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Regional variation in otolith geochemistry of juvenile Atlantic cod (*Gadus morhua*) in coastal Newfoundland

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Abstract: We examined spatial variation in otolith geochemistry as a natural tag in juvenile Atlantic cod (*Gadus morhua*) to resolve geographic patterns during early life history. Individuals from 54 inshore sites spanned five embayments in eastern Newfoundland. Otolith composition differed at all spatial scales and related inversely to spatial scale. Classification analysis revealed increasing discrimination at coarser spatial scales: site (26%–58%), bay (49%), and coast (76%). Assignment success declined by $\sim 10\%$ per added site with increasing sampling sites per bay, demonstrating fine-scale (<100 km) variation. When we partitioned environmental variability from observed otolith chemistry using predictive models, assignment success improved by 56%, 14%, and 5% for site, bay, and coast, respectively. Our results demonstrate environmental influence on spatial structure of otolith chemistry and illustrate the importance of resolving baseline variability in otolith chemistry when conducting assignment tests. Collectively, our results describe the potential utility of juvenile otolith composition in evaluating contributions of subpopulations to the Northwest Atlantic cod stock and highlight important limitations imposed by environmental variation at scales less than 100 km.

Résumé : Nous avons examiné la variabilité spatiale de la géochimie des otolites comme marqueur naturel chez les morues (*Gadus morhua*) juvéniles, pour cerner les motifs de répartition géographique au début de leur cycle de vie. Les individus provenaient de 54 sites côtiers dans cinq échancrures de l'est de Terre-Neuve. La composition des otolites variait à toutes les échelles spatiales et était inversement reliée à l'échelle spatiale. L'analyse de classification a révélé une augmentation de la discrimination parallèlement à celle de l'échelle spatiale, comme suit : site (26–58 %), baie (49 %) et côte (76 %). Le succès d'affectation diminuait de ~10 % pour chaque site ajouté à mesure qu'augmentait le nombre de sites d'échantillonnage par baie, ce qui démontre une variation à petite échelle (<100 km). Une fois la variabilité environnementale exclue de la chimie des otolites à l'aide de modèles prédictifs, le succès d'affectation augmentait de 56 %, 14 % et 5 % à l'échelle du site, de la baie et de la côte, respectivement. Nos résultats démontrent l'influence du milieu sur la structure spatiale de la chimie des otolites. Collectivement, nos résultats décrivent l'utilité potentielle de la composition des otolites de juvéniles pour évaluer les contributions de sous-populations au stock de morues du nord-ouest de l'Atlantique et ils font ressortir d'importantes limites imposées par les variations des conditions environnementales à des échelles inférieures à 100 km. [Traduit par la Rédaction]

Introduction

The extent of dispersal and survival to successful reproduction collectively determine the geographic scale of natural populations (Cowen et al. 2006; Hastings and Botsford 2006; Pineda et al. 2010). Together these processes dictate ecological connectivity and the evolutionary persistence of populations and species (Hastings and Botsford 2006; Hilborn et al. 2003). Accordingly, the successful management of aquatic resources requires identification of population links, or paths of recruitment, and their relative contributions to population persistence (Botsford et al. 2009; DiBacco et al. 2006; Grant et al. 2010). Such knowledge facilitates stock identification (e.g., Cadrin et al. 2005), marine reserve design (e.g., Almany et al. 2009; Berglund et al. 2012; Sale et al. 2005), and the protection of marine biodiversity (Ruzzante et al. 2006). However, the multitude of physical and biological processes that potentially influence the distributions of marine populations with dispersive larval and adult stages complicates understanding of connectivity among habitats and geographic regions (Pineda et al. 2010). Future successful management of marine ecosystems requires the development of methodologies that permit estimates of marine dispersal and connectivity at the scale of individual population components.

Multiple empirical approaches have been developed to quantify connectivity and dispersal in marine environments (see reviews in Botsford et al. 2009; DiBacco et al. 2006; Leis et al. 2011; Levin 2006; Thorrold et al. 2002). Molecular genetic approaches effectively test population divergence, individual assignment, or parentage (e.g., Bradbury and Bentzen 2007; Hedgecock 2010; Jones et al. 2007), but their effectiveness requires stable isolation or extremely small

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Fig. 1. Map of surveyed locations for juvenile Atlantic cod in coastal Newfoundland 2007. Inset shows location of eastern Newfoundland with respect to eastern Canada. Black dots represent sites where sufficient numbers (n = 17) and white dots represent sites with insufficient numbers (n = 38) of juvenile Atlantic cod were available for discrimination analysis. See Table 1 for sample size information.



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population sizes (Saenz-Agudelo et al. 2009). Alternatively, markers in calcified structures (e.g., otoliths) may distinguish population structure within large numbers of individuals and may therefore provide effective metrics of dispersal, even in high gene flow scenarios (Campana 1999; Elsdon et al. 2008; Thorrold et al. 2001). Fish otoliths are metabolically inert calcified structures that grow continuously throughout development and integrate at least some elements from the surrounding water into the calcium carbonate matrix in proportion to ambient concentrations. In regions with sufficient spatial variation in water chemistry and environmental conditions (e.g., water temperature and salinity), analysis of otolith geochemical composition can provide a natural marker of both time and space to determine differences in spatial use by marine populations. The identification of species and habitats that provide fine-scale, geochemical-based otolith tags will substantially advance understanding of marine dispersal and connectivity and resolve fish movement in a way not possible with other approaches (e.g., Thorrold et al. 2001).

Atlantic cod (*Gadus morhua*), a common demersal species, occupies coastal and continental shelf environments throughout the North Atlantic. After a 1- to 2-month pelagic egg and larval phase (Pepin and Helbig 1997), larvae metamorphose into benthic juveniles, primarily inhabiting shoal waters near the coast (Bulatova 1963; Laurel et al. 2003). Adults move in variable patterns (Rose et al. 2011), often migrating many hundreds of kilometres (D'Avignon and Rose 2013; Rose 1993), yet an estimated 44% of all known Atlantic cod spawning groups do not migrate extensively and are characterized as small, inshore groups (Robichaud and Rose 2004).

Our study evaluates the potential use of otoliths as a natural tag to discern fine-scale natal patterns of Atlantic cod in coastal Newfoundland. Specifically, we evaluate how environmental variability within an assessment unit can influence the application of assignment tests using otolith chemistry over fine geographic scales (10-100 km). We develop this evaluation using spatial subsampling and a complementary laboratory rearing study (Stanley et al. 2015), asking whether biogenic variation in otolith geochemistry in field populations provides a sufficient signal to differentiate among otolith compositions of fish from different natal sites and which factors might influence this distinction. Previous population studies based on adult Atlantic cod otoliths (Campana et al. 1994; D'Avignon and Rose 2013) and genetics (Bradbury et al. 2011; Hutchinson et al. 2001; Neat et al. 2014) distinguished spawning stocks at large spatial scales (>1000 km). However, neither approach provided a mechanism to link these aggregations with juvenile nursery habitat. Our study builds on this body of work by determining variability in otolith geochemistry of newly settled juvenile fish at fine spatial scales (within and among coastal embayments) and thus evaluates the potential for future assignments of adults to critical coastal nursery habitats. Our objectives are to (i) measure spatial distributions of juvenile cod in coastal Newfoundland and their association with known spawning locations; (ii) examine spatial heterogeneity in geochemical composition of juvenile (age-0) cod otoliths from five embayments in eastern Newfoundland; and (iii) quantify the success with which juveniles may be assigned, based on otolith geochemistry, to known site, bay, or coast of capture; and (iv) evaluate how small-scale geographic sampling and environmental variation influence assignment power. Quantification of discrete nursery signatures represents the first step in developing geochemical signatures to measure connectivity and dispersal in marine systems at finer spatial scales (<100 km) than previous studies.

Materials and methods

Sample collection

To collect juvenile cod, we deployed a 25 m seine from a small boat in shallow water (usually <10 m); two people then hauled the seine onshore. The stretched mesh size of the beach seine was \sim 10 mm, retaining fish as small as 20 mm standard length. Each haul swept an area of approximately 880 m² (16 m alongshore by 55 m offshore), sampling only the bottom 2 m of the water column. We collected juvenile fish using two hauls in adjacent but nonoverlapping locations at each site in rapid succession. In total we sampled 55 sites (Fig. 1) spanning approximately 800 km of coastline in eastern Newfoundland, Canada. Sites were classified based on the embayment (n = 5) and coast of capture (n = 2). Coasts were classified as north and south of the Avalon Peninsula in eastern Newfoundland, where St. Mary's and Placentia Bay constitute "southeast" coast sites (Fig. 1). All sites were sampled between October and November 2007. We collected a maximum of 50 juvenile cod from each site, measured total length of all individuals, and preserved them in 95% ethanol. We included only sites where we captured a minimum of five individuals in further analyses. Salinity was recorded at each site using water collected at the surface and a YSI 55 probe.

Otolith preparation and geochemical analysis

Juveniles 80 mm in length or less were selected for otolith extraction, assuming that size range would represent new (age-0) recruits. Both sagittal and lapillar otoliths were removed, cleaned with ultrapure water, air-dried, and stored in acid-washed glass vials. All otoliths were then mounted on glass microscope slides and polished to the core using 0.3 μ m lapping film. Mounted otoliths were cleaned again with a nylon brush, triple-rinsed in ultrapure water, and sonified for 2 min. Otoliths were then air-dried in a laminar flow hood, transferred to clean Petri dishes, and transported to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility for analysis. We selected one lapillus per fish at random for laser ablation and one sagitta at random for δ^{13} C and δ^{18} O

analysis because they provided sufficient material for analysis. We chose lapilli for laser ablation because their clearly distinguishable annuli helped us standardize sampling of otolith material among individuals. We selected one lapillus and sagitta at random from each fish for analysis.

We measured otolith elemental composition using a Thermo Finnigan Element2 inductively coupled plasma mass spectrometer (ICP-MS) coupled with an ArF excimer 193 nm laser ablation system. We chose to quantify five isotopes (25Mg, 55Mn, 138Ba, 88Sr, ⁴³Ca) that were consistently higher than blank replicates during analyses. Assays, based on a 50 μ m spot size, consisted of a 250 μ m raster along the edge of the otolith. Laser repetition rate was set at 5 Hz for all analyses using a dwell time of 60 s and a scan speed of 5 μ m·s⁻¹ for spot and line analyses, respectively. The ICP-MS method consisted of 38 passes per analysis with a dwell time of 0.1 s per isotope on each pass and a total dwell time of 3.8 s per isotope during each analysis. Otolith sampling targeted the outer edge of the otolith to measure elemental composition laid down in the early larval period. Otoliths were randomized for analysis to eliminate potential bias associated with temporal changes in instrument precision. To correct for instrument bias and drift, we used certified reference material (CRM) consisting of powdered otoliths (Yoshinaga et al. 2000), dissolved in 2% HNO₃ (SeaStar) and diluted to a Ca concentration of 40 µg·g⁻¹, following Thorrold and Swearer (2009). External precision was estimated by analyzing a second otolith CRM (Sturgeon et al. 2005), also dissolved in 2% HNO₃ (SeaStar) and diluted to a Ca concentration of 40 $\mu g \cdot g^{-1}$, periodically throughout the laser analyses. We determined analytical accuracy from concentrations of Japanese otolith and National Research Council standards, averaged across all samples. We found that accuracy exceeded 98% for all otolith constituents measured, including isotopic ratios described below. We calculated limits of detection as five times the mean intensity of blanks (2% HNO₃), run every ten assays for each otolith constituent. Any measurements below the respective limit of detection were excluded from analysis. We conducted all statistical analyses using otolith elemental compositions converted to molar values and standardized to calcium concentrations (Me:Ca). External precision estimates, based on the relative standard deviation values of the second CRM, were 0.40% for Sr:Ca, 1.4% for Ba:Ca, 4.2% for Mg:Ca, and 17.9% for Mn:Ca.

We measured δ^{13} C and δ^{18} O values in sagittal otoliths of each individual 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 near-edge material. Stable isotopes were measured using an isotope ratio mass spectrometer and techniques outlined by Ostermann and Curry (2000). We report values of both isotopes (‰) relative to Vienna Pee Dee Belemnite. Long-term precision estimates based on the use of NBS-19 are ±0.07‰ for δ^{18} O and ±0.03‰ for δ^{13} C (Ostermann and Curry 2000).

Statistical analysis

Fish otoliths likely incorporate trace elements, such as Mg, Mn, Ba, and Sr, as substitutes for Ca (Campana 1999); therefore, we expressed all elemental concentrations as ratios to Ca (Me:Ca). Data for each element and isotope were assessed for normality by visual inspection and log-transformed as necessary. We examined the utility of geochemical signatures for spatial assignment to multiple geographic scales with quadratic discriminant function analyses (QDFA) using the "MASS" package in R (Venables and Ripley 2002). Assignment success of each QDFA was assigned using jack-knifed cross-validation with a set training subset of seven randomly chosen individuals per site. To evaluate the influence of small-scale heterogeneity on classification success, we generated all possible unique combinations of sites for bays with at least four sample sites (Bonavista, Trinity, and Placentia Bay) and ran a QDFA for each subset. Here we consider embayment as the scale of interest because it is generally the scale at which management

delineates coastal stocks and the smallest scale at which we can evaluate the potential influence of sampling spatial coverage on assignment success. We limited our comparison of classification success to four sites per bay or fewer because that was the number available for all sites except Placentia Bay. Based on changes in assignment success, we evaluated a priori hypotheses about the interpretation of assignment at this scale relative to the spatial coverage of sampling within embayments, inferring that (*i*) no significant change signifies that spatial heterogeneity in otolith chemistry contributes little to patterns observed, (*ii*) significant reduction signifies significant, fine-scale variability within embayment, which mediates assignment success, and (*iii*) significant improvement suggests low variability among sites within an embayment and that more samples improve the estimation of the multi-elemental centroid and thus assignment success.

To describe the utility of the assignment tests, we compared the proportion of successfully assigned individuals at each spatial scale and data source with the proportion expected if samples were randomly distributed among sites, using contingency tables and χ^2 proportional comparisons in R.

Complementary laboratory experiments (see Stanley et al. 2015 for details) tested the relationship between environment and juvenile cod otolith chemistry based on a controlled temperature (5-12 °C) by salinity (25-32 psu) experimental design that reflected the range of conditions expected of juvenile habitat sampled in this study (Supplemental Table S11). Experiments revealed significant environmental influence on otolith chemistry for all elements and isotopes tested (Stanley et al. 2015). From these analyses, we developed a predictive mode that employed canonical ordination and a redundancy analysis (RDA) based on Euclidean distance dissimilarity matrices (see Legendre and Anderson 1999). The redundancy model enables prediction of baseline scaling relationships between multivariate dependant and independent environmental variables, in our case, otolith chemistry as a function of temperature and salinity. As with field otolith data, otolith constituent estimates were checked for normality and log-scaled prior to fitting the RDA model. Models were fitted using RDA functions available as part of the Vegan community ecology package available in R (Stephens et al. 2010). Using predicted compositions from this model, we ran QDFAs based on residual differences (composition observed in the field minus predicted otolith chemistry for each constituent) and compared assignment success. Here we assumed the model would capture the baseline influences of variable environmental conditions observed among sample sites and therefore could help to partially elucidate the "residual" variation in otolith chemistry within the system. Based on changes in assignment success, we evaluated a priori hypotheses about the influence of abiotic and biotic variables on otolith chemistry where we infer (i) no significant change signifies that environmental heterogeneity contributes little to patterns in otolith chemistry or the model and (or) inputs were not suitable, (ii) significant reduction signifies that most of the discrimination signal could be attributed to environmental heterogeneity, and (iii) significant improvement signifies a robust residual signal exists, external to environmental heterogeneity, and reflects local abiotic and biotic complexity. We explored changes in assignment at three different spatial scales: site, bay, and coast of origin, basing inputs of salinity for the redundancy model on measurements averaged from multiple locations within the sample sites from ~ 1 m depth at the time of fish sampling. Sampling did not coincide with major rain events, and therefore we assume our data provide a relative index of salinity conditions among sample sites. Temperature inputs were collected from sea surface temperature satellite telemetry measurements (NOAA ETOPO11 min global relief) taken within 25 km

of each sample site and averaged for 2 months prior to collection (see Supplemental Table S1¹), representing the approximate period sampled by the otolith assays and well before fish were likely to disperse from nursery areas in their second year (Shapiera et al. 2014). Surface measurements were assumed to represent reasonable proxies of benthic juvenile habitat, given that we anticipated little vertical temperature–salinity structure over the depths sampled (<10 m).

We examined general associations between otolith chemistry and environment observed in the field for each otolith constituent in relation to water salinity, surface temperature, and location of sample site. Correlative relationships were tested in R using permutational Pearson correlations according to Legendre (2005). Associations between otolith chemistry and standard length were explored using a mixed effects model with the sample site treated as a random factor. To control for family-wise error rates and multiple comparisons, we based the significance of each test on Benjamini–Hochberg adjusted *p* values (Benjamini and Hochberg 1995) using the *p* value adjustment methods available in base R (R Core Team 2015).

All statistical analyses assume deposition of otolith material during the juvenile period in the sampled nursery habitat. Micromilling the edge of the otolith ensured we measured elements and isotopes recently deposited during the juvenile life history. Home ranges for age-0 cod have been estimated at less than 2.5 km² (Clark and Green 1990) and 3.5 km² (Shapiera et al. 2014) in coastal Newfoundland, considerably less than the spatial scales tested, suggesting that between-site movements are highly unlikely.

Results

Spatial distribution of juveniles

Juvenile cod abundance varied significantly across the survey area, and juveniles occurred at only 32% of the 54 sites surveyed (Table 1). Catches averaged 32 juvenile cod per tow; highest catches were observed along the northern side of Trinity Bay and in southern Bonavista Bay (Fig. 2), where we collected as many as 500 juveniles per tow. Fish ranged from 30 to 100 mm in standard length. Placentia Bay and St. Mary's Bay cod were ~10 mm smaller on average than other bays (ANOVA, df_{num} = 1, df_{den} = 325, *F* = 78.25, *p* < 0.001), but were not different from each other (ANOVA, df_{num} = 1, df_{den} = 175, df = 1, *F* = 0.028, *p* = 0.87; Fig. 3). Placentia Bay cod displayed a bimodal size frequency distribution with significantly higher average size for sites at the mouth of the bay near Spanish Room (ANOVA, df_{num} = 1, df_{den} = 126, *F* = 11.95, *p* = 0.001, *n* = 129; Fig. 1).

Otolith geochemistry

Otolith chemistry varied within and among sites (Fig. 4) for all elements. The largest differences among sample sites for otolith constituents were observed for Sr:Ca, Mn:Ca, Ba:Ca, and δ^{13} C, particularly among bays and coasts of capture (Fig. 5).

We tested relationships between otolith geochemical constituents with juvenile cod length and environmental factors and found significant patterns in only seven of 30 possible combinations. Body length was significantly related to $\delta^{13}C$ (r = 0.03, p < 0.0001) and weakly related to Mg:Ca (r = -0.002, p = 0.046). Significant environmental associations were found between salinity and both Ba:Ca (r = 0.2, p = 0.001) and Sr:Ca (r = -0.17, p = 0.002). We also observed significant, albeit weak, correlations with the latitude of each sample site for $\delta^{18}O$ (r = -0.19, p < 0.0001), Sr:Ca (r = 0.21, p < 0.0001), and Ba:Ca (r = -0.16, p = 0.003). No significant correlations were observed between any otolith constituent and temperature or longitude.

ID	Site name	Site code	n (element)	n (isotope)
Placen	tia Bay (PB)			
1	Great Brule	GB	20	19
2	St. Bernard's Cove	BC	14	14
3	Woody Island	WE	17	16
4	Spanish Room	SR	19	12
5	Harbour Buffett	HB	22	13
St. Ma	ry's Bay (SMB)			
6	Colinet Island	CI	33	32
Conce	ption Bay (CB)			
7	Harbour Grace	HG	20	19
8	Holyrood	HR	18	18
9	Bristol's Hope	BH	5	5
Trinity	v Bay (TB)			
10	Little Mosquito Cove	LMC	20	18
11	Smith Sound	SS	19	19
12	Dildo	DL	20	20
13	Tappers Cove	TC	18	17
Bonavi	ista Bay (BB)			
14	Canning's Cove	CC	19	17
15	Bread Cove	BC	20	20
16	Piper's Cove	PC	20	20
17	Indian Bay	IB	20	20
Total			324	200

Note: See Fig. 1 for geographic positions.

Fig. 2. Map of juvenile cod abundance from a beach seine survey of eastern Newfoundland in 2007.



Classification success

We evaluated the impact of low numbers of individuals in several samples on observed patterns. First, we examined the scale of correct assignment using all sites, excluding Bristol's Hope, where we sampled only five individuals. The QDFA of elemental and

Fig. 3. Boxplots of juvenile length from each of the five bays surveyed: PB, Placentia Bay; SMB, St. Mary's Bay; CB, Conception Bay; TB, Trinity Bay; BB, Bonavista Bay. Box shading denote results of ANOVA Tukey's post hoc tests for differences in length among bays at the α = 0.05 level. Raw data are presented as points underlying the boxplots. Sample sizes (*n*) represent the number of fish captured at that location.



isotope data produced correct assignments ranging from 5% to 48% per site, with highest correct assignment for the Colinet Island samples (Table 2). For misassigned samples, over 54% were correctly assigned to bay and 78% to coast of origin. Assignment to an individual site might have been partially limited by the number of available samples. Overall, we had fewer than 20 individuals with complete isotopic and elemental data for the majority of sites (Table 1), which potentially limited the ability of discrimination tests to differentiate among sites.

We also evaluated assignment success for pooled sites both at the natal bay and coast scale. Because St. Mary's Bay contained only one sample (Colinet Island), we pooled it with adjacent Placentia Bay. Correct assignments using elemental data alone varied from 24% to 77% for each of the four bay groupings, with lowest values for Conception Bay and highest values for Placentia Bay (Table 3). Correct assignments to the north and south coasts were 88% and 61%, respectively (Table 3). Assignments based on isotope ratios revealed similar patterns of relative assignment success as the element data, with lowest bay-scale assignments for Conception Bay (27%) and highest for Placentia Bay (77%). Correct assignments to the north and south coasts were 91% and 58%, respectively (Table 3). Combining elemental and isotopic data generally improved assignments on average; however, in some cases assignment success decreased with the inclusion of isotopic data compared with elemental data alone (Table 3). Correct assignment estimates with all data averaged \sim 50% and ranged from 26% (Trinity Bay) to 75% (Placentia Bay). Correct assignment to the north and south coasts were 66% and 86%, respectively, using both ele-



Fig. 4. Boxplots of measured otolith elements and isotopes per sample site. Horizontal lines of each box represent the median value; edges of the box are the 25th and 75th percentiles; the whiskers indicate the 95th and 5th percentiles. Solid dots represent outliers outside the 90th percentile range. Refer to Table 1 for key to abbreviations.

mental and isotopic data. Collectively, assignment success decreased with the scale of resolution (Table 4). Irrespective of the analysis scale or data, fish length had no significant effect on assignment success (Table 4).

The impact of small-scale heterogeneity was apparent in Placentia Bay sites, which varied widely in correct assignment success (5% to 42%). Placentia Bay fish also displayed a bimodal size distribution, with inner bay fish 10 mm smaller on average than those from the outer, more exposed site (Spanish Room) (ANOVA: $df_{num} = 1$, $df_{den} = 92$, F = 11.95, p = 0.001). By separating Placentia Bay into inner and outer bay samples, assignment success increased in the outermost site by ~10%. When we repeated the bay-scale QDFA with Placentia Bay size modes separated, the inner bay (small individuals)

samples correctly assigned with 93% accuracy and the outermost site increased to 42% accuracy.

To evaluate the influence of among-sample site heterogeneity on bay-scale assignment, we generated all possible permutations of sites for bays with four or more sampling sites (Table 1). Correct assignment declined with increasing number of samples, on average by 10% for every within-bay sample added (linear regression: slope = -10.21, $r^2 = 0.38$, p < 0.0001), ranging from 90% to 100% with one sample per bay to $\sim 60\%$ -70% with four samples per bay (Fig. 6).

The multivariate predictive model (RDA) based on results from Stanley et al. (2015) was highly significant (ANOVA: $df_{num} = 1$, $df_{den} = 305$, F = 55.023, p < 0.00001), with an adjusted coefficient of determination (r^2) of 0.49, partitioned into 0.30 and 0.19 for tem-



Fig. 5. Boxplots of measured otolith elements and isotopes per bay and coast of capture. North (N) and south (S) coastal data are shaded grey. Horizontal lines of each box represent the median value; edges of the box are the 25th and 75th percentiles; the whiskers indicate the 95th and 5th percentiles. Solid dots represent outliers outside the 90th percentile range. Refer to Table 1 for key to abbreviations.

perature and salinity, respectively (see Supplemental Fig. S1 for model summary plot¹). Though all redundancy axes were significantly related to otolith geochemistry (RDA₁ — ANOVA: df_{num} = 1, df_{den} = 305, F = 80.73, p < 0.0001; RDA₂ — ANOVA: df_{num} = 1, df_{den} = 305, F = 29.306, p < 0.0001), variability and small treatment effects between the intermediate and warm temperature conditions likely reduced the explained variance (49% of the model).

Distribution of residuals (Fig. 7) differed in overall pattern and magnitude from that of the raw otolith chemistry (Fig. 4). Classification success based on residuals (observed otolith chemistry minus predicted otolith chemistry from RDA model) improved assignment success at all scales (Tables 2 and 3; Fig. 8). The majority of observed changes in assignment success (residuals versus observed) were greater than zero, averaging (\pm 1 standard error, *n*) 57% (\pm 5.9%, 17), 14% (\pm 6.9%, 5), and 5% (\pm 0.3%, 2) at the site, bay, and coast, respectively (Fig. 7). Assignment to Bonavista Bay using only isotopic data was the only classification that significantly decreased postcorrection (~40% decrease; Table 3). The addition of

length to the assignment analysis did not significantly change assignment success (ANOVA: $df_{num} = 1$, $df_{den} = 4$, F = 0.004, p = 0.95; Fig. 8).

Though the relative assignment success varied among sites and spatial scales, χ^2 proportional comparisons revealed that the majority of tests were significantly greater than classification based on a random assignment of fish among all sites (Tables 2 and 3).

Discussion

Successful management and conservation strategies for exploited marine species hinge upon understanding the extent of dispersal and connectivity in marine organisms (Botsford et al. 2009; Laurel and Bradbury 2006; Sale et al. 2005). Characterization of dispersal kernels and connectivity matrices requires measurements of movements that integrate across life history stages (Hastings and Botsford 2006). Our evaluation of otolith geochemistry as a natural tag using a suite of elements and stable isotopes showed sig-

ID	Location name	n	Raw	Residual	
Placent	tia Bay (PB)				
1	Great Brule	20	0.42	0.79	
2	St. Bernard's Cove	14	0.28	1.00	
3	Woody Island	17	0.27	1.00	
4	Spanish Room	19	0.08*	0.63	
5	Harbour Buffett	22	0.17	1.00	
St. Mar	y's Bay (SMB)				
6	Colinet Island	33	0.48	1.00	
Concep	otion Bay (CB)				
7	Harbour Grace	20	0.32	1.00	
8	Holyrood	18	0.28	1.00	
Trinity	Bay (TB)				
10	Little Mosquito Cove	20	0.28	0.83	
11	Smith Sound	19	0.26	0.63	
12	Dildo	20	0.35	0.95	
13	Tappers Cove	18	0.38	0.50	
Bonavi	sta Bay (BB)				
14	Canning's Cove	19	0.24	0.47	
15	Bread Cove	20	0.25	0.35	
16	Piper's Cove	20	0.32	1.00	
17	Indian Bay	20	0.45	0.95	
Mean			0.30	0.82	

Table 2. Proportion of juveniles correctly assigned to sampling site, bay, and coast of origin using otolith elemental composition and a quadratic discriminant function analysis.

Note: Analyses presented are the product of observed field chemistry and residual chemistry (observed-predicted) from laboratory environmental corrections. Proportions flagged with an asterisk (*) denote assignment tests that were not significantly greater than random at $\alpha = 0.01$. Sample size (*n*) is indicated.

nificant spatial variation in juvenile Atlantic cod among sites in coastal Newfoundland. Individuals successfully classified to bay of capture with 54% accuracy on average or to coast (south versus north) with 78% accuracy on average, which substantially exceeded the accuracy rates possible using techniques that require large spatial scales (e.g., parasite loading - Khan et al. 2011; genetics - Ruzzante et al. 2000; morphometrics - Templeman 1981), anadromy (e.g., Avigliano et al. 2014), or by random chance (20% for bays (n = 5) and 50% for coasts (n = 2)). Otolith geochemistry also varied significantly at finer spatial scales, with assignment success to the correct embayment and coast exceeding 80% for some sites (93% in inner Placentia Bay), highlighting the potential for fine-scale environmental variability to limit assignment success. Indeed, we found an inverse relationship between successful discrimination and the number of sample sites within an embayment, suggesting that unresolved variation could lead to misclassifications. Similarly, when we accounted for small-scale variation in environmental conditions among sites with a laboratorybased predictive model, assignment success improved significantly across all spatial scales tested. Our work adds to a growing literature that documents significant spatial variation in otolith geochemistry of coastal marine fishes (Clarke et al. 2009). The presence of significant spatial variation in otolith geochemistry and subsequent relatively high rates of spatial discrimination support its potential as a tool to delineate movements and spatial patterns of Atlantic cod in coastal Newfoundland, at scales considerably smaller than previously tested (see Campana 1999; D'Avignon and Rose 2013 for examples using adult cod). We posit the potential role for geochemical approaches in the conservation and management of this species and emphasize the complexities imposed by natural environmental variability at small spatial scales, often overlooked by assignment studies.

Many studies have used otolith composition to track movement patterns across species and habitats (Campana 2005; Elsdon et al. 2008). Studies of variation in otolith composition have successfully discriminated among fish from local rivers (e.g., Kennedy et al. 2002), estuaries (e.g., Bradbury et al. 2008; Thorrold et al. 1998), reefs (Swearer et al. 1999), coastal regions (Campana et al. 1999; Correia et al. 2012; Warner et al. 2005), and spawning aggregations (D'Avignon and Rose 2013; Thorisson et al. 2011), with varying degrees of accuracy. Our geochemical data provided levels of successful classification comparable to previous coastal applications. Clarke et al. (2009) reported classification accuracies of 70%–77% for Atlantic silversides (Menidia menidia) along the eastern United States using a similar suite of elements and stable isotopes. On a broader scale, Rooker et al. (2008) documented >88% correct classification of bluefin tuna to nursery locations throughout the Atlantic, and D'Avignon and Rose (2013) documented accuracies of 66%-78% for Atlantic cod over broad spatial scales (>500 km) in the Northwest Atlantic. Continuous distributions of individuals, spatial-temporal variability in environment, and relatively weak spatial heterogeneity in otolith composition pose substantive challenges to assignment of coastal marine fishes on small scales. Though variable within the scale of assignment, our results nonetheless suggest variability in juvenile Atlantic cod otolith chemistry at small spatial scales (>100 km), which could be employed to increase spatial resolution of retrospective analysis, particularly at scales (e.g., bay or coast) relevant to management (e.g., DFO 2013).

We observed variation at the scale of sample site for most elements and stable isotopes. The largest differences among our samples were in Sr:Ca, Mn:Ca, Ba:Ca, and δ^{18} O, which provided much of the discriminatory power, particularly among bays and coast of capture. The identification of strong Sr:Ca, Ba:Ca, and δ^{18} O differences among sites, and their significant correlation with ambient salinity, supports the hypothesis that concomitant variability in water chemistry potentially generates regional composition in otolith chemistry through direct mediation (Elsdon and Gillanders 2004; Stanley et al. 2015). River runoff typically has lower Sr:Ca and δ^{18} O values and higher Ba:Ca values than seawater (Gillanders 2005a). Previous work documented consistently lower salinity at the head of Placentia Bay (Bradbury et al. 2000), near an important spawning and nursery location, where we noted high assignment success. In addition to elemental analysis, stable isotopes have been used extensively to reconstruct environmental histories, trophic interactions, and movement patterns in marine fishes (Jones and Campana 2009; Rooker et al. 2008, Weidman and Millner 2000). The fractionation of $\delta^{13}C$ and $\delta^{18}O$ in otolith aragonite is also well-studied in relation to biological and hydrographic variation (e.g., Thorrold et al. 1997). Stable oxygen isotopes are thought to be deposited close to isotopic equilibrium with ambient water and thus reflect a combination of δ^{18} O of the ambient water and water temperature at the time of deposition (Jamieson et al. 2004; Jones and Campana 2009; Thorrold et al. 1997; Weidman and Millner 2000). The correlation we observed here between δ^{18} O and latitude confirms a similar pattern reported for the eastern Atlantic (Weidman and Millner 2000) linking δ¹⁸O to bottom temperature and to relationships we evaluated in the lab (Stanley et al. 2015). Warmer temperatures characterize the southern portion of the survey area and the inner reaches of embayments such as Placentia Bay (Bradbury et al. 2000), potentially contributing to observed otolith variation within and among bays.

The spatial scale of analysis can clearly impact assignment success in geographic comparisons. We observed significant variation in otolith composition at multiple spatial scales, with improved classification success at increasing spatial scale. We also observed several examples of significant small-scale variation, where individual sampling sites classified with a high level of success. These sites included western Trinity Bay, where the cod spawning-overwintering aggregation represents one of the largest remaining in eastern Newfoundland (Rose et al. 2011; Stanley et al. 2013). Despite these few specific sites with high classification success, overall classification to sampling site was relatively poor

or isotopes and a quadratic discriminant function analysis with cross validation.								
Scale	Location	n	Elements		Isotopes		Otolith	
			Observed	Laboratory	Observed	Laboratory	Observed	Laboratory
Bay	Placentia	92	0.77	0.76	0.77	0.75	0.75	0.75
	Conception	38	0.24*	0.65	0.27*	0.41	0.32*	0.62
	Trinity	77	0.32*	0.38	0.26*	0.44	0.27*	0.48
	Bonavista	79	0.59	0.66	0.51	0.11*	0.6	0.62
	Mean	_	0.48	0.61	0.45	0.43	0.49	0.62
Coast	South	125	0.61	0.66	0.58	0.62	0.66	0.67
	North	194	0.88	0.9	0.91	0.87	0.86	0.89
	Mean		0.75	0.78	0.75	0.75	0.76	0.78

Table 3. Proportion of juveniles correctly assigned to pooled sites using otolith geochemical composition, elements, or isotopes and a quadratic discriminant function analysis with cross validation.

Note: Analyses were conducted using observed data and residuals from predicted laboratory composition. Proportions flagged with an asterisk (*) denote assignment tests that were not significantly greater than random at α = 0.01. Sample size (*n*) is indicated.

Table 4. Results of analyses of variance (ANOVA) examining differences in assignment success as a function of spatial scale, addition of length, and lab standardization.

Index	df	MS	F	р	Post hoc
Scale	2	2333	12.040	0.0004	Site < bay = coast
Length	1	1085	1.421	0.2360	
Standardization	1	9208	14.77	0.0002	Standardization > raw

Note: Post hoc Tukey's tests presented are significant at α = 0.05 level.

 $(\sim\!42\%$ overall) with, for example, only 8% success for the Spanish Room site, our most exposed sample site. Poor assignment to the Spanish Room site was a function of highly variable otolith composition that prevented reliable assignment in the discrimination analysis, which likely reflected reduced stability in environmental conditions. Similarly, Fodrie and Levin (2008) noted lower assignment success rates for halibut at exposed sample sites relative to halibut collected within embayments, and Jamieson et al. (2004) noted higher variability in Atlantic cod otolith δ^{13} C in offshore relative to inshore habitats. Poor assignment in such sites may suggest errors in characterization, reducing assignment success collectively for all sites. Nonetheless, the majority of misclassifications (including Spanish Room) assigned successfully to embayment of capture, resulting in classification success at the bay and coast scales. Importantly, analysis restricted to the two dominant spawning areas in eastern Newfoundland — the head of Placentia Bay and northwestern Trinity Bay - produced classification successes of 77% and 85%, respectively.

Accurate assignment of individuals, populations, or locations normally requires sampling all possible geographic sources in a region because failure to do so may result in major errors during mixture analysis or assignment (Campana 1999; Gillanders 2005b). We addressed this concern by evaluating the relationship between number of samples examined and assignment accuracy for the embayment of capture. The significant declines in correct assignment we observed with increasing number of samples are consistent with increased misassignments when spatial variation in otolith composition is accurately described. When comparing assignment success at different spatial scales, we used different training data sets (same number but with different allocations). We considered this limitation a trade-off for the need to incorporate the variability nested within the system, as highlighted by declining assignment success with an increased number of sample sites. Indeed, the degree to which baseline variability has been quantified remains a challenge for assignment studies, but the consequences of an incomplete baseline are potentially important.

We are confident that our experimental design effectively sampled most juvenile habitat within the study region. Elevated juvenile density and survival in Atlantic cod occur in structured habitats, primarily eelgrass (*Zostera marina*) (Laurel et al. 2003). Our survey targeted eelgrass habitats using published records of **Fig. 6.** Discriminant function assignment success aggregated at three spatial scales (site, bay, and coast) as a function of the number of sites within each bay. Analysis was restricted to bays (i.e., Bonavista, Trinity, and Placentia) with four or more sites. Raw data are presented as points underlying the boxplots.



cod sampling in nearshore Newfoundland (Methven et al. 1998), which allowed us to sample the dominant potential nursery areas on the north and south coasts, respectively. Moreover, the overall spacing of sample sites at \sim 70 km between bays and <30 km within bays should resolve much of the inherent spatial variation, given relatively limited expected movement in age-0 cod (Laurel et al. 2004; Ryan et al. 2012). Interestingly, the geochemical similarities we observed between distant sites within our study area (e.g., outer Placentia and eastern Trinity Bay) reduced our classification success. This observation suggests that geochemical similarities among distant sites could easily lead to inference of movement among unconnected sites in the absence of extensive sampling to characterize accurately the probability of assignment. Environmental conditions experienced by fish considerably influence the rate of incorporation of otolith constituents in addition to other mediating factors that characterize the ecosystem (e.g., genetics, diet, ambient water chemistry). Work with juvenile black bream (Acanthopagrus butcheri) (Elsdon and Gillanders 2002), Chinook salmon (Oncorhynchus tshawytscha) (Miller 2011), and juvenile Atlantic cod (Hoie et al. 2004; Stanley et al. 2015) all document



Fig. 7. Boxplots of measured otolith elements and isotopes per bay and coast of capture. Horizontal lines of each box represent the median value; edges of the box are the 25th and 75th percentiles; the whiskers indicate the 95th and 5th percentiles. Solid dots represent outliers outside the 90th percentile range. Refer to Table 1 for key to abbreviations.

significant and complex interactive influences of temperature and salinity on incorporation rates of elements and isotopes into otoliths. The observation of consistent decreased assignment success as sites were "added" to each bay during discriminant analysis likely relates to environmental heterogeneity within the scale of assessment that reduced discrimination. Spatial-temporal variability in environmental conditions characterizes many coastal environments (Craig and Colbourne 2004), especially at the small spatial scales and short time periods (~2 months) such as those investigated in our analysis.

To further illustrate the impact of environmental heterogeneity, we partitioned the variance in otolith chemistry attributable to environmental conditions using a predictive laboratory model and observations of environmental conditions in the field. Residual discriminant analyses significantly increased assignment success for all spatial scales we tested. Increased assignment success suggests that even with removal of environmental influence (temperature and salinity), a residual "signal" remains that presumably reflects the remaining abiotic and biotic variables that characterize the system. This residual signal appears robust, with discrimination among some areas greatly improving. High assignment success to sites such as inner Placentia Bay, for example, could be attributed to terrestrial influences (e.g., Palmer and Edmond 1992), as these areas are characterized by higher riverine runoff than other sample sites and thus create the potential for a land-based signal. Collectively, these results describe how environmental variability at small scales (10–100 km) could potentially diminish or accentuate spatial pattern signal characteristic of the system.

The inability of conservation and management plans to evaluate significant small-scale spatial complexity of coastal fish species presents a clear threat to the persistence and stability of coastal stocks (Botsford et al. 2009; Hilborn et al. 2003). Our survey area was located within multiple management areas for Atlantic cod that are often subject to different management decisions.



Fig. 8. Discriminant function assignment success as a function of scale for raw data and laboratory-corrected residuals. Open boxes are assignments using geochemistry data, while shaded boxes represent data using a combination of fish length and geochemistry.

Placentia Bay is thought to support both resident and migratory stocks, which are each subject to local commercial harvest (Mello and Rose 2005; Robichaud and Rose 2004). Clearly, the application of approaches such as otolith geochemical analysis, which can at least partially resolve some fine-scale spatial patterns, as we have shown here, may be critical to successful conservation and management of rebuilding stocks with complex population structure.

Summary

We observed spatial variation in otolith composition of juvenile cod from coastal Newfoundland and describe the potential of this variation to partially resolve the spatial structure of juvenile habitat at scales smaller than previously available. Moreover, we demonstrate how variation in environmental conditions at scales less than 100 km could confound assignment through mediation of otolith composition and the alteration of natural spatial variance, stressing the importance of resolving baseline variability in otolith chemistry when conducting assignment tests. Natural geochemical tags recorded in otolith geochemistry provide an important tool to facilitate the management and protection of genetic diversity in this commercial species, which until recently (see trends reported in DFO 2013) was considered at risk of commercial extinction in Canadian waters (Bradbury 2010).

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