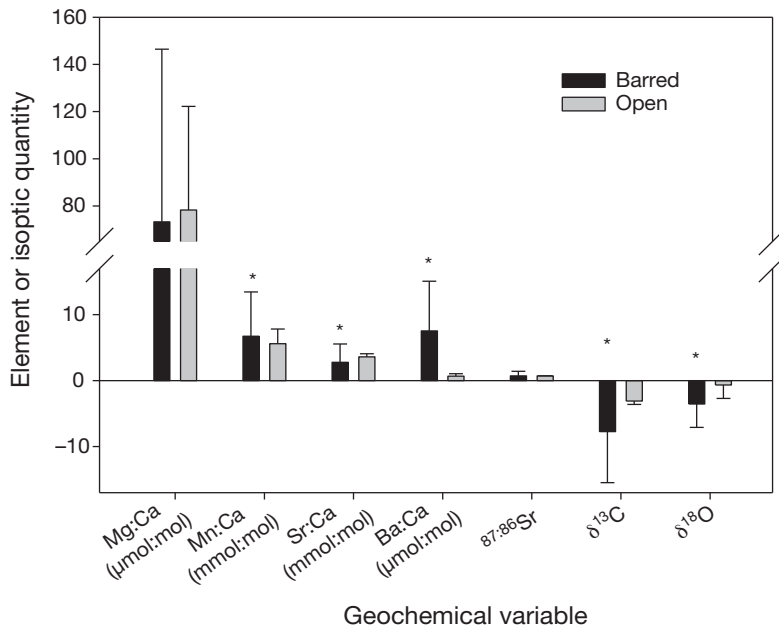
Fig. 5. Elements and isotopes (average ± SD) measured in rainbow smelt otoliths from both barred and open estuaries. *significant differences, p < 0.001

values in otoliths have also been found to correlate with salinity (Kerr et al. 2007), as have the remaining 4 geochemical signatures (Sr:Ca, Ba:Ca, $^{87:86}$Sr, and δ^{18}O), which also vary spatially (Thorrold et al. 1997, Elsdon et al. 2008). The significant correlations among these elements and isotopes support the hypothesis of a common environmental influence such as ambient salinity. The nature of the correlations observed likely reflects differences or similarities in the relative availability of elements in marine and freshwater environments (e.g. Elsdon et al. 2008). The link with estuarine structure and salinity is further supported by the clear distinction in otolith composition between barred and open estuaries observed in multiple elemental signatures. Unfortunately, we lack an estimate of long-term trends in salinity at each of the sites sampled. It also seems reasonable to hypothesize that estuarine structure (i.e. whether open or closed) could influence early-life dispersal (e.g. Bradbury et al. 2006) or adult movements, linking the presence of discrete otolith tags with limited connectivity. Although it remains untested, this hypothesis may be explored using tags presented here as well as molecular genetic approaches (e.g. Bradbury et al. 2008c).

The geochemical signatures in individuals from Garnish River were most distinct, deviating significantly from the other samples for 5 of the 7 chemicals examined. This divergent geochemical signature may be associated with an unusual migratory behavior in this population. Contrary to the spring spawning migrations to the head of the tide characteristic of smelt, Gar-

nish River smelt migrate upstream in the fall and overwinter in a headwater lake, after which they spawn in the lake inflows before migrating back to the estuary in the spring (I. R. Bradbury et al. unpubl. data). Larvae are present in the estuary by early fall, indicating downstream movement sometime in spring and summer. This freshwater residency is likely responsible for the large divergence we observed in otolith composition. Transects of Sr:Ca and $^{87:86}$Sr across the first year of life reveal individual variation in the time of appearance in the estuary. Most individuals examined displayed consistent regions of freshwater otolith signatures (i.e. non-marine $^{87:86}$Sr values) at the core, indicative of lake residency for several weeks to months (I. R. Bradbury unpubl. data). In addition to the Garnish River population, longer freshwater exposure and greater dependence on zooplankton for food likely contributed to the unique chemical composition of otoliths in smelt from Holyrood Pond, on St. Mary's Bay (Bradbury et al. 2009a,b). This population is morphologically discrete and matures at an early age and size (Bradbury et al. 2009b).

Geochemical differences allowed correct assignment of individuals to location of capture. Assignment accuracy varied with the geochemical parameter, and increased significantly (~20%) with the use of the isotope data, as noted elsewhere (e.g. Clarke et al. 2009). We observed the highest rates of accurate assignment for isotope values alone, which increased assignment success by ~10% for locations near the head of St. Mary's Bay. Overall, accurate assignment averaged 87% for the isotope data alone, and all estimates of accurate assignment exceeded 80%. Similar estimates of accurate assignment in estuarine and anadromous fish have been reported in weakfish (90%; Thorrold et al. 1998) and in American shad (91%; Walther et al. 2008). Exceedingly high rates of accurate assignment were primarily associated with some of the barred estuaries such as Garnish River (100% accuracy), Salt Pond (95% accuracy), and Holyrood Pond (95% accuracy). North Harbour (Placentia Bay) displayed the lowest rates of correct assignments despite its clear geographic isolation. The reason for this remains unclear and will require further examination.

Our observations of distinct tags may be used to provide estimates of movement across the life history of rainbow smelt in subsequent studies. Previous work indicates that dispersal potential in smelt may vary dramatically with ontogeny. Upon hatching, the trans-

port of larvae to the estuary depends heavily on local hydrography, although the role of vertical migration behavior may increase with development (Bradbury et al. 2006). Larval and juvenile stages largely associate with low-salinity water in the upper reaches of estuaries, although some reports offer evidence of dispersal between neighboring estuaries (Bradbury et al. 2008b,c). For adult stages, McKenzie (1964) and Murawski et al. (1980) both observed straying between spawning locations in a single estuary. Similarly, Rupp (1968) reported mixing between spawning locations within a freshwater system over 6 to 7 km. In contrast, Magnin & Beaulieu (1965) reported average displacement rates for the St. Lawrence estuary of 150 km, with some individuals moving as far as 300 km, and a daily displacement of 42 km d^{-1} in the St. Lawrence estuary. Interestingly, evidence of straying over fine spatial scales (larvae and adults) does not seem to influence population divergence, at least in one instance. Bradbury et al. (2008b,c, 2009a,b) report evidence of larvae and adult straying among populations that display evidence of genetic isolation using microsatellite loci. The persistence of isolation in the face of dispersal may reflect local adaptation and strong selection against straying (Bradbury et al. 2008c). The ability to identify dispersed individuals will allow the examination of the strength of selection against migrating individuals and quantification of the contribution of various structuring forces.

SUMMARY

We observed significant spatial variation in otolith elemental and isotopic composition, which permitted the accurate assignment of individuals to location of origin. Such natural tags will permit the characterization of dispersal kernels in smelt across the entire life history, and help disentangle the roles of dispersal, survival, and adaptation on population structure. At present, the lack of understanding of the scale of dispersal and connectivity in most marine and estuarine species presents a real obstacle for conservation and management efforts. The use of otolith geochemistry as a geo-referenced tag represents a significant advance in tracking the movements of fish in coastal and oceanic habitats, and in defining spatial habitat usage across the entire life history.

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