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Estimating contemporary early life-history dispersal in an estuarine fish: integrating molecular and otolith elemental approaches

I. R. BRADBURY,* S. E. CAMPANA† and P. BENTZEN*
*Marine Gene Probe Laboratory, Biology Department, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada, B3H4J1,†Marine Population Division, Bedford Institute of Oceanography, PO Box 1006, Dartmouth, Nova Scotia, Canada, B2Y4A2

Abstract

Dispersal during the early life history of the anadromous rainbow smelt, *Osmerus mordax*, was examined using assignment testing and mixture analysis of multilocus genotypes and otolith elemental composition. Six spawning areas and associated estuarine nurseries were sampled throughout southeastern Newfoundland. Samples of adults and juveniles isolated by > 25 km displayed moderate genetic differentiation (F_{ST} ~ 0.05), whereas nearby (< 25 km) spawning and nursery samples displayed low differentiation (F_{ST} < 0.01). Self-assignment and mixture analysis of adult spawning samples supported the hypothesis of independence of isolated spawning locations (> 80% self-assignment) with nearby runs self-assigning at rates between 50 % and 70%. Assignment and mixture analysis of juveniles using adult baselines indicated high local recruitment at several locations (70–90%). Nearby (< 25 km) estuaries at the head of St Mary's Bay showed mixtures of individuals (i.e. 20–40% assignment to adjacent spawning location). Laser ablation inductively coupled mass spectrometry transects across otoliths of spawning adults of unknown dispersal history were used to estimate dispersal among estuaries across the first year of life. Single-element trends and multivariate discriminant function analysis (Sr:Ca and Ba:Ca) classified the majority of samples as estuarine suggesting limited movement between estuaries (< 0.5%). The mixtures of juveniles evident in the genetic data at nearby sites and a lack of evidence of straying in the otolith data support a hypothesis of selective mortality of immigrants. If indeed selective mortality of immigrants reduces the survivorship of dispersers, estimates of dispersal in marine environments that neglect survival may significantly overestimate gene flow.

Keywords: assignment testing, dispersal, larval fish, otolith elemental composition, rainbow smelt, selection

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Introduction

Marine species are often characterized by high gene flow, weak genetic structure, and large genetic neighbourhoods (Palumbi 2004). The scale of larval dispersal is often considered a major determinant of spatial pattern, connectivity, and metapopulation structure (Sinclair 1988; Hastings & Botsford 2006). Dispersal may be directly related to evolutionary dynamics, stability and persistence of marine communities (Sinclair 1988). Effective dispersal is the end result of immigration and subsequent survival. Measuring supply alone has proven challenging, given the scale of potential dispersal and dynamics of marine pelagic stages. As a result, present understanding of marine dispersal is biased towards low-dispersal species (Laurel & Bradbury 2006). Even less is known about the success (i.e. mortality) of dispersing larvae and the role of immigrant inviability in marine species (see Gilg & Hilbish 2003 for an exception). Disentangling these processes requires contemporary estimates of dispersal and reconstructions of dispersal history at various ontogenetic stages. This knowledge gap remains one of the largest obstacles to marine conservation (Palumbi 2004; Sale *et al*. 2005).

Correspondence: I. R. Bradbury, Fax: (902) 494 3736; E-mail: ibradbur@dal.ca

The last decade has seen dramatic technological improvements for tracking contemporary larval dispersal in marine environments (e.g. Thorrold *et al*. 2006; Bradbury & Bentzen 2007). Molecular approaches, such as those based on multilocus mixture analysis or assignment testing, present great potential for tracking dispersal (e.g. Manel *et al*. 2005; Hauser *et al*. 2006). Assignment methods (e.g. Rannala & Mountain 1997; Cornuet *et al*. 1999; Pritchard *et al*. 2000) have proven effective in measuring dispersal in terrestrial species, with moderate to high genetic differentiation (e.g. Berry *et al*. 2004), allowing estimates of dispersal unachievable by other methods. In marine species, assignment-based methods remain relatively untested and may be limited by high connectivity, high gene flow, and low genetic structure (Cornuet *et al*. 1999; Manel *et al*. 2005). Accurate assignment at low differentiation ($F_{ST} \sim 0.015$) using Bayesian approaches with baseline data may be possible (Hauser *et al*. 2006), suggesting that assignment approaches will be effective in some marine species. Ruzzante *et al*. (2006) successfully used Bayesian mixture analysis to identify spatial structure in herring populations at low levels of differentiation ($F_{ST} \sim 0.027$). The performance of these methods under moderate to high gene flow requires further evaluation to delineate the lower limit of successful application if they are to be used effectively in aquatic organisms (Hauser *et al*. 2006; Waples & Gaggiotti 2006).

In contrast, otolith-based techniques have proven successful in resolving dispersal and mixture separation in high gene flow systems where environmental heterogeneity (i.e. ambient water composition, temperature, etc.) exists (e.g. Campana *et al*. 1999; Agler *et al*. 2001; Thorrold *et al*. 2001). Otolith elemental approaches are based on the facts that otoliths are largely metabolically inert and otolith growth is continuous throughout development. Elements are integrated into the calcium carbonate matrix based on ambient concentrations, although rates of accretion of some elements seem dependent on physiology (Campana 1999). The success of otolith-based technologies is dependent on distinct background environmental differences and may be of limited use at small geographical scales where spatial environmental variance is low. Yet, movements at small scales are potentially most interesting because the likelihood of mixing and connectivity is high, such as within single watersheds, or nearby marine sites. One approach to maximize resolution using otolith-based technologies is to examine connectivity in estuarine systems where species exist along a salinity gradient, which may be used to reconstruct movements between estuaries and coastal areas (Gillanders 2005). Movements into coastal environments may be evident in the concentrations of elements such as Sr (e.g. Kraus & Secor 2004) and Ba (Elsdon & Gillanders 2005), which allow reconstruction of possible movements between estuaries (e.g. Crook *et al*. 2006).

Rainbow smelt, *Osmerus mordax* (Mitchill), 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 and the larvae develop in downstream estuaries (e.g. Bradbury *et al*. 2004). Smelt typically mature at 2–3 years of age and may live to 4–5 years, usually spawning up to 3 years consecutively (McKenzie 1964). The close association of multiple life-history stages with estuarine habitat and the potential for active (i.e. through vertical migration) estuarine retention of larvae has led several studies to conclude that population structure is likely associated with estuaries or retention areas (e.g. Bernatchez & Martin 1996; Bradbury *et al*. 2006a, b). Recent work based on spatial patterns of early life-history stages has suggested that larval dispersal may be limited to a single estuary (Bradbury *et al*. 2006b) and that local adaptation may occur on the scale of the local estuary (Bradbury *et al*. 2006a).

The overall goal of this work was to compare both contemporary molecular and otolith-based estimates of early life-history dispersal in rainbow smelt. First, assignment testing and genetic mixture analysis of multilocus genotypes were used to track dispersal of rainbow smelt early life-history stages between estuaries in coastal Newfoundland. Mixtures of juveniles sampled in estuarine nurseries were assigned to spawning locations, using baseline data of adult spawning groups. Second, LA-ICP-MS (i.e. laser ablation inductively coupled mass spectrometry) otolith transects of the first year of life from each of these six spawning groups was used to estimate marine vs. estuarine residency. Finally, both techniques were compared and connectivity during the early life history of anadromous smelt assessed.

Methods

Sampling locations and collections

Sampling locations were typically small coastal streams generally less than 5 m in width and 1 m depth (see Bradbury *et al*. 2006a, 2006b). Adult fish were collected with dip and fyke nets during the spawning period at six spawning locations in St Mary's and Placentia Bays (Fig. 1) between 2003 and 2006, with two sites sampled in multiple years (Table 1). Pectoral or caudal fin clips were taken and immediately placed in 95% ethanol. Juvenile young-of-the-year smelt were sampled in downstream estuaries during the autumn (October and November) of 2004 at each of the six sites. Sites were chosen based on accessibility by small craft and the presence of eelgrass habitat. A 25-m demersal seine with 19 mm stretched mesh was deployed 50 m from shore. At each site, a minimum of 100 juveniles (30–60 mm total length) were collected, measured and placed in 95% ethanol.

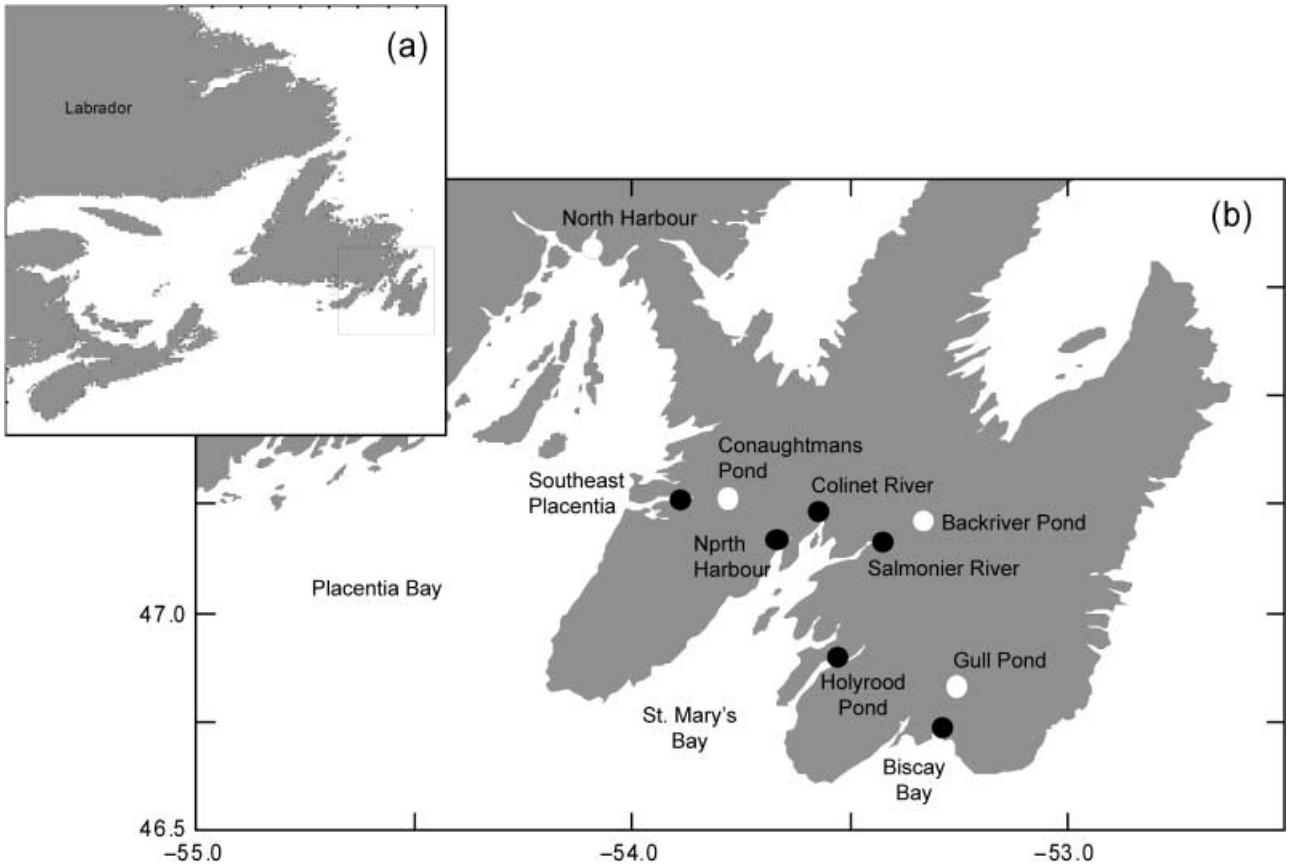

Fig. 1 Map of sample locations, inset (a) shows location of southeast coast of Newfoundland (b) with respect to eastern Canada. Open circles represent baseline sample locations. Closed circles represent sampled anadromous smelt spawning locations.

Microsatellite analysis

DNA was extracted following the protocol of Elphinstone *et al*. (2003), modified to work with a 96-well filter plate and automated on a robotic liquid handling system (Perkin Elmer). Nine microsatellite loci were used as follows: Omo1, Omo2, Omo3, Omo4, Omo5, Omo9, Omo11, Omo15, and Omo16 (Coulson *et al*. 2006). Individuals were genotyped using polymerase chain reaction (PCR) conditions of 5-μL or 10-μL volumes containing 20–100 ng DNA, 1.5 mM $MgCl_2$, 80 μM each dNTP, 0.5 U *Taq* DNA polymerase (New England Biolabs), 0.3 μM of each primer (forward primers were end-labelled with HEX, or ROX dye), and 1× PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl). Two temperature profiles were used for touchdown to allow for the possibility of multiplex PCRs. Touchdown PCR conditions were as follows: 94 °C for 2 min, followed by four to five cycles of 94 °C for 30 s, program-specific touchdown annealing temperatures (T_a) of –1 °C per cycle for 30 s, 72 °C for 30 s, followed by 25–26 cycles where the T_a was held constant at 4 °C below the starting temperature. A final extension was held at 72 °C for 5 min. Reactions were run on Eppendorf thermocyclers and imaged on an FMBioII system (Hitachi Genetic Systems). See Coulson *et al*. (2006) for further details regarding PCR conditions.

Molecular statistical analysis

Data were checked for the presence of null alleles and scoring errors using MICRO-CHECKER (van Oosterhout *et al*. 2004). Genetic polymorphism was quantified by examination of the number of alleles, and observed and expected heterozygosities using GENETIX (version 4.05.2, Belkhir *et al*. 2004). Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were done using FSTAT (version 2.9.3.3, Goudet 1995). *F*-statistics and significance were calculated using FSTAT and ARLEQUIN (Schneider *et al*. 2000). Analysis of molecular variance (AMOVA) was used to partition observed genetic variance into components associated with nearby and isolated locations and was conducted using ARLEQUIN.

Given the difficulty in predicting which assignment/clustering approach may be best suited to each specific situation (e.g. Hauser *et al*. 2006), and the fact that this

Table 1 Details for genetic samples of adults and juveniles from locations throughout southeastern Newfoundland. See Fig. 1 for site locations. Adult samples were collected from spawning events, whereas juvenile samples were collected from estuaries downstream of a given spawning location. H_E and H_O refer to expected and observed heterozygosities, respectively

Population (year)	Location	Sample size	H_E	H_O	Average No. of alleles
Adult samples					
Salmonier River (2002)	St Mary's Bay	80	0.73	0.71	10.44
Salmonier River (2003)	St Mary's Bay	94	0.71	0.70	9.89
Salmonier River (2006)	St Mary's Bay	93	0.71	0.74	10.22
Colinet River (2003)	St Mary's Bay	94	0.72	0.73	10.78
North Harbour River (2004)	St Mary's Bay	94	0.71	0.73	10.33
Biscay Bay River (2003)	St Mary's Bay	94	0.71	0.69	9.56
Biscay Bay River (2006)	St Mary's Bay	93	0.70	0.67	9.00
Holyrood Pond Brook (2005)	Holyrood Pond (SMB)	94	0.74	0.66	10.22
Holyrood Pond Park (2004)	Holyrood Pond (SMB)	94	0.73	0.71	9.89
Deer Pond Brook (2004)	Holyrood Pond (SMB)	41	0.71	0.71	8.44
Pathend Brook (2005)	Holyrood Pond (SMB)	94	0.73	0.72	10.00
Southeast Placentia (2005)	Placentia Bay	94	0.67	0.66	9.33
Juvenile samples					
Salmonier River estuary (2004)	St Mary's Bay	94	0.73	0.73	11.11
Colinet River estuary (2004)	St Mary's Bay	94	0.76	0.69	11.00
North Harbour estuary (2004)	St Mary's Bay	94	0.73	0.73	11.44
Biscay Bay estuary (2004)	St Mary's Bay	94	0.73	0.72	10.33
Holyrood Pond (2004)	St Mary's Bay	94	0.72	0.70	9.33
Southeast Placentia estuary (2004)	Placentia Bay	94	0.67	0.67	10.22

SMB, St Mary's Bay.

study likely encompassed multiple gene flow regimes, multiple approaches were used and compared. Bayesian clustering without baseline data was done using STRUCTURE version 2.0 (Pritchard *et al*. 2000) and used to examine whether predictions of structuring at the scale of spawning run were consistent with multilocus genetic data. This approach assumes HWE and linkage equilibria among loci, introduces population structure, and assigns populations that are not in linkage equilibrium using an MCMC (Markov chain Monte Carlo) algorithm to estimate the number of populations (K). The algorithm was run 10 times for each K to ensure convergence of values, and with a burn-in of 50 000 repetitions, 200 000 repetitions after burn-in, and K = 1–8. STRUCTURE was initially run with 1 million repetitions to ensure 200 000 was sufficient. The optimal K was determined visually from the value at which L P(D) plateaus as well as using ΔK method of Evanno *et al*. (2005). A second STRUCTURE analysis was then completed with K = 6 and the Q values (admixture coefficient) of most likely assignment examined. Frequency histograms of these values were examined using adults and juveniles for the locations at the head of the bay without regard for assigned cluster, to evaluate assignment confidence at low differentiation. Q value frequency distributions for adults and juveniles were compared using a G-test. Bayesian individual assignment using baseline data was conducted in GENECLASS version 2.0 (Piry *et al*. 2004) and was taken as the potential source showing the highest probability using the approach of Rannala & Mountain (1997). Mixture analysis was conducted using the GMA Windows-based computer program of Kalinowski (2003), which uses a Bayesian approach and baseline data to perform genetic mixture analysis. Both self-assignment and assignment with baseline data were done for each individual and mixture analysis. For the mixture analysis, simulated mixtures of baseline data were used to assess the ability of baselines to differentiate between samples following Ruzzante *et al*. (2006).

Otolith elemental analysis

Sagittal otoliths were extracted from 10 randomly selected spawning adults from each of the six spawning rivers (Fig. 1). In addition, baseline data were acquired using otoliths from smelt from three freshwater ponds distributed across the study range (Fig. 1). An estuarine baseline sample was obtained from juveniles sampled at North Harbour Placentia Bay, and a marine baseline was obtained from fish held at the Ocean Sciences Centre of Memorial University for 1 year in seawater (32 ppt). In all baseline samples, approximately 10 individuals were sampled from each locality. Otoliths were cleaned using Super Q water and stored in acid-washed vials. Otoliths were embedded in epoxy and sectioned using an Isomet saw. Sections were

imaged and aged. The approximate width of the first annulus was confirmed using a sample of 1-year-old fish taken from one of the estuaries (i.e. Biscay Bay) in June 2004. The width of each consecutive yearly annulus was measured and compared individually for each year of growth using analysis of variance (ANOVA) to detect significant differences in growth among regions.

Otolith elemental composition was measured using LA-ICP-MS. All assays were conducted at WRL laboratories at Arkansas State University, using protocols described in detail elsewhere (Coghlan *et al*. 2007). A CETAC LSX500 (260 nm) laser-ablated otolith material and was coupled to a Perkin-Elmer DRCII Dynamic Reaction Cell ICP-MS for detection and measurement. All assays were based on a 25 µm spot size and collected using DIGI-LAZ software. Samples ($n = 10$) were taken in triplicate evenly spaced across the first year to the end of the first winter. At the beginning of each day of sampling, the argon gas flow and lens voltage was adjusted to optimize sensitivity. In the estuarine and marine baseline samples, only the edge of the otolith was assayed. In the freshwater samples, assays were conducted at the core, middle and edge in order to examine ontogenetic variance in a constant environment.

Micro-analytical carbonate standard 1 (MACS-1, US Geological Survey) was used as a standard reference material to calibrate and control for drift every five to nine samples. Concentrations of each 35 analytes were estimated from each otolith sample using the GEO-PRO software (Cetac Technologies). ^{42}Ca was used as an internal standard as it is 38% by weight in MACS-1 and otoliths. ^{43}Ca was measured. The limit of quantification was estimated as three times the standard deviation for seven blank replicates for each of the 35 analytes. Analysis revealed multiple detectable elements, but ^{86}Sr and ^{136}Ba were chosen because they have been shown to be most related to salinity (Elsdon & Gillanders 2005). Limits of detection for each element were as follows: Sr, 12.13 ng/g; Ba, 37.62 ng/g; Ca, 102 ng/g. Data analysis followed Campana (2005). To control for the amount of ablated material, all concentrations were expressed in comparison to the amount of Ca in ppt. All replicates were averaged and examined for the presence of outliers using mean-variance plots. To validate the interpretation of otolith composition reflecting ambient concentrations, water samples were collected at each of the baseline locations, acidified with ultrapure nitric acid, and subsequently analysed for trace element concentrations using solution ICP-MS (assays conducted by RPC, Fredericton, NB, Canada). To assign samples as one of the three baselines (freshwater, estuarine, marine), two approaches were used. First, single-element comparisons using Sr:Ca and Ba:Ca were made with each salinity baseline. Second, linear discriminant function analysis (DFA) was applied on mean element ratios. Using stepwise selection to identify elements of interest in separating baseline samples, Sr:Ca and Ba:Ca were then chosen to assign unknown samples to a baseline group. Baseline discrimination allowed separation habitats using these two elements and sample sizes with 97% accuracy.

Results

Size and growth

All sampled juveniles were between 40 mm and 80 mm in total length (Fig. S1, Supplementary material). Most sites were characterized by individuals less than 70 mm, with the exception of those in Holyrood Pond, which commence spawning 4–6 weeks earlier than those at any of the other locations and subsequently contained individuals ranging from 50 to 80 mm total length. As differing growth rates may also influence otolith composition independent of habitat, growth rates during the first year of life were compared using the size of the first annulus in adult samples across all sites. ANOVA indicated no significant differences ($P = 0.128$) among locations and allowed for further comparison.

Molecular analysis

Average heterozygosities and the average numbers of alleles within populations were 0.64–0.83 and 8–14, respectively (Table 1 and A1). No evidence of linkage disequilibrium was detected, and there was only one significant deviation from HWE, Omo15 at a single location. Instances of null alleles estimated using MICRO-CHECKER (van Oosterhout *et al*. 2004) were rare and not consistently associated with a specific locus or population. Nulls were estimated to be present in ~5% of all comparisons (loci × location) and when present the estimated frequency was usually less than 5%.

Adult global F_{ST} was 0.053 ± 0.014 and indicative of significant structuring ($P < 0.001$). F_{ST} between spawning runs ranged from 0.003 to 0.087 and comparisons between estuarine juvenile samples ranged from 0.003 to 0.085. Spawning runs separated by less than 25 km displayed low differentiation ($F_{ST} = 0.003$–0.005), whereas spawning locations separated by > 25 km displayed substantially greater differentiation ($F_{ST} = 0.05$–0.10). Similarly, juvenile samples showed moderate structure overall with $F_{ST} = 0.058$ ± 0.014. AMOVA of both adult and juvenile samples indicated significant ($P < 0.05$) variance, which was explained by separating nearby locations and isolated locations, as well as at the sample level (Table S2, Supplementary material). Bayesian clustering indicated similar levels of structuring in both juveniles (Fig. 2a) and adult samples (Fig. 2b). In both data sets, STRUCTURE analysis indicate Ln[P(D)] values plateaued at approximately six clusters (Fig. 2), with the largest increase (i.e. ΔK) in Ln[P(D)]

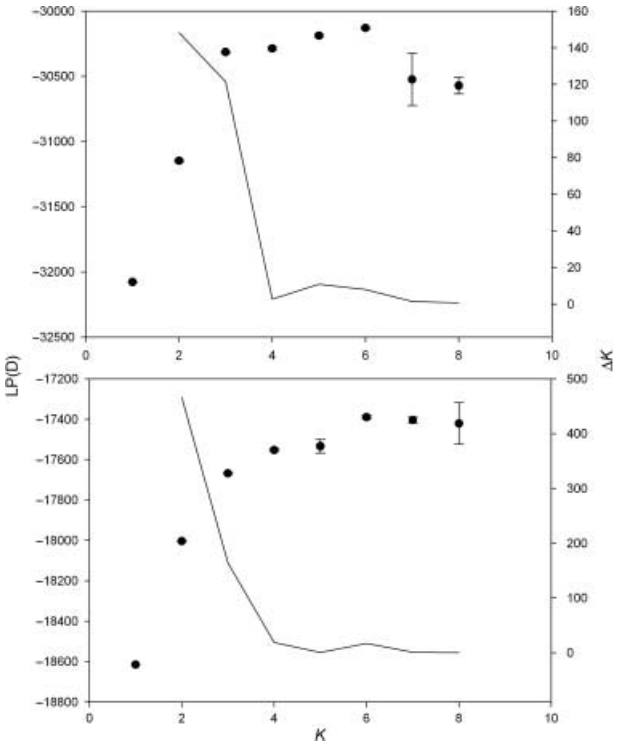

Fig. 2 STRUCTURE clustering of adult (a) and juvenile (b) microsatellite genotypes from six spawning and estuarine nursery locations throughout southeastern Newfoundland. Closed circles indicate mean LP(D) for 10 replicate runs with standard deviation run with $K = 1–8$. Solid line represents ΔK calculated following Evanno *et al*. 2005). See Fig. 1 for sample location details.

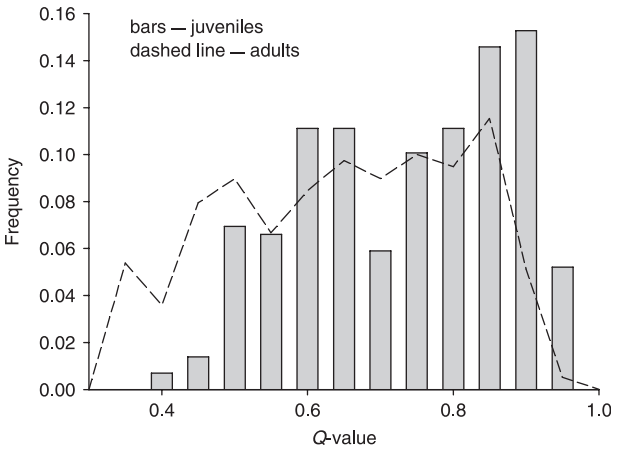

Fig. 3 *Q*-value frequency distribution from STRUCTURE analysis of adult (dashed line) and juvenile (bars) microsatellite data from head of St Mary's Bay assignments. See Fig. 1 for site locations. *Q* values based on most likely assignment for each individual regardless of assignment location, and analysis with $K = 6$.

observed at $K = 2$. Despite low genetic differentiation, individual *Q* values among estuaries in the head of St Mary's Bay were relatively high, consistent with the presence of distinct clusters and significant assignment (Fig. 3) and were not significantly different from adult *Q* values ($P > 0.05$)

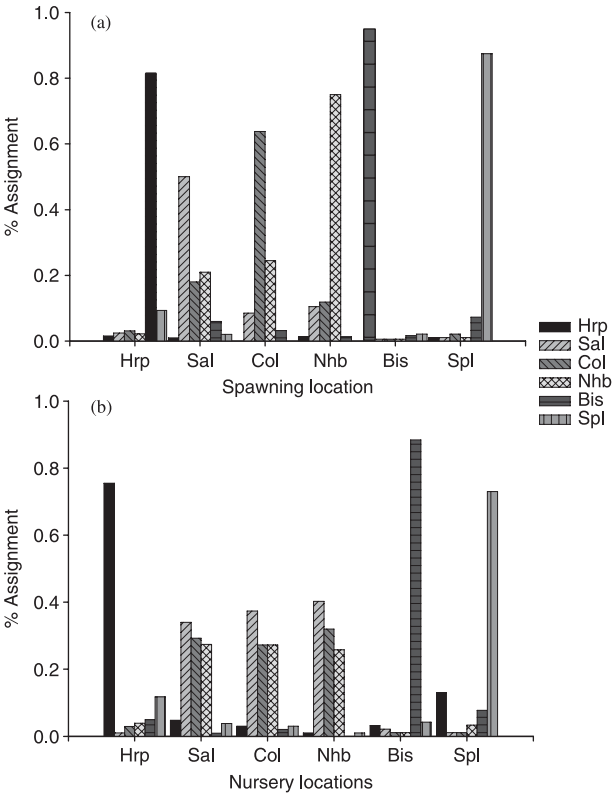

Fig. 4 Assignment of rainbow smelt (a) spawning adults (self-assignment) and (b) juveniles from six spawning locations throughout southeastern Newfoundland. Assignment using GENECLASS 2.0 (Piry *et al*. 2004) and most likely baseline location taken as assignment. (Hrp, Holyrood Pond; Sal, Salmonier; Col, Colinet; Nhb, North Harbour; Bis, Biscay Bay; Spl, Southeast Placentia).

Assignment testing using GENECLASS yielded self-assignment of adults to the source populations with 50–90% accuracy (Fig. 4a). The estuaries closest in proximity (i.e. those at the head of St Mary's Bay) generally displayed the lowest self-assignment (50–75%). Juvenile estuarine samples assigned with frequencies of 33–84% to adjacent spawning locations (Fig. 4b). Again, nearby estuaries in the head of St Mary's Bay displayed the lowest assignment to associated spawning runs (~33%), with relatively high assignment to neighbouring spawning sites (20–30%; Fig. 4b). Genetic mixture analysis using the method of Kalinowski (2003) produced greater than 80% self-assignment of adults for all populations (Fig. 5a, Table S3, Supplementary material). Mixture analysis of isolated nursery samples (> 25 km from nearest neighbour) using adult baselines yielded assignment rates of > 75% to the adjacent spawning run, suggesting little dispersal of larvae from the

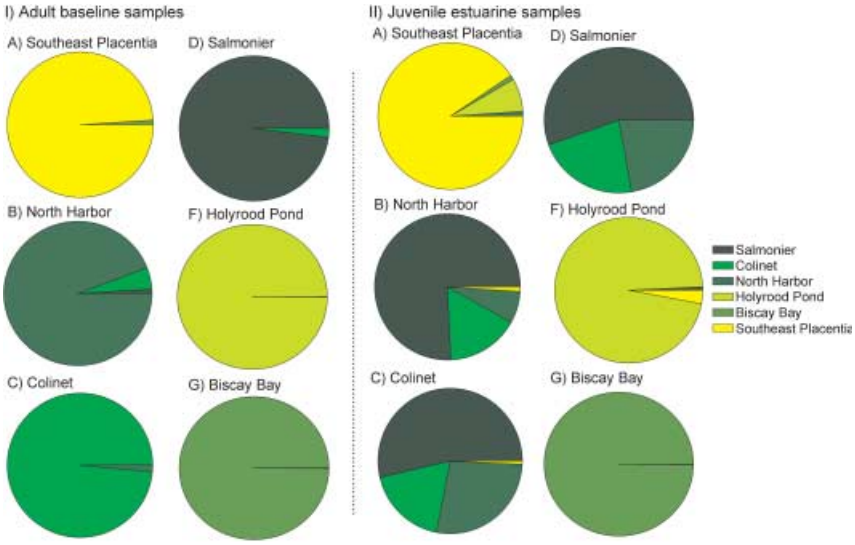

Fig. 5 Genetic mixture analysis of rainbow smelt (I) spawning adults (i.e. self assignment) and (II) juveniles from six spawning locations throughout southeastern Newfoundland using GMA 1.0 (Kalinowski 2003). Values represent mean mixture estimates; see Supplementary material for error associated with values.

Table 2 Summary statistics for otolith Sr:Ca, Ba:Ca, and DFA1 for baseline samples from fresh, estuarine, and marine environments

	Baseline differences		
	Freshwater	Estuarine	Marine
Sr	0.0038 ± 0.001	0.0078 ± 0.0005	0.011 ± 0.003
Ba	0.00012 ± 0.000012	$1.28 \times 10^{-6} \pm 4.35 \times 10^{-6}$	$3.5 \times 10^{-6} \pm 3.5 \times 10^{-6}$
DFA1	1.55 ± 0.59	–2.72 ± 0.44	–5.40 ± 2.48

estuary of the natal stream (Fig. 5b; Table S4, Supplementary material). Mixture analysis of juvenile samples within the head of St Mary's Bay (< 25 km apart), however, suggested that juveniles in each nursery comprised a mixture of the three source populations, and that in each location, juveniles from Salmonier comprised the largest component of the mixture (Fig. 5b, Table S4).

Mixtures were simulated from baseline data and analysed using GMA (see Table S5, Supplementary material). Overall accuracy and precision were high as indicated by the narrow variation, and the observation that the 95% confidence intervals encapsulated the actual proportion in 75% of all cases. All exceptions were associated with samples from the head of St Mary's Bay. The simulations suggested that locations isolated by > 25 km self-assigned correctly for 97–99% of individuals. Similarly, the simulations indicate that populations at the head of St Mary's Bay could be distinguished from others correctly on average 70% with a maximum standard deviation of 0.01 (Table S5). Accordingly, the simulations indicated that in the high gene flow situation, correct assignment was about 70%, such that if there was 100% self-recruitment, the assignment values would be 74 ± 0.08% for Colinet, 71 ± 0.09% for North Harbour; and 66 ± 0.10% for Salmonier. In contrast, correct assignment for the locations characterized by low gene flow (Southeast Placentia, Holyrood Pond, Biscay Bay) were consistently over 95% in each simulation.

Otolith chemistry

Ambient water concentrations (i.e. Ba:Ca and Sr:Ca) differed among each of the baseline salinity treatments with Sr:Ca increasing and Ba:Ca decreasing with salinity (Fig. S2a–b, Supplementary material). Similar trends were observed within the otolith baseline samples (Fig. S2c–d), and we observed positive associations between baseline otolith samples and ambient water concentrations. DFA of baseline samples produced a first axis that allowed 97% correct assignment of samples (Fig. S3, Supplementary material). Average values (± standard deviation) for Sr:Ca, Ba:Ca, and DFA1 for the baseline samples are included in Table 2. The majority of DFA1 values for the marine baseline were < –4, which was subsequently used as a conserva-tive transition between estuary and marine residency (Fig. S3).

Single-element ratios (i.e. Sr:Ca and Ba:Ca) revealed signatures indicative of estuarine residency (Sr, Fig. S4, Supplementary material; and Ba, Fig. 5a). Trends across the first year indicated little variation among individuals at most sites, with Biscay Bay and North Harbour displaying some variation both among and within individuals. Biscay

Table 3 Results of discriminant function analysis and assignments of all samples across the first year of life in fresh, estuarine, or marine environments based on otolith elemental composition

Location	Total samples	No. assigned		
		Freshwater	Estuarine	Marine
Biscay Bay	100	0	99	1
Salmonier River	90	4	86	0
Colinet River	110	10	100	0
North Harbour	90	10	78	2
Holyrood Pond	90	4	86	0
Southeast Placentia	100	2	98	0

Bay and North Harbour were the two locations where single-element ratios approached the marine baseline for a few samples, and these locations were also characterized by exceptionally high levels of Ba:Ca (Fig. S5, Supplementary material). The site that displayed the least variation overall was the partially landlocked site Holyrood Pond, which with the exception of enrichment (i.e. elevated Sr:Ca and Ba:Ca) at the core, was essentially uniform within and among all individuals.

DFA classified the majority of otolith samples as estuarine (94.5%), with 5% freshwater assignment, and 0.5% marine (Table 3, Fig. S3) and was consistent with single-element trends. Most of the freshwater assignments were associated with Colinet and North Harbour (Table 3). Only Biscay Bay and North Harbour included any marine classifications with a total of three individuals showing marine signatures. Similar trends in variation were observed in DFA1 (Fig. 6) as compared with single-element patterns (see above). Again, Holyrood Pond showed little variation between individuals or across transects. Biscay Bay and North Harbour displayed the most variation both along transects and among individuals (Fig. 6).

Discussion

Dispersal during the early life history of marine organisms may be extensive (Scheltema 1971) and likely plays a regulatory role in marine population dynamics and connectivity (Sinclair 1988; Hastings & Botsford 2006). Generalizations of broad-scale dispersal across large ocean distances based on the duration of the pelagic larval period are likely to be inappropriate for species in which behaviour or mortality significantly curtail dispersal (e.g. Bradbury *et al*. 2006b). In this study, we contrast estimates of early life-history dispersal in an estuarine fish obtained using molecular genetic and otolith elemental approaches. Low connectivity and self-recruitment was observed over scales of tens of kilometres using assignment of multilocus genotypes despite high dispersal potential. Otolith elemental composition during the first year of life revealed little evidence of marine occupancy indicating that > 95% of individuals are retained in estuarine waters. These findings add to a growing body of literature documenting self-recruitment and limited dispersal in marine and estuarine organisms including those with extensive pelagic larval periods (e.g. Thorrold *et al*. 2001; Taylor & Hellberg 2003; Jones *et al*. 2005). In this context of structured adult spawning populations, the evidence that juveniles from multiple spawning locations at the head of St Mary's Bay formed mixed assemblages was unexpected but consistent with the hypotheses of natal homing and/or selection against juveniles that disperse.

Both the molecular and otolith approaches support the hypothesis of limited broad-scale dispersal despite a preflexion larval period of up to a month. Behavioural contributions to larval and juvenile smelt transport have been examined extensively (Ouellet & Dodson 1985; Bradbury *et al*. 2006b) and suggest that ontogenetic shifts in vertical migration behaviour may allow local retention and recruitment. Passive transport based on local currents could result in transport on the scale of hundreds of kilometres (Bradbury *et al*. 2000). Nonetheless, most of the estuarine samples isolated by > 25 km assigned with high rates (> 80%) to the local spawning locations. The results of high self-recruitment and limited dispersal for smelt are consistent with observed strong spatial associations between estuarine habitats and larval (e.g. McKenzie 1964; Bradbury *et al*. 2006b) or adult stages (Bradbury *et al*. 2006a).

Bayesian clustering of the microsatellite data supported the presence of six discrete populations, with both spawning adult and juvenile samples displaying probability values that plateaued at $K = 6$. The close similarity of the results for adults and juveniles supports the stability of these genetic groups across life-history stages. Interestingly, the largest change in probability (i.e. ΔK) was observed at $K = 2$, a pattern consistent with evidence that the southern Avalon Peninsula represents a zone of secondary contact for two glacial races of smelt (authors' unpublished data). For the sites that displayed the highest gene flow (head of St Mary's Bay), individual Q values of the most likely assignment were high, indicating confident assignment of many individuals. Surprisingly, low Q values appeared more common in the adult sample than in the juvenile sample. Low Q values in the juvenile stages might be

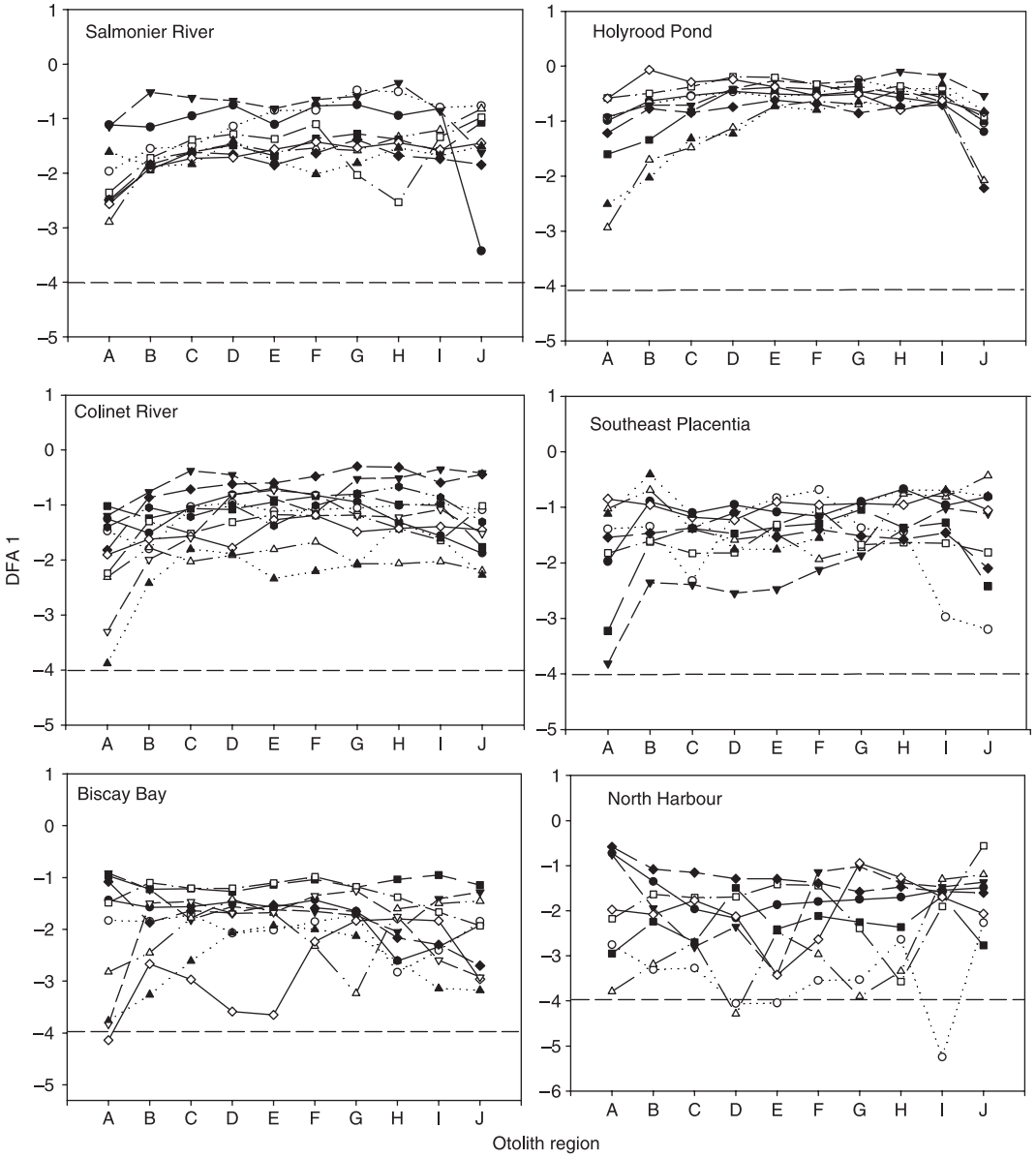

Fig. 6 Transects of the first axis of the discriminant function analysis across first year of life (A–J) in individual rainbow smelt otoliths from six locations throughout southeastern Newfoundland. Dashed horizontal lines represent standard deviation of seawater baseline. See Fig. 1 for sample locations. See Table 2 for baseline values. Each transect represents a single individual.

expected if some interbreeding were occurring between straying fish and residents, yet this was not observed and we observed no significant difference between adult and juvenile *Q*-value distributions.

Genetic assignment of individual multilocus genotypes and mixture analysis both suggested limited dispersal over large scales and population admixture of juveniles at small scales. Sites separated by > 25 km displayed higher self-assignment of adults and assignment of juveniles than nearby sites at the head of St Mary's Bay. On a small-scale (< 25 km), assignment tests and mixture analysis identified mixtures of offspring from various nearby spawning groups (head of St Mary's Bay). Assignment of juveniles from all three nurseries in the head of St Mary's Bay identified Salmonier as the dominant source of offspring, suggesting that Salmonier may act as a source population, supplying recruits to adjacent sites. This suggestion is challenged by the observation that otolith composition was not consistent with movement between estuaries, suggesting that that offspring which do disperse between nearby estuaries may have very low survival rates. Alternatively, the juveniles and adults examined in this study may have

experienced differing levels of straying during their early life histories, and temporal variation in dispersal and episodic dispersive events (e.g. Knutsen *et al*. 2004) cannot be ruled out as an explanation for the discordant estimates of realized dispersal in juveniles and adults.

The power of assignment and genetic mixture analysis to distinguish among populations that experience moderate to high gene flow may be inherently low and further constrained by low numbers of loci. Previous authors have suggested that assignment success declines below $F_{ST} \sim 0.05$ (Cornuet *et al*. 1999; Berry *et al*. 2004). Hauser *et al*. (2006) extended this lower limit suggesting accurate assignment under circumstances of moderate gene flow ($F_{ST} \sim 0.015$) using eight loci. In marine species with low differentiation ($F_{ST} < 0.01$), the utility of assignment tests remains uncertain. This inability to validate assignments in marine species results from the logistical constraints of tagging pelagic egg and larval stages, and typically large N_e values (e.g. Jones *et al*. 2005; Hauser *et al*. 2006).

One alternative to assess assignment power is to utilize a simulation-based approach that generates simulated mixtures of baseline data and explores accurate assignment. Ruzzante *et al*. (2006) used this approach to explore likely assignment success of Atlantic herring and successfully identified structure at relatively high levels of gene flow. Using a similar simulation-based approach, we found that the expected accuracy of assignment among the populations at the head of St Mary's Bay (see Table 2) is approximately 70%. The observation that generally less than 30–40% of the juveniles at the head of St Mary's Bay assign to the nearest estuary supports the hypothesis that mixtures are present and not merely an artefact of assignment at low differentiation. Admittedly, the accuracy of juvenile assignments may be diminished if genetic drift has occurred between baseline and mixture collections (Hauser *et al*. 2006); however, since all of the samples were collected within the span of a single 3-year generation, the effects of drift are likely to be very small.

The application of otolith trace element chemistry to track movements associated with diadromy between fresh- and seawater is now widespread (Campana & Thorrold 2001; Gillanders 2005). Although few studies have documented movements between estuaries and the sea (but see Milton *et al*. 1997; Thorrold *et al*. 1997), discrimination between estuarine and coastal habitats with elemental composition has been successful (e.g. Gillanders & Kingsford 1996; Forester & Swearer 2002). We were able to discriminate clearly between the three salinity regimes on the basis of Sr:Ca and Ba:Ca ratios, reflecting patterns observed in ambient water samples. The hypothesis of limited marine excursions and straying is supported by otolith chemistry, indicating primarily estuarine residency throughout the first year of life. In fact, in only 0.5% of assays was there any evidence of a marine signature. The partially landlocked site, Holyrood Pond, was characterized by relatively homogenous spatial structure with little variation in salinity. The observed low variation in otolith composition observed among and within Holyrood Pond individuals supports the predictive link between hydrography and otolith chemistry.

Several studies have used otolith elemental composition to provide insight into early life-history dispersal in marine fish (Jones *et al*. 1999; Hamer *et al*. 2005; Sandin *et al*. 2005). The ability to resolve movements between distinct habitats is dependent on the size of the assay spot relative to the temporal scale of movement (i.e. days, weeks, months). The spot size used in this study corresponds to approximately 30 days of growth based on measurement and counts of daily annuli (authors' unpublished data), and periods of habitat residency less than the 30-day threshold may be misclassified. The minimum marine residency time required to achieve a marine assignment is 9–12 days based on simple mixing equations (Stewart *et al*. in press). The degree of straying between nearby estuaries that could go undetected will be dependent on swimming ability and the distances involved. Based on maximum swim speeds for larval smelt (Bradbury *et al*. 2006b), the largest movement possible in 12 days is ~50 km. Given that sustained swim speeds are usually significantly less than maximum swim speeds, it is unlikely that larval horizontal swimming would contribute much to mixing on temporal scales below detection. Post-flexion juvenile swimming abilities likely exceed larval abilities, and movements between estuaries over short time periods could potentially connect estuaries at temporal scales below the detection threshold, particularly if water currents speed dispersal. However, if such movements were frequent, then the number of marine assignments would be expected to be higher than 0.5%, unless dispersers experience poor survival to the adult stage.

The genetic evidence of relatively high dispersal of juveniles at small scales in the head of St Mary's Bay appears unequivocal. Although the level of differentiation among these populations was below the threshold recommended for accurate genetic assignment, the simulations indicated that reasonably accurate genetic assignment should be possible, and this was empirically demonstrated with the higher rates of self-assignment observed for adults. Assignments of the other three locations (Biscay Bay, Southeast Placentia; Holyrood Pond) indicated similar levels of separation among adult and juvenile samples, suggesting that the influence of genetic drift on assignment accuracy was minimal. Finally, the predominance in the other estuaries at the head of St Mary's Bay of juveniles inferred to have dispersed from Salmonier River is consistent with an order of magnitude of higher spawning adult abundance in Salmonier (authors' unpublished data) relative to the other estuaries and the general circulation pattern in St

Mary's Bay, which moves from east to west in a counterclockwise gyre.

The genetic evidence that juveniles mix between nearby estuaries at a much greater rate than is evident in adult samples is consistent with two nonmutually exclusive possibilities: (i) homing of juveniles to natal spawning sites or (ii) selection against immigrant individuals prior to reproduction. Evidence from the otolith composition data that spawning adults have not left the natal estuary appears more consistent with the latter possibility that larvae or juveniles that disperse from the natal estuary are subject to high mortality. This inference is subject to two caveats. First, it is possible that during times of increased runoff, nearby estuaries may be connected allowing movements between areas without producing detectable marine signatures in otoliths. Some evidence exists that the freshwater surface layer in Salmonier estuary extends beyond Salmonier Arm in autumn, yet it remains unclear to what degree these estuaries become connected, or for how long. Extensive freshwater plumes that substantially diminish the gaps between estuaries are unlikely through the summer months and early autumn when river outflow is low and dispersal potential highest. Resolution of this issue will require further hydrographical surveys in the head of St Mary's Bay for examination. Second, given the numbers examined for otolith chemistry (approximately 10 samples from each estuary), our ability to resolve low rates of marine excursions is limited and may require increased sample sizes. Yet, the levels of mixing observed in juvenile genetic assignment are consistent with frequent marine movements and should have been easily detected with the sample sizes examined.

Whether or not some dispersing juveniles successfully return to their natal estuary, selection against migrants may also contribute to the weak structure observed (i.e. Gilg & Hilbish 2003). In fact, recent work has suggested that selection against migrants may indeed be one of the more prevalent forms of reproductive isolation in nature (Hendry 2004; Nosil *et al*. 2005). Previous work examining morphological divergence and gene flow within these populations observed high morphological divergence and no association between genetic and morphological divergence (Bradbury *et al*. 2006a) supporting the hypothesis of evolutionary independence of these spawning locations. Furthermore, a tagging study (authors' unpublished data) has documented annual straying of adults between spawning locations (up to 10%) at scales of 100–150 km where genetic differentiation is moderate to high and not consistent with successful straying.

Summary

The scale of effective early life-history dispersal in marine species remains one of the largest knowledge gaps in the understanding of marine dynamics. Using both microsatellite and microchemistry data, we observed significant self-recruitment in estuarine rainbow smelt populations despite an extensive larval period. The observation of small-scale mixing during the juvenile stage from genetic data and no evidence of a marine signature in successful adult otoliths supported a hypothesis of local selection against immigrants. Yet, the influence of low differentiation and estuarine structure on elemental and molecular assignment methods requires further evaluation. If selection reduces the survivorship of dispersers, estimates of dispersal based on nongenetic approaches in marine species may significantly overestimate gene flow.

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Supplementary material

The following supplementary material is available for this article:

Fig. S1 Length frequency plots for each of the nursery estuaries sampled throughout southeastern Newfoundland autumn 2004.

Fig. S2 Ambient water ratios (a, b) and otolith ratios (c, d) for Sr:Ca (a, c) and Ba:Ca (b, d) from baseline samples for marine, estuarine, and three freshwater ponds.

Fig. S3 Histograms of otolith first discriminant function axis for three salinity baselines: marine, estuarine, and freshwater.

Fig. S4 Transects of Sr:Ca across first year of life in rainbow smelt, *Osmerus mordax*, otoliths.

Fig. S5 Transects of Ba:Ca across first year of life in rainbow smelt otoliths from six locations in southeastern Newfoundland.

Table S1 Genetic descriptive statistics of rainbow smelt spawning adults and juveniles from six spawning locations and estuarine locations throughout southeastern Newfoundland using

Table S2 AMOVA results comparing genetic variation between nearby locations sampled at the head of St Mary's Bay with other locations

Table S3 Genetic mixture analysis of rainbow smelt spawning adults (i.e. self assignment) from six spawning locations throughout southeastern Newfoundland using GMA 1.0 (Kalinowski 2003)

Table S4 Genetic mixture analysis of rainbow smelt juvenile estuary samples using adult spawning baselines from six spawning locations throughout southeastern Newfoundland using GMA 1.0 (Kalinowski 2003)

Table S5 Assignment of simulated mixtures of baseline individuals. Simulations and genetic mixture analysis using GMA (Kalinowski 2003)

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