Low genetic connectivity in an estuarine fish with pelagic larvae

I.R. Bradbury, S.E. Campana, and P. Bentzen

Abstract: We evaluated the spatial scale of metapopulation structure and genetic connectivity in rainbow smelt, *Osmerus mordax*, using eight microsatellite loci at 22 spawning locations throughout Newfoundland and Labrador. Consistent with low gene flow and limited dispersal, significant genetic structuring ($F_{ST} \approx 0.11$) was present at small spatial scales (<200 km). Moreover, strong isolation by distance (IBD, $P < 0.001$, $r^2 = 0.47$) was observed, which was linear at small scales and nonlinear at large distances (>200 km). We hypothesized that despite high dispersal potential associated with a pelagic larval stage, behaviours restricting gene flow may result in structuring at the estuary scale. Multidimensional scaling and neighbour-joining of multilocus genotypes indicate some bay-scale associations. However, a comparison of F_{ST} values and IBD residuals at both estuary and bay scales indicated low structure within and elevated structure among estuaries. Estuarine structuring was further supported by the presence of significant small-scale IBD within several coastal embayments (50–100 km), as well as Bayesian clustering consistent with estuarine-scale independence. Finally, estimates of dispersal based on the IBD relationship are consistent with local estuarine recruitment (<1.5 km·generation^{-1}). We conclude that the unexpectedly high genetic structure observed is consistent with behavioral influences reducing dispersal, supporting previous work implicating active larval retention.

Résumé : Nous avons déterminé l'échelle spatiale de la structure de la métapopulation et de la connectivité génétique chez l'éperlan arc-en-ciel, *Osmerus mordax*, à l'étude de huit locus microsatellites à 22 sites de fraie répartis sur l'ensemble de Terre-Neuve-et-Labrador. En accord avec le faible flux génique et la dispersion limitée, il y a une structuration génétique importante ($F_{ST} \approx 0{,}11$) à de petites échelles spatiales (<200 km). De plus, nous observons une fort isolement par distance (IBD, $P < 0{,}001$, $r^2 = 0{,}47$) qui est linéaire à de petites échelles et non linéaire sur de grandes distances (>200 km). Nous posons en hypothèse que, malgré le potentiel élevé de dispersion associée au stade larvaire pélagique, des comportements qui restreignent le flux génique entraînent une structuration à l'échelle de l'estuaire. Un cadrage multidimensionnel et une analyse du plus proche voisin des génotypes à plusieurs locus indiquent l'existence de certaines associations à l'échelle de la baie. Cependant, une comparaison des valeurs de F_{ST} et des résidus de l'IBD, tant à l'échelle de l'estuaire que de la baie, révèle une structure faible à l'intérieur des estuaires et une structure plus importante entre les estuaires. La structuration au niveau des estuaires est, de plus, établie par la présence d'une IDB significative à petite échelle à l'intérieur de plusieurs baies côtières (50–100 km), de même que par un regroupement bayésien compatible avec une indépendance à l'échelle de l'estuaire. Finalement, les estimations de la dispersion basées sur les relations des IBD sont compatibles avec un recrutement estuarien local (<1,5 km·génération^{-1}). Nous concluons que la structure génétique plus importante que prévue que nous observons est compatible avec l'existence d'influences comportementales qui réduisent la dispersion, ce qui est en accord avec des recherches antérieures qui concluent à une rétention active des larves.

[Traduit par la Rédaction]

Introduction

Connectivity among demes in fragmented habitats is a key factor promoting the stability and persistence of metapopulations (e.g., Hanski 2001; Johst et al. 2002). Highly connected metapopulations may be guarded against extinction through the rescue effect (Brown and Kodrick-Brown 1977) or recolonization (Hanski 1999; Schtickzelle et al. 2006). The scale of connectivity is ultimately determined by habitat heterogeneity (i.e., structural connectivity) and dispersal patterns and mortality (i.e., functional connectivity). The degree of connectivity directly influences the scale of adaptive potential (Wright 1931), temporal persistence (Hanski 2001; Hastings and Botsford 2006), and ecosystem structure (e.g., Cowie and Holland 2006). Although measures of metapopulation dynamics and connectivity are com-

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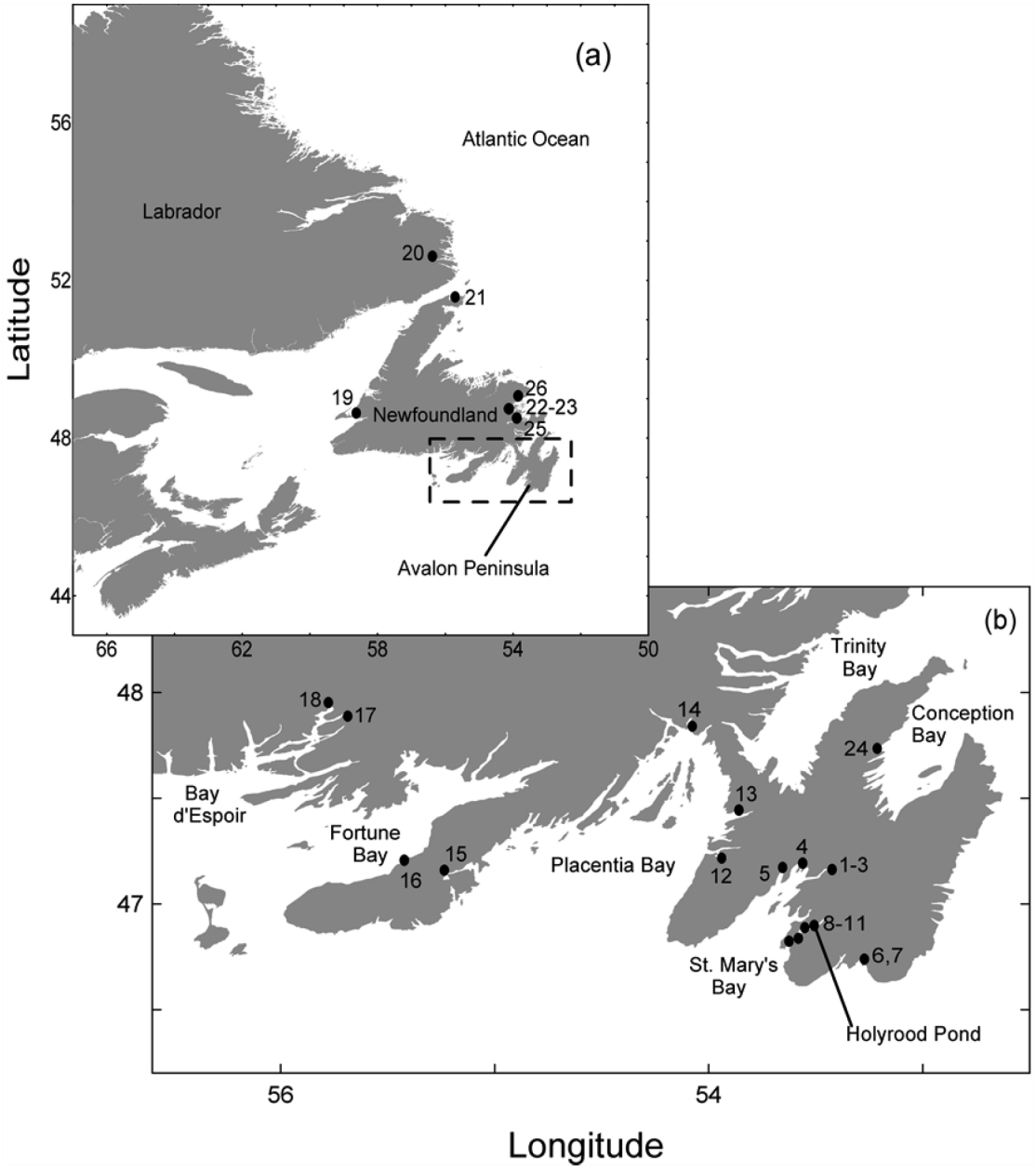
Fig. 1. (*a*) Map of study locations in Newfoundland and Labrador on the east coast of Canada. Inset (*a*) shows location of southeast coast of Newfoundland. Location numbers refer to locations in Table 1.



mon for terrestrial organisms such as insects (Hanski 1999), birds (Opdam 1991) or amphibians (Stevens et al. 2006), similar studies are comparatively rare for many marine environments (see Laurel and Bradbury 2006).

In marine species, the bipartite life history and pelagic larvae stage confer high dispersal potential (Palumbi 2003). Despite high dispersal potential and long-standing assumptions regarding the ubiquity of long-distance dispersal during the larval stage (e.g., Scheltema 1971), examples of marine species with strong genetic structure and low connectivity are increasingly common (e.g., Taylor and Hellberg 2003; Jones et al. 2005; Ruzzante et al. 2006). Alternate hypotheses regarding the prevalence of broad-scale gene flow are that resultant gene flow may be constrained or modified by behavioral contributions (e.g., Bradbury et al. 2003) or mortality (Cowen et al. 2000). Subsequently, predictions based on passive larval durations may substantially overestimate dispersal. Accordingly, high interspecific differences in the scale of marine connectivity and dispersal may be expected, despite the widespread presence of a pelagic larval stage (Hellberg et al. 2002).

Rainbow smelt (*Osmerus mordax*) is a small pelagic fish that is widely distributed in coastal and freshwater systems in 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 (Ouellet and Dodson 1985; Bradbury et al. 2004). Prior work suggests important behavioural contributions to early life-history pattern in the form of synchronized hatch (Bradbury et al. 2004) and estuarine larval retention through diel vertical migration (Laprise and Dodson 1989; Bradbury et al. 2006*a*). The close association of smelt with estuarine

Table 1. Locations, details, and summary genetic statistics for rainbow smelt (*Osmerus mordax*) samples from coastal Newfoundland and Labrador 2002–2006.

Population (year)	Location	Sample size	H_e	H_o	Mean no. of alleles
1. Salmonier River (2002)	St. Mary's Bay	80	0.76	0.76	11.00
2. Salmonier River (2003)	St. Mary's Bay	94	0.75	0.75	10.38
3. Salmonier River (2006)	St. Mary's Bay	93	0.74	0.78	10.63
4. Colinet River (2003)	St. Mary's Bay	94	0.74	0.76	11.63
5. North Harbour River (2004)	St. Mary's Bay	94	0.74	0.77	10.88
6. Biscay Bay River (2003)	St. Mary's Bay	94	0.72	0.71	9.88
7. Biscay Bay River (2006)	St. Mary's Bay	93	0.70	0.67	9.00
8. Holyrood Pond Brook (2005)	Holyrood Pond (SMB)	94	0.77	0.68	10.63
9. Holyrood Pond Park (2004)	Holyrood Pond (SMB)	94	0.75	0.74	10.13
10. Deer Pond Brook (2004)	Holyrood Pond (SMB)	41	0.72	0.73	8.88
11. Pathend Brook (2005)	Holyrood Pond (SMB)	94	0.75	0.74	10.25
12. Southeast Placentia (2005)	Placentia Bay	94	0.72	0.71	10.00
13. Long Harbour Brook (2004)	Placentia Bay	79	0.71	0.73	7.75
14. North Harbour River (2003)	Placentia Bay	80	0.70	0.66	8.75
15. Salt Pond Brook (2003)	Placentia Bay	92	0.78	0.73	10.25
16. Garnish River (2004)	Fortune Bay	74	0.81	0.77	11.38
17. Conne River (2004)	Bay d'Espoir	94	0.75	0.72	11.13
18. Little River (2004)	Bay d'Espoir	94	0.73	0.75	10.13
19. Smelt Brook, Point Amal (2004)	Port aux Port Penninsula	80	0.84	0.83	14.25
20. Mary's Harbour (2004)	Labrador	55	0.69	0.68	8.63
21. Pistolet Bay	Northern Peninsula	76	0.80	0.73	9.63
22. Gambo River (2003)	Bonavista Bay	80	0.77	0.66	11.38
23. Gambo River (2005)	Bonavista Bay	80	0.78	0.64	11.00
24. Salmon Cove River (2005)	Conception Bay	94	0.70	0.69	11.50
25. Chuff Brook (2006)	Bonavista Bay	93	0.71	0.64	11.13
26. Traverse Pond (2006)	Bonavista Bay	93	0.72	0.67	11.00

Note: For Holyrood Pond samples, SMB indicates St. Mary's Bay. H_e, expected heterozygosity; H_o, observed heterozygosity.

habitat and the potential for active (i.e., vertical migration) estuarine retention of larvae has lead several studies to conclude that population structure is likely linked to individual estuaries or retention areas (Bernatchez and Martin 1996). Nonetheless, studies to date have primarily used morphological (Frechet et al. 1983*a*), allozyme (Copeman 1977), or mtDNA (Taylor and Bentzen 1993) variation to examine smelt population structure. Moreover, population structure of smelt in mainland Canada is likely influenced by heavy exploitation or habitat degradation (e.g., McKenzie 1964). In contrast, the currently very limited commercial or recreational harvest within Newfoundland and Labrador offers a unique opportunity to study the species where anthropogenic influences have been minimal. Examination of genetic differentiation within the Pacific osmerid, eulachon (*Thaleichthys pacificus*), which possesses a similar life history, suggests divergence may be weak over large spatial scales (McLean and Taylor 2001; Beacham et al. 2005), contrary to hypotheses of estuarine isolation.

We utilize spatial variation at eight microsatellite loci to examine smelt population structuring and the degree of functional connectivity among spawning runs. We hypothesize that, in contrast with expectations for a species with an extensive pelagic larval period (~30 days at preflexion or the upward flexion of the notochord), populations are structured at the scale of individual estuaries in accordance with member–vagrant theory (e.g., Sinclair 1988; Bernatchez 1997). The overall goal here is to explore the potential consequences of behavioural retention in a species with an extensive pelagic larval period and mobile adults (Bradbury et al. 2006*a*) for the structuring of smelt metapopulations and the long-term evolutionary dynamics of the species.

Materials and methods

Study area

In total, 22 locations around Newfoundland and Labrador were sampled from 2003 to 2006 (Fig. 1, Table 1). Oceanographic and circulation conditions vary dramatically across the study; although the Labrador Current dominates coastal Newfoundland transport (Bradbury et al. 2000), there is considerable variation associated with different bays. The Cabot Strait, which is approximately 110 km across and 500 m deep, separates Newfoundland from the mainland to the south, and the Strait of Belle Isle (average width 18 km) separates Newfoundland from Labrador. Newfoundland's coastline is characterized by numerous large (>100 km in at least one axis) embayments (Fig. 1) for which limited data suggest potential retention times for pelagic eggs or larvae on the order of 20–40 days (e.g., deYoung and Sanderson 1995; Bradbury et al. 2000).

Genetic analysis and differentiation statistics

Fish were collected with dip nets or fyke nets during the spawning period. Pectoral or caudal fin clips were taken and immediately placed in 95% ethanol. DNA was extracted fol-

Table 2. Pairwise F_{ST} above the diagonal and p value below the diagonal for 26 samples of rainbow smelt (*Osmerus mordax*) from 22

	1	2	3	4	5	6	7	8	9	10	11	12
1	—	0.005	0.008	0.006	0.003	0.053	0.068	0.052	0.050	0.058	0.057	0.066
2	0.99	—	0.007	0.007	0.003	0.049	0.064	0.052	0.043	0.048	0.047	0.055
3	0.99	*	—	0.000	0.002	0.053	0.069	0.068	0.069	0.074	0.072	0.084
4	0.86	*	0.66	—	0.004	0.058	0.077	0.077	0.073	0.079	0.080	0.086
5	0.99	0.53	0.40	0.01	—	0.054	0.069	0.063	0.062	0.066	0.065	0.077
6	*	*	*	*	*	—	0.005	0.090	0.086	0.097	0.083	0.082
7	*	*	*	*	*	0.836	—	0.105	0.099	0.112	0.096	0.099
8	*	*	*	*	*	*	*	—	0.010	0.013	0.014	0.059
9	*	*	*	*	*	*	*	0.966	—	0.001	0.000	0.027
10	*	*	*	*	*	*	*	*	0.998	—	0.004	0.034
11	*	*	*	*	*	*	*	*	0.998	0.112	—	0.027
12	*	*	*	*	*	*	*	*	*	*	*	—
13	*	*	*	*	*	*	*	*	*	*	*	*
14	*	*	*	*	*	*	*	*	*	*	*	*
15	*	*	*	*	*	*	*	*	*	*	*	*
16	*	*	*	*	*	*	*	*	*	*	*	*
17	*	*	*	*	*	*	*	*	*	*	*	*
18	*	*	*	*	*	*	*	*	*	*	*	*
19	*	*	*	*	*	*	*	*	*	*	*	*
20	*	*	*	*	*	*	*	*	*	*	*	*
21	*	*	*	*	*	*	*	*	*	*	*	*
22	*	*	*	*	*	*	*	*	*	*	*	*
23	*	*	*	*	*	*	*	*	*	*	*	*
24	*	*	*	*	*	*	*	*	*	*	*	*
25	*	*	*	*	*	*	*	*	*	*	*	*
26	*	*	*	*	*	*	*	*	*	*	*	*

Note: An asterisk (*) indicates comparisons were significant at $p < 0.001$.

lowing 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). Individuals were genotyped using polymerase chain reaction (PCR) conditions of 5 or 10 μL volumes containing 20–100 ng DNA, 1.5 mmol·L^{-1} $MgCl_2$, 80 μmol·L^{-1} each dNTP, 0.5 U (1 U ≈ 16.67 nkat) *Taq* DNA polymerase (New England Biolabs), 0.3 μmol·L^{-1} for each primer (forward primers were end-labelled with HEX, or ROX dye), and 1× PCR buffer (10 mmol·L^{-1} Tris–HCl, pH 8.3; 50 mmol·L^{-1} 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 4–5 cycles of 94 °C for 30 s, program-specific touchdown annealing temperatures (T_a) minus 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). Eight microsatellite loci were used as follows: Omo1, Omo2, Omo3, Omo4, Omo5, Omo9, Omo15, and Omo16. See Coulson et al. (2006) for further details.

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). Relationships among locations were visualized using two approaches. First, pairwise F_{ST} values were visualized in two-dimensional space using multidimensional scaling (MDS) conducted in Primer 5.2.2 (Clarke and Gorley 2001). Second, a neighbour-joining tree based on Cavalli Sforza and Edward chord distances and bootstrapped across loci (1000 replicates) was generated using Populations (Langella 1999) and visualized using TreeView version 1.6.6 (Page 1996).

Spatial analysis

An analysis of molecular variance (AMOVA) partitioned observed genetic variance into components associated with geographic regions (i.e., bays) and was conducted using ARLEQUIN. The presence of isolation by distance (IBD) was assessed using least squares regression of linearized F_{ST} against pairwise geographic distance. To assess the scale of linearity, regressions were done at increasing spatial scales and slope plotted against the maximum size of the spatial bin (e.g., Castric and Bernatchez 2003; Bradbury and Bentzen 2007). Geographic distance between each site was measured as the shortest distance following within 5 km of the coastline using charts and Google Earth 2004. All distances were measured three times and averaged.

Spatial autocorrelation measures were calculated independently using PASSAGE (Rosenberg 2001). Alleles present in less than 5% of samples were excluded because rare events may substantially bias autocorrelation metrics (Legendre and Legendre 1998). Two autocorrelation metrics were used: Moran's I and Geary's c. Moran's I (Moran 1950) is similar to Pearson's correlation coefficient in that it is based on product moment formulation and usually varies

locations from coastal Newfoundland and Labrador (see Table 1 for sample locations and names).

13	14	15	16	17	18	19	20	21	22	23	24	25	26
0.145	0.071	0.104	0.090	0.144	0.134	0.055	0.098	0.072	0.120	0.112	0.138	0.171	0.146
0.141	0.061	0.116	0.092	0.149	0.142	0.064	0.103	0.066	0.118	0.112	0.136	0.170	0.142
0.162	0.083	0.120	0.098	0.147	0.148	0.072	0.110	0.081	0.134	0.127	0.147	0.179	0.161
0.159	0.085	0.12	0.100	0.151	0.149	0.074	0.113	0.083	0.139	0.131	0.149	0.185	0.168
0.156	0.081	0.120	0.099	0.152	0.148	0.069	0.111	0.078	0.130	0.123	0.142	0.176	0.156
0.145	0.075	0.112	0.103	0.133	0.122	0.080	0.108	0.095	0.128	0.120	0.134	0.186	0.163
0.163	0.090	0.113	0.108	0.142	0.132	0.097	0.115	0.109	0.143	0.136	0.145	0.201	0.181
0.128	0.080	0.099	0.085	0.134	0.122	0.046	0.114	0.062	0.079	0.068	0.122	0.140	0.088
0.122	0.048	0.092	0.080	0.134	0.126	0.049	0.118	0.046	0.084	0.077	0.121	0.151	0.099
0.146	0.059	0.106	0.084	0.148	0.136	0.061	0.125	0.057	0.096	0.087	0.138	0.165	0.108
0.128	0.046	0.103	0.087	0.141	0.139	0.050	0.126	0.046	0.084	0.078	0.124	0.150	0.099
0.137	0.023	0.124	0.095	0.145	0.140	0.070	0.143	0.056	0.112	0.105	0.138	0.172	0.128
—	0.139	0.126	0.115	0.131	0.152	0.085	0.179	0.115	0.127	0.124	0.092	0.187	0.159
*	—	0.117	0.104	0.150	0.146	0.081	0.156	0.069	0.138	0.130	0.142	0.2	0.157
*	*	—	0.070	0.095	0.107	0.083	0.137	0.075	0.118	0.120	0.135	0.179	0.161
*	*	*	—	0.073	0.087	0.076	0.090	0.057	0.101	0.096	0.116	0.150	0.140
*	*	*	*	—	0.032	0.108	0.137	0.103	0.118	0.118	0.109	0.171	0.156
*	*	*	*	*	—	0.113	0.122	0.122	0.117	0.109	0.112	0.181	0.153
*	*	*	*	*	*	—	0.122	0.046	0.047	0.041	0.088	0.102	0.071
*	*	*	*	*	*	*	—	0.139	0.127	0.129	0.167	0.214	0.178
*	*	*	*	*	*	*	*	—	0.074	0.068	0.107	0.127	0.101
*	*	*	*	*	*	*	*	*	—	0.001	0.076	0.078	0.049
*	*	*	*	*	*	*	*	*	0.99	—	0.078	0.062	0.040
*	*	*	*	*	*	*	*	*	*	*	—	0.144	0.113
*	*	*	*	*	*	*	*	*	*	*	*	—	0.051
*	*	*	*	*	*	*	*	*	*	*	*	*	—

between 1 (positive) and –1 (negative) autocorrelation values (Legendre and Legendre 1998). Geary's c is usually inversely related to Moran's I, though differences have been described (Legendre and Legendre 1998). Geary's c values of 0–1 indicate positive correlation, >1 indicates negative correlation, and $c = 1$ indicates no correlation.

Bayesian clustering

Bayesian clustering was done using STRUCTURE version 2.0 (Pritchard et al. 2000) and tested whether predictions of structuring at the scale of spawning run were consistent with multilocus genetic data. This approach uses assumptions of HWE and linkage equilibria among loci, introduces population structure, and uses a Markov chain Monte Carlo (MCMC) algorithm to estimate the number of populations (K). The algorithm was run three times for each K to ensure convergence of values and with a burn-in of 50 000 replications, 200 000 replications after burn-in, and K ranging from 1 to 22.

Dispersal distance estimation

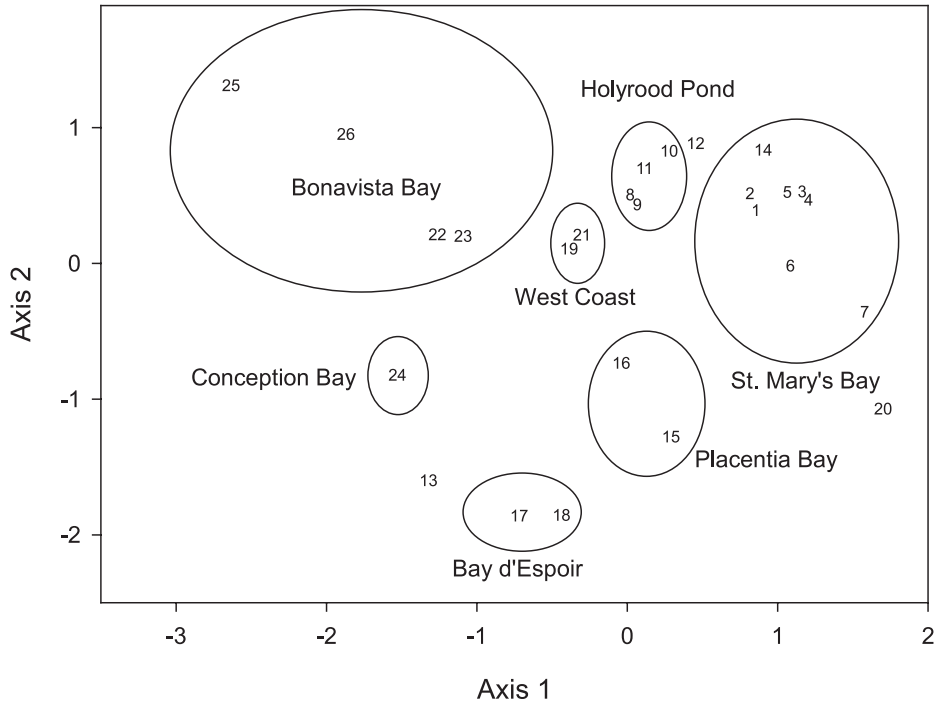
Dispersal distance was estimated using the approach outlined in Rousset (1997) and Bradbury and Bentzen (2007). The product of effective organism density and dispersal distance (d) may be estimated using the slope (b) of the regression of $F_{ST}/(1 - F_{ST})$ against geographic distance. Specifically, $1/b = 4D\sigma^2$, where D is the effective adult density (individuals·km^{-1}), and σ^2 is the variance of parent–offspring axial distance. Assuming symmetrical exponential dispersal, the IBD slope approximated by $4D\sigma^2$ was converted to d using $d = 1/\sqrt{(2/\sigma^2)}$ (Buonaccorsi et al. 2004). This estimate was compared with the relationship from Kinlan and Gaines (2003), based on the equation $d = 0.0016(\text{IBD slope})^{-1.0001}$. The IBD slope was taken as the maximum slope from the iterative regression analysis to account for nonequilibrium conditions. Previous work suggests census estimates of smelt density in these habitats are approximately 5040 individuals·km^{-1} (I.R. Bradbury, unpublished data). However, in the absence of effective population estimates, and because effective population size and census size usually differ by two–three orders of magnitude in marine and estuarine fish (Hansen et al. 2002; Arden and Kapuscinski 2003), a value of 50.40 individuals·km^{-1} was also used and likely more representative of actual effective densities. In all cases, estimate variance was calculated as the standard deviation using the distribution and standard deviation of possible slope values and subsequently converted to dispersal distance using both approaches presented above.

Results

Genetic differentiation

Summary statistics for genetic samples are included in Table 1. An average of 86 (range = 41–94) fish were genotyped per location. The eight microsatellite loci revealed substantial genetic variation, with an average expected heterozygosity within populations of 0.74 (range = 0.64–0.83), and an average of 10.43 alleles within populations

Fig. 2. Multidimensional scaling of multilocus genetic data for 22 spawning locations of anadromous rainbow smelt (*Osmerus mordax*) from Newfoundland and Labrador. Refer to Table 1 and Fig. 1 for sample and site details.



(range = 8–14). No evidence of linkage was observed. Deviations from HWE were observed at six locations and were primarily associated with Omo3 and Omo16. Tests for the presence of null alleles and scoring errors using MICRO-CHECKER (van Oosterhout et al. 2004) suggest that the presence of null alleles is rare (<5%) and primarily associated with Omo3 and Omo16. Because removal of either locus had no major effect on observed trends, they were included in further analyses. The estimate of global F_{ST} was 0.11, which is indicative of significant structuring. Pairwise estimates of F_{ST} (Table 2) among locations ranged from 0.002 to 0.23, and the majority of values were significant (>95%) with the exception of temporal replicates or locations from within a single estuary (e.g., Holyrood Pond). In all three locations with temporally replicated samples, comparisons were not significant among years (Table 2).

Spatial analysis

MDS (Fig. 2) suggested the tendency for some spatial association (Kruskal stress = 0.15, r^2 = 0.87), although strict bay-scale associations were weak and limited to St. Mary's Bay. Neighbour-joining analysis of chord distances (Fig. 3) revealed that >50% bootstrap support was generally associated with nodes linking locations at small spatial scales, such as a Holyrood Pond group and a head of St. Mary's Bay group. Overall, there was limited evidence of bay-scale structure (i.e., clustering of all locations within an embayment). AMOVA results based on groups and outlier locations identified in MDS analysis suggest significant structuring at all levels (Table 3), and among-group variance explained 5.8% of the total variance ($p < 0.001$).

Significant isolation by distance was present at multiple spatial scales and was assessed using spatial autocorrelation and least squares regression. Autocorrelation metrics indicated significant positive correlations at small spatial scales. Moran's *I* ranged from 0.8 to 0.2 at small distances, with a global estimate of 0.6. Values became negative between 100 and 150 km (Fig. 4*a*). Similarly, Geary's *c* suggested significant positive correlations at small scales and reached a value of 1 at ~150 km (Fig. 4*b*), indicative of no association.

Fig. 3. Neighbour-joining dendrogram based on Cavalli Sforza and Edwards chord distances. See Table 1 for location key. Numbers on each node represent percentages of bootstrap support for each node.

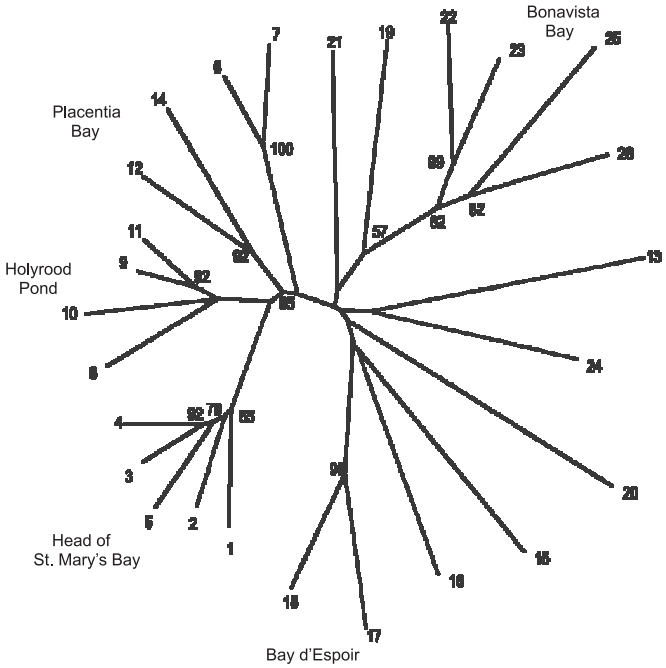
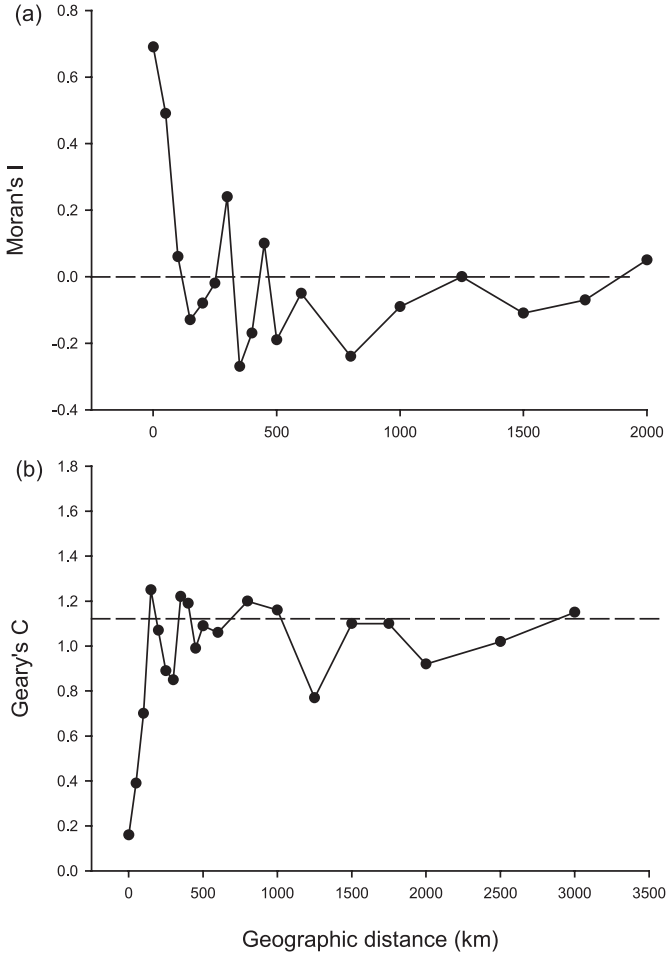


Table 3. Analysis of molecular variance (AMOVA) results comparing genetic variation among groups of rainbow smelt (*Osmerus mordax*) identified in multidimensional scaling analysis (Fig. 2) of populations sampled throughout Newfoundland and Labrador.

Source of variation	df	Sum of squares	Variance component	Percentage of total	F_{ST}	F_{SC}	F_{CT}
Among groups	9	701.366	0.151 82	5.75	0.1073***		
Among populations within groups	17	434.688	0.131 53	4.98		0.05283***	
Within populations	4460	10 518.41	2.358 39	89.27			0.05747***
Total	4485	11 654.47	2.641 74	100			

Note: Asterisks (***) indicate significance at $\alpha = 0.05$.

Fig. 4. Moran's *I* (*a*) and Geary's *c* (*b*) correlograms for 22 locations of anadromous rainbow smelt from coastal Newfoundland and Labrador based on eight microsatellite loci.



Overall, regression between genetic differentiation and geographic distance indicated a nonlinear association (r^2 = 0.35, Fig. 5*a*). Isolation-by-distance linearity, measured as the r^2 of the linear regression, increased with scale of analysis until approximately 200 km, and the IBD slope decreased with increasing spatial scale, as would be expected, with a nonlinear trend at larger distances (Fig. 5*b*). To evaluate the scale of isolation, IBD was examined individually within three of the embayments (Fig. 6). In two of the comparisons (St. Mary's and Bonavista bays) significant IBD was present within each bay, and in the third comparison (Placentia Bay), elevated differentiation was present overall, although there was no increase with distance (Fig. 6). To examine the role of estuarine and bay structure in regulating gene flow, genetic differentiation was compared at both the bay and estuary scale (Fig. 7). Comparison of F_{ST} values and residuals from the IBD plot suggested little structure among sites that share an estuary (i.e., low F_{ST} and negative IBD residuals) but large structural differences between sites within an embayment (i.e., high F_{ST} and higher residuals).

Bayesian clustering

STRUCTURE analysis indicated a high number of populations based on Bayesian clustering. The values of –log Pr(*X*/*K*), representing the estimated posterior probabilities of *K*, reached a plateau at 14 before becoming unstable (Fig. 8) and was consistent over multiple runs (n = 3, Fig. 8).

Dispersal distance estimation

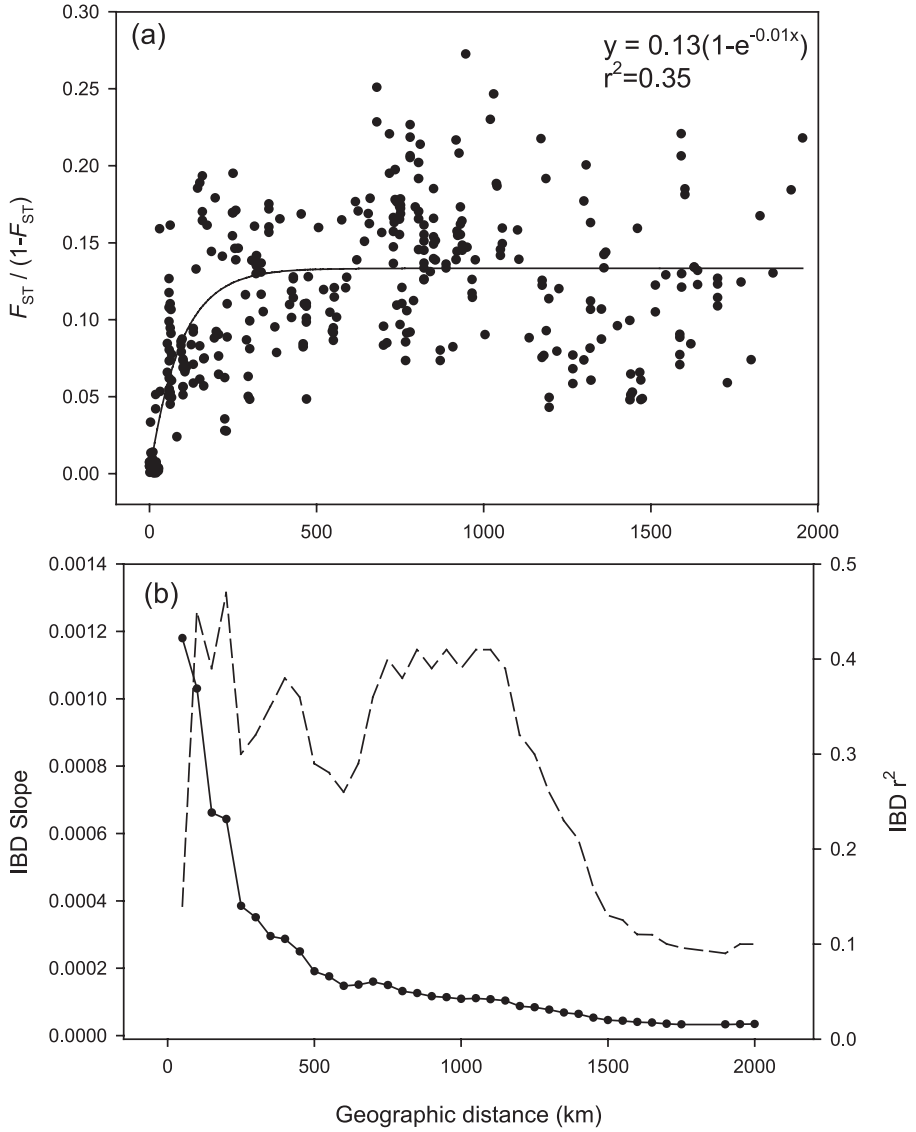
Using an effective density estimate of 5040 individuals·km^{-1}, the dispersal estimate was 0.15 km·generation^{-1}, which increased using a more realistic and lower effective density of 50.40 individuals·km^{-1}, to an estimate of 1.4 ± 2.94 km·generation^{-1}. Using the relationship from Kinlan and Gaines (2003), we obtained an estimate of 1.33 ± 5.37 km·generation^{-1}.

Discussion

The stability and resilience of marine species is likely linked to the connectivity of marine metapopulations (e.g., Hanski 1999; Johst et al. 2002; Kritzer and Sale 2006). Marine populations may be connected by the exchange of individuals either through pelagic larval transport or adult movement. Despite the ubiquity of a pelagic larval stage and inferred high dispersal potential, active retention or natal philopatry may have dramatic consequences for the connectivity and long-term evolutionary dynamics of marine ecosystems. We demonstrate significant small-scale genetic structuring and limited realized dispersal in rainbow smelt in coastal Newfoundland and Labrador, despite an extensive pelagic larval stage (i.e., approximately 30 days from hatch until flexion) and mobile adults. The results support the hypothesis that smelt populations are structured at the scale of the local estuary, which is consistent with the member–vagrant hypothesis and with a behavioural role in regulation of connectivity.

The linkage of smelt population structure to retention areas has been hypothesized by previous authors (e.g., Frechet et al. 1983*a*; Baby et al. 1991; Bernatchez and Martin 1996).

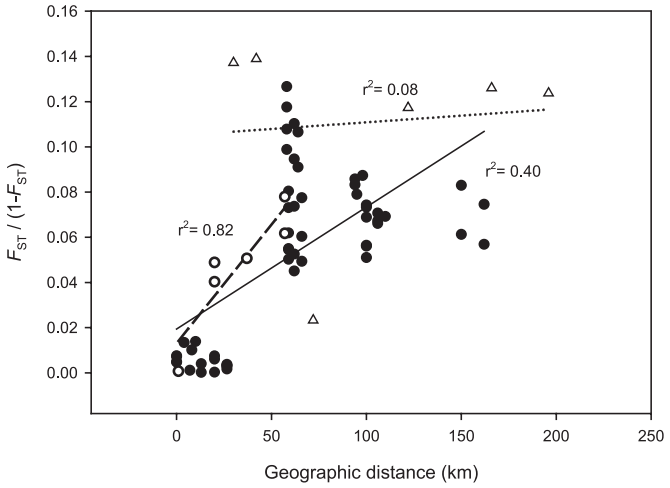
Fig. 5. (*a*) Isolation by distance (IBD) plot of linearized genetic divergence (F_{ST}) with geographic distance. Solid line represents nonlinear regression. (*b*) IBD slope (solid line) and r^2 (broken line) from iterative linear regression analysis at increasing bin sizes.



Spatial associations with morphology (Frechet et al. 1983*a*), parasites (Frechet et al. 1983*b*), and mtDNA (Bernatchez and Martin 1996) all support linkages to estuarine structure. Bradbury et al. (2006*b*) observed strong phenotypic differences among 10 locations along the south coast of Newfoundland and support the evolutionary independence of local estuaries. This present work supports these earlier findings and resolves the spatial scale of structuring and the delineation of predominant forces. In addition to the observed fine-scale genetic correlation, an absence of drift dispersal equilibrium was observed at large scales as indicated by the nonlinearity of the IBD relationship. This observation suggests a threshold of ~200 km, below which dispersal may influence spatial structure but beyond which genetic drift may dominate (Slatkin 1993).

The various clustering approaches suggest considerable fine-scale structuring, with only weak evidence of large-scale structure. Nodes suggested by clustering indicate all of the strongly supported branches are terminal and consistent with small-scale (e.g., estuarine) comparisons. The lack of support for the major branches supports the hypothesis of structuring at the scale of individual estuaries. The STRUCTURE analysis suggested the presence of ~14 significant clusters or populations. This number of clusters is inconsistent with structuring at the scale of spawning location. Given the presence of nonsignificant F_{ST} values among sample sites sharing estuaries (e.g., Holyrood Pond and St. Mary's Bay), such an estimate may support structuring at the estuary scale, though even combining nearby locations that may share an estuary results in 16 clusters and not the observed 14. Nonetheless, this hypothesis of estuarine structure is further supported by the observation of significant isolation by distance within local embayments at scales of <100 km in both St. Mary's Bay and Bonavista Bay. Within Placentia Bay, this pattern was not observed; nonetheless, differentiation within Placentia Bay was substantial (F_{ST} = 0.13). Similar trends were observed with the MDS analysis, where Placentia Bay lacked the genetic cohesion observed in some

Fig. 6. Isolation by distance (IBD) relationships for three bays included in study analyzed separately. Solid circles represent St. Mary's Bay, open circles represent Bonavista Bay, and triangles represent Placentia Bay. Lines (solid, St. Mary's Bay; dashed, Bonavista Bay; dotted, Placentia Bay) represent least squares regression for each comparison.



of the other bays. These results may be linked to the fact that Placentia Bay is the largest bay and the samples were spaced further apart, making it difficult to resolve fine-scale differences.

Our estimates of dispersal distance coincide with dispersal restricted to single estuaries. The use of IBD relationships in the estimation of marine dispersal is gaining widespread use, yet it is subject to several assumptions and limitations (for a review see Bradbury and Bentzen 2007). Our estimates of dispersal using the approaches of Kinlan and Gaines (2003) and Rousset (1997) appear similar, with both approaches suggesting dispersal estimates of <1.5 km·generation^{-1}. Such estimates of dispersal are in direct agreement with estimates of larval transport in these habitats (Bradbury et al. 2006*a*) and with estimates of adult straying (McKenzie 1964).

As such, it seems behavioral modifications to dispersal and metapopulation connectivity likely result from either active larval retention or natal philopatry. Vertical migration in areas of strong shear has been implicated as a mechanism for larval smelt retention (Ouellet and Dodson 1985; Laprise and Dodson 1989). Bradbury et al. (2006*a*) examined larval transport within Salmonier estuary and suggested ontogenetic shifts in behavior may interact with seasonal hydrography to restrict larval transport to a single estuary. In contrast, adult tagging studies to date have primarily addressed spawning site fidelity and not natal philopatry. McKenzie (1964) documented annual homing rates of 90% to three streams within the Miramichi River. Nonetheless, estimates of annual site fidelity may differ dramatically from spawning natal fidelity, particularly if larval export is substantial. The role that natal homing may play in regulating smelt population structure is unclear. The genetic similarity observed here among locations within a single retention area or estuary (e.g., Holyrood Pond) suggests that if homing occurs within estuaries, its contribution may be secondary to that of the retention area. This is supported by a related study utilizing otolith elemental composition, which indi-

Fig. 7. Average (+ variance) F_{ST} (*a*) and isolation by distance (IBD) residuals (*b*) for pairwise comparisons between sample locations sharing estuaries and bays. Data is based on linear portion of IBD (0–200 km).

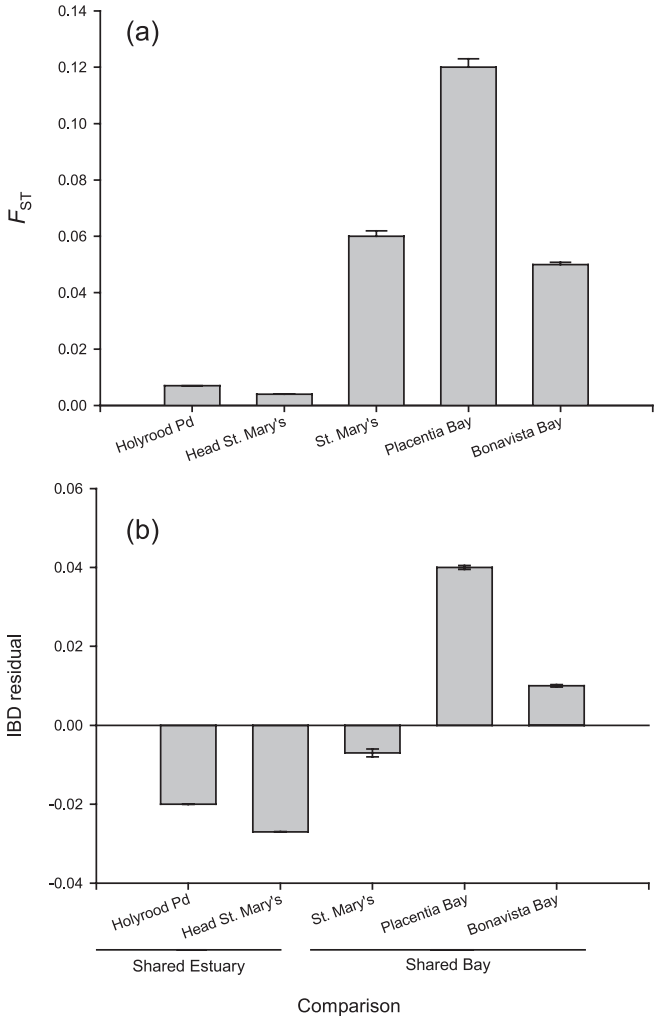
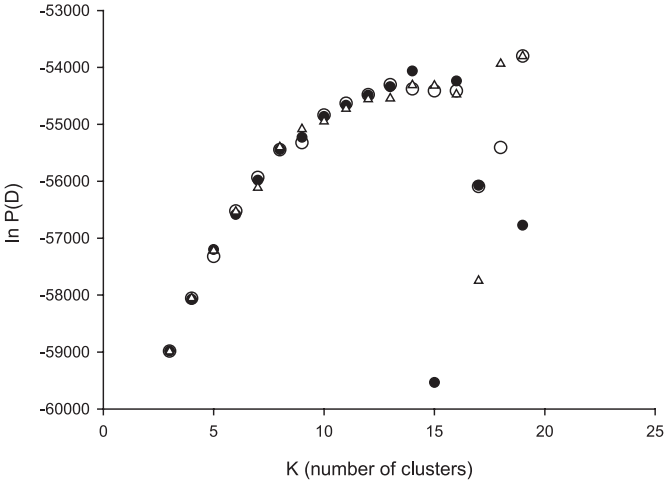


Fig. 8. Results from STRUCTURE analysis, ln $P(D)$ with various values of K (1–22) for three replicates with 50 000 iterations burn-in and 200 000 after burn-in.



cated that high rates of multiyear spawning site fidelity of smelt in these habitats results from strong habitat associations and not annual homing, as elemental composition suggests smelt rarely leave local estuaries (I.R. Bradbury, unpublished data).

Within other species of the family Osmeridae, the level of geographic differentiation typically seems low. McLean and Taylor (2001) and McLean et al. (1999) document spatial genetic structure over large distances (>2000 km) in a Pacific osmerid (*Thaleichthys pacificus*) with an anadromous life history and observed F_{ST} values of 0.045 and 0.023 for microsatellite loci and mitochondrial sequence variation, respectively. Similarly, Beacham et al. (2005) used microsatellite loci in the same species and observed weak structure as well ($F_{ST} < 0.01$). Low estimates of divergence were also observed in capelin (*Mallotus villosus*) in the North Atlantic (Røed et al. 2003) with comparisons between the east and west Atlantic showing $F_{ST} = 0.03$ and a lack of significant differentiation within each region. Interestingly, even for rainbow smelt, the level of gene flow observed within Newfoundland and Labrador waters may be anomalously low (Bradbury et al. 2006*b*), and smelt from the mainland display genetic structuring indicative of marine species and other osmerids. These comparisons with other osmerids and rainbow smelt from elsewhere support a hypothesis of unusually high divergence and low connectivity for Newfoundland smelt. As such, it seems regional variation in gene flow and connectivity within a species or life history may be large, making generalizations difficult.

Admittedly, estimates of differentiation here are on par with other anadromous fish species (e.g., Hendry et al. 2004; Bradbury and Bentzen 2007). Comparisons of Atlantic salmon (*Salmo salar*) along the coast of Newfoundland encompassing many of the same sites indicate levels of divergence equal to our observations here (Palstra and Ruzzante 2007). However, as smelt lack the typical salmonid life history and are characterized by short upstream migrations and small pelagic marine larvae that exit the river within hours of hatching, this level of differentiation is unexpected. Examples of elevated genetic isolation and low connectivity have been noted in several marine and estuarine invertebrate (e.g., Lessios 1998; Perrin et al. 2004) and fish species (Adams et al. 2006). Nonetheless, the degree of structure observed here remains unexpectedly high ($F_{ST} \approx 0.12$). Reports of differentiation in other estuarine fishes suggest moderate ($F_{ST} \sim 0.003$–0.02) levels of connectivity (Beheregaray and Sunnucks 2001; Adams et al. 2006); and in marine fishes, low ($F_{ST} < 0.01$) differentiation and relatively high connectivity appear the norm (Bentzen et al. 1996; Jorgensen et al. 2005). Levels of gene flow and differentiation observed within other osmerids (e.g., McLean and Taylor 2001; Beacham et al. 2005) are consistent with these examples of marine and estuarine species, again highlighting the elevation of differentiation among Newfoundland smelt samples. Whether the low connectivity of smelt populations in Newfoundland waters is a result of local adaptation in the form of larval behavior, adult homing abilities, or some other cause is unknown and ultimately requires further evaluation.

The interaction of genetic connectivity and spatial scale can allow inference of dominant evolutionary processes (Slatkin 1993). At dispersal drift equilibrium, a monotonic relationship between genetic differentiation and geographic distance should be present (Slatkin 1993). The scale at which equilibrium will be apparent will be dependent both on the dispersal kernel of the species and the time since colonization. The observation of significant isolation by distance over small to moderate geographic scales suggests that the 10 000 years since the last glacial retreat in eastern Canada has been sufficient for equilibrium to be established on scales of up to 200 km. Moreover, the observation of a maximum F_{ST} at scales larger than 200 km suggests nonequilibrium conditions at larger scales, likely as a result of the combined effects of limited dispersal and recent colonization. Similar trends in a declining IBD slope with distance have been observed elsewhere (e.g., Castric and Bernatchez 2003; Bradbury and Bentzen 2007).

The consequences of low connectivity for the stability and persistence of metapopulations can be profound (Hanski 1999). Classical metapopulation models suggest that persistence is dependent on a balance between dispersal–colonization rate and extinction rate (Hanski 1999). Connectivity directly determines the contribution of recolonization of extinct, vacant habitats and buffering of stochastic environmental influences in occupied habitats. Without the stabilizing influences of gene flow, local populations may be extremely vulnerable to disturbance and extinction pressure. In light of the limited dispersal that connects these smelt spawning locations and given the ephemeral nature of smelt spawning habitat, it appears that smelt populations in Newfoundland and Labrador may be more susceptible to disturbance than other marine or anadromous species. Furthermore, it seems that connectivity and metapopulation dynamics beyond the scale of local embayments may be quite limited in smelt in Newfoundland, as dispersal at these scales appears uncommon and the likely scenario is one of a series of disconnected metapopulations within local embayments.

In conclusion, these results show significant genetic structuring and low connectivity in rainbow smelt over large and small spatial scales. The observed high genetic isolation appears at odds with pelagic larval dispersal. These results contribute to a growing body of evidence suggesting that large interspecific variation exists in spatial complexity and genetic structure within marine species, despite the dispersal potential of the pelagic larval stage. Strong heterogeneity in population spatial structure among marine and estuarine species suggests substantial differences in the processes and mechanisms that maintain metapopulations. We suggest that studies such as this, which identify the critical scale of connectivity within a marine species, will be essential to the understanding and management of marine ecosystems.

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References

Adams, S.M., Lindmeier, J.B., and Duvernell, D.D. 2006. Microsatellite analysis of the phylogeography, Pleistocene history and secondary contact hypotheses for the killifish, *Fundulus heteroclitus*. Mol. Ecol. **15**(4): 1109–1123.

Arden, W.R., and Kapuscinski, A.R. 2003. Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. Mol. Ecol. **12**: 35–49.

Baby, M.C., Bernatchez, L., and Dodson, J.J. 1991. Genetic structure and relationships among anadromous and landlocked populations of rainbow smelt, *Osmerus mordax* Mitchill, as revealed by mtDNA restriction analysis. Can. J. Fish. Aquat. Sci. **39**(Suppl. A): 61–68.

Beacham, T.D., Hay, D.E., and Le, K.D. 2005. Population structure and stock identification of eulachon (*Thaleichthys pacificus*), and anadromous smelt in the Pacific Northwest. Mar. Biotechnol. **7**: 363–372.

Beheregaray, L.B., and Sunnucks, P. 2001. Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. Mol. Ecol. **10**: 2849–2866.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. 2004. Genetix 4.02, logiciel sous Windows pour la genetique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II, Montpellier, France.

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**(12): 2706–2721.

Bernatchez, L. 1997. Mitochondrial DNA analysis confirms the existence of two glacial races of rainbow smelt *Osmerus mordax* and their reproductive isolation in the St Lawrence River estuary (Québec, Canada). Mol. Ecol. **6**(1): 73–83.

Bernatchez, L., and Martin, S. 1996. Mitochondrial DNA diversity in andromous rainbow smelt, *Osmerus mordax* Mitchill: a genetic assessment of the member–vagrant hypothesis. Can. J. Fish. Aquat. Sci. **53**(2): 424–433.

Bradbury, I.R., and Bentzen, P. 2007. Non-linear genetic isolation by distance: implications for dispersal estimation in anadromous and marine populations. Mar. Ecol. Prog. Ser. **340**: 245–257.

Bradbury, I.R., Snelgrove, P.V.R., and Fraser, S. 2000. Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland. Can. J. Fish. Aquat. Sci. **57**(9): 1761–1772.

Bradbury, I.R., Snelgrove, P.V.R., and Pepin, P. 2003. Passive and active behavioural contributions to patchiness and spatial pattern during the early life history of marine fishes. Mar. Ecol. Prog. Ser. **257**: 233–245.

Bradbury, I.R., Campana, S.E., Bentzen, P., and Snelgrove, P.V.R. 2004. Synchronized hatch and its ecological significance in rainbow smelt *Osmerus mordax* in St. Mary's Bay, Newfoundland. Limnol. Oceanogr. **49**(6): 2310–2315.

Bradbury, I.R., Gardiner, K., Snelgrove, P.V.R., Campana, S.E., Bentzen, P., and Guan, L. 2006*a*. Larval transport, vertical distribution, and localized recruitment in anadromous rainbow smelt (*Osmerus mordax*). Can. J. Fish. Aquat. Sci. **63**(12): 2822–2836.

Bradbury, I.R., Coulson, M., Campana, S.E., and Bentzen, P. 2006*b*. Morphological and genetic differentiation in anadromous rainbow smelt: disentangling the effects of geography and morphology on gene flow. J. Fish. Biol. **69**: 95–114.

Brown, J.H., and Kodrick-Brown, A. 1977. Turnover rates in insular biogeography, effect of immigration on extinction. Ecology, **58**: 445–449.

Buonaccorsi, V.P., Westerman, M., Stannard, J., Kimbrell, C., Lynn, E., and Vetter, R. 2004. Molecular genetic structure suggests limited larval dispersal within grass rockfish *Sebastes rastrelliger*. Mar. Biol. **145**: 779–788.

Castric, V., and Bernatchez, L. 2003. The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics, **163**: 983–996.

Clarke, K.R., and Gorley, R.N. 2001. Primer version 5.0. User manual/tutorial. Primer-E Limited, Plymouth, UK.

Copeman, D.G. 1977. Population differences in rainbow smelt, *Osmerus mordax*: multivariate analysis of mensural and meristic data. J. Fish. Res. Board Can. **34**(8): 1220–1229.

Coulson, M.W., Paterson, I.G., Green, A., Kepkay, R., and Bentzen, P. 2006. Characterization of di- and tetranucleotide microsatellite markers in rainbow smelt (*Osmerus mordax*). Mol. Ecol. Notes, **6**(3): 942.

Cowen, R.K., Lwiza, K.M.M., Sponaugle, S., Paris, C.B., and Olson, D.B. 2000. Connectivity of marine populations: open or closed? Science (Washington, D.C.), **287**(5454): 857.

Cowie, R.H., and Holland, B.S. 2006. Dispersal is fundamental to biogeography and evolution of diversity on oceanic islands. J. Biogeogr. **33**: 193–198.

deYoung, B., and Sanderson, B. 1995. The circulation and hydrography of Conception Bay, Newfoundland. Atmos.–Ocean, **33**: 135–162.

Elphinstone, M.S., Hinten, G.N., Anderson, M.J., and Nock, C.J. 2003. An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. Mol. Ecol. **3**: 317–320.

Frechet, A., Dodson, J.J., and Powles, H. 1983*a*. Use of variation in biological characters for the classification of anadromous rainbow smelt (*Osmerus mordax*) groups. Can. J. Fish. Aquat. Sci. **40**(6): 718–727.

Frechet, A., Dodson, J.J., and Powles, H. 1983*b*. Parasites of the anadromous American smelt (*Osmerus mordax*) of Quebec and their usefulness as biological tags. Can. J. Zool. **61**: 621–626.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. J. Hered. **86**: 485.

Hansen, M.M., Ruzzante, D.E., Nielsen, E., Bekkevold, D., and Mensberg, K.L.D. 2002. Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. Mol. Ecol. **11**: 2523–2535.

Hanski, I. 1999. Metapopulation ecology. Oxford University Press, Oxford, UK.

Hanski, I. 2001. Spatially realistic theory of metapopulation ecology. Naturwissenschaften, **88**: 372–381.

Hastings, A., and Botsford, L.W. 2006. Persistence of spatial populations depends on returning home. Proc. Natl. Acad. Sci. U.S.A. **103**(15): 6067–6072.

Hellberg, M.E., Burton, R.S., Neigel, J.E., and Palumbi, S.R. 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. **70**: 254–271.

Hendry, A.P., Castric, V., Kinnison, M.T., and Quinn, T.P. 2004. The evolution of philpatry and dispersal. *In* Evolution illuminated: salmon and their relatives. *Edited by* A.P. Hendry and S.C. Stearns. Oxford University Press, Oxford, UK. pp. 52–91.

Johst, K., Brandl, R., and Eber, S. 2002. Metapopulation persistence in dynamic landscapes: the role of dispersal distance. Oikos, **98**: 263–270.

Jones, G.P., Planes, S., and Thorrold, S. 2005. Coral reef fish larvae settle close to home. Curr. Biol. **15**: 1314–1318.

Jorgensen, H.B.H., Hansen, M.M., Bekkevold, D., Ruzzante, D.E., and Loeschcke, V. 2005. Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. Mol. Ecol. **14**(10): 3219–3234.

Kinlan, B.P., and Gaines, S.D. 2003. Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology, **84**(8): 2007–2020.

Kritzer, J.P., and Sale, P.F. 2006. Marine metapopulations. Elsevier Press, Burlington, Mass.

Langella, O. 1999. Populations 1.2.30. Available from bioinformatics.org/~tryphon/populations [accessed January 2007; updated May 2002].

Laprise, R., and Dodson, J.J. 1989. Ontogeny and importance of tidal vertical migrations in the retention of larval smelt *Osmerus mordax* in a well-mixed estuary. Mar. Ecol. Prog. Ser. **55**(2–3): 101–111.

Laurel, B.J., and Bradbury, I.R. 2006. "Big" concerns with high latitude marine protected areas (MPA): trends in connectivity and MPA size. Can. J. Fish. Aquat. Sci. **63**: 2603–2607.

Legendre, P., and Legendre, L. 1998. Numerical ecology. Elsevier Science BV, Amsterdam, The Netherlands.

Lessios, H.A. 1998. The first stage of speciation as seen in organisms separated by the Isthmus of Panama. *In* Endless forms: species and speciation. *Edited by* D. Howard and S. Berlocher. Oxford University Press, Oxford, UK. pp. 186–201.

McKenzie, R.A. 1964. Smelt life history and fishery in the Miramichi River, New Brunswick. Fish. Res. Board Can. Bull. 144.

McLean, J.E., and Taylor, E.B. 2001. Resoltuion of population structure in a species with high gene flow: microsatellite variation in the eulachon (Osmeridae: *Thaleichthys pacificus*). Mar. Biol. **139**: 411–420.

McLean, J.E., Hay, D.E., and Taylor, E.B. 1999. Marine population structure in an anadromous fish: life history influences patterns of mitochondrial DNA variation in the eulachon, *Thaleichthys pacificus*. Mol. Ecol. **8**: S143–S158.

Moran, P.A.P. 1950. Notes on continuous stochastic phenomena. Biometrika, **37**: 17–23.

Nellbring, S. 1989. The ecology of smelts (genus *Osmerus*): a literature review. Nord. J. Freshw. Res. **65**: 116–145.

Opdam, P. 1991. Metapopulation theory and habitat fragmentation: a review of holarctic breeding bird studies. Landsc. Ecol. **5**(2): 93–106.

Ouellet, P., and Dodson, J.J. 1985. Dispersion and retention of anadromous rainbow smelt (*Osmerus mordax*) larvae in the middle estuary of the St. Lawrence River. Can. J. Fish. Aquat. Sci. **42**(2): 332–341.

Page, R.D.M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. **12**: 357–358.

Palstra, F.P., O'Connell, M.F., and Ruzzante, D.E. 2007. Population structure and gene flow reversals in Atlantic salmon (*Salmo salar*) over contemporary and long-term temporal scales: effects of population size and life history. Mol. Ecol. **16**(11): 4504–4522. doi: 10.1111/j.1365–294X.2007.03541.x.

Palumbi, S.R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. **13**(1): 146–158.

Perrin, C., Wing, S.R., and Roy, M.S. 2004. Effects of hydrographic barriers on population genetic structure of the sea star *Coscinasterias muricata* (Echinodermata, Asteroidea) in the New Zealand fiords. Mol. Ecol. **13**(8): 2183–2195.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**(2): 945–959.

Røed, K.H., Fevolden, S.E., Christiansen, J.S., and Midthjell, L. 2003. Isolation and characterization of DNA microsatellites for capelin *Mallotus villosus* population genetic studies. Mar. Ecol. Prog. Ser. **262**: 299–303.

Rosenberg, M.S. 2001. PASSAGE. Pattern Analysis, Spatial Statistics, and Geographic Exegesis. Department of Biology, Arizona State University, Temple, Ariz.

Rousset, F. 1997. Genetic differentiation and estimation of gene. flow from F-statistics under isolation by distance. Genetics, **145**: 1219–1228.

Ruzzante, D.E., Mariani, S., Bekkevold, D., André, C., Mosegaard, H., Clausen, L.A.W., Dahlgren, T., Hutchinson, W.F., Hatfield, E.M.C., Torstensen, E., Brigham, J., Simmonds, E.J., Laikre, L., Larsson, L.C., Stet, R.J.M., Ryman, N., and Carvalho, G.R. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proc. R. Soc. Lond. B Biol. Sci. **273**(1593): 1459–1464.

Scheltema, R.S. 1971. The dispersal of the larvae of shoalwater benthic invertebrate species over long distances by ocean currents. *In* Proceedings of the 4th European Marine Biology Symposium, Bangor, North Wales, UK, 14–20 September 1969. *Edited by* D. Crisp. Cambridge University Press, London, UK. pp. 7–28.

Schneider, S., Roessli, D., and Excoffier, L. 2000. ARLEQUIN, Version 2.0: a software for population genetic analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schtickzelle, N., Mennechez, G., and Baguette, M. 2006. Dispersal depression with habitat fragmentation in the bog fritillary butterfly. Ecology, **87**(4): 1057–1065.

Sinclair, M. 1988. Marine populations. An essay on population regulation and speciation. Washington Sea Grant, Seattle, Wash.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution, **47**: 264–279.

Stevens, V.M., Verkenne, C., Vandewoestijne, S., Wesselingh, R.A., and Baguette, M. 2006. Gene flow and functional connectivity in the natterjack toad. Mol. Ecol. **15**(9): 2333–2344.

Taylor, E., and Bentzen, P. 1993. Molecular genetic evidence for reproductive isolation between sympatric populations of smelt *Osmerus mordax* in Lake Utopia, south-western New Brunswick, Canada. Mol. Ecol. **2**: 345–357.

Taylor, M.S., and Hellberg, M.E. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science (Washington, D.C.), **5603**: 109.

van Oosterhout, C., Hutchison, W.F., and Wills, D.M.P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, **4**: 535.

Wright, S. 1931. Evolution in Mendelian populations. Genetics, **16**: 97–159.