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# Structural and functional connectivity of marine fishes within a semi-enclosed Newfoundland fjord

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The interplay between structural connectivity (*i.e.* habitat continuity) and functional connectivity (*i.e.* dispersal probability) in marine fishes was examined in a coastal fjord (Holyrood Pond, Newfoundland, Canada) that is completely isolated from the North Atlantic Ocean for most of the year. Genetic differentiation was described in three species (rainbow smelt *Osmerus mordax*, white hake *Urophycis tenuis* and Atlantic cod *Gadus morhua*) with contrasting life histories using seven to 10 microsatellite loci and a protein-coding locus, *Pan*I (*G. morhua*). Analysis of microsatellite differentiation indicated clear genetic differences between the fjord and coastal regions; however, the magnitude of difference was no more elevated than adjacent bays and was not enhanced by the fjord's isolation. *Osmerus mordax* was characterized by the highest structure overall with moderate differentiation between the fjord and St Mary's Bay ($F_{ST}$ *c*. 0·047). In contrast, *U. tenuis* and *G. morhua* displayed weak differentiation ($F_{ST} < 0{\cdot}01$). Nonetheless, these populations did demonstrate high rates (<75%) of Bayesian self-assignment. Furthermore, elevated differentiation was observed at the *Pan*I locus in *G. morhua* between the fjord and other coastal locations. Interestingly, locus-specific genetic differentiation and expected heterozygosity were negatively associated in *O. mordax*, in contrast to the positive associations observed in *U. tenuis* and *G. morhua*. Gene flow in these species is apparently unencumbered by limited structural connectivity, yet the observed differentiation suggests that population structuring exists over small scales despite high dispersal potential.



Key words: cod; dispersal; microsatellite DNA; pantophysin; smelt; white hake.

## INTRODUCTION

Connectivity among local populations is a fundamental component of metapopulation dynamics that often regulates species persistence, stability and adaptive potential (Crooks & Sanjayan, 2006). Spatial structuring in wild populations is influenced by

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both dispersal probabilities of organisms (*i.e.* functional connectivity) and by habitat homogeneity and discontinuity (*i.e.* structural connectivity). Resultant gene flow is a function of both components of connectivity (Gustafson & Gardener, 1996) because fragmented landscapes may restrict dispersal, whereas gap-crossing abilities may allow movements between available patches. Examinations of the relative roles of structural and functional processes are rare because of the difficulties in quantifying dispersal kernels and habitat connectivity in a single study (Taylor *et al*., 2006).

In marine species, the role of habitat and structural connectivity is often assumed to be limited because of the apparent lack of physical barriers and high potential gene flow. Dispersal distances are thought to be highly variable among species and may range from 10s to 100s of km (Kinlan & Gaines, 2003; Palumbi, 2004; Bradbury *et al*., 2008*a*). Theoretically, reductions in structural connectivity may occur with limited effect on metapopulation dynamics until some key connectivity or dispersal threshold value is reached, below which demes quickly become disconnected (Crooks & Sanjayan, 2006; Frankham, 2006). This threshold should be directly tied to the dispersal probability of a species in a particular habitat. In light of recent work that suggests limited dispersal ($\leq$100s of km) and local recruitment in many marine species despite a prevalent pelagic larval stage (Levin, 2006; Bradbury *et al*., 2008*a*), the threshold at which marine demes become disconnected may be lower than has been thought historically. Furthermore, examination of the balance between structural and functional connectivity in marine species is problematic because large-scale manipulations of populations or habitats are logistically and ethically difficult, if not impossible.

Holyrood Pond is a largely landlocked fjord on the south-east coast of the island of Newfoundland, Canada, which measures *c*. 22 km $\times$ 1·5 km, with a maximum depth of 100 m, and a surface area of 2031 ha (O'Connell *et al*., 1984). The fjord is isolated from the ocean for most of the year by a gravel beach barrier *c*. 100 m wide. Connection to the ocean occurs through a small channel (maximum depth 4 m, width 20–40 m) that is closed by the prevailing south-west winds and currents and opened for short periods annually by human mechanical intervention in order to prevent coastal flooding. On average, this connection is maintained for only two to three periods in a year, typically during the late spring or autumn and for a period of days to weeks at a time. The maximum continuous connection reported is *c*. 90 days, and the average annual total open period is 55 days. As a result of this exchange, the fjord is largely salt water or brackish and supports a marine fish community (J. Negrijn & G. Whelan, unpubl. data). Little is known regarding the degree to which resident fish populations exchange individuals with demes outside the fjord, and the degree to which resident populations are self-sustaining. Movements of adults of several species of marine fishes both into and out of the fjord, however, have been noted (O'Connell *et al*., 1984; J. Negrijn & G. Whelan, unpubl. data), and given that the opening of the fjord often coincides with reported peak spawning times for marine fishes such as Atlantic cod *Gadus morhua* L. (Hutchings *et al*., 1993), exchange of eggs and larvae seems likely.

This work encompasses two main objectives. First, a biological and physical (conductivity–temperature–depth, CTD) oceanographic survey of Holyrood Pond was conducted to provide insight into the fjord ecosystem and hydrodynamics. Oceanographic and circulation conditions vary dramatically throughout coastal Newfoundland, and the Labrador Current plays a dominant role through an inshore

 

branch, which enters several coastal embayments and drives counterclockwise circulation patterns (Sheng & Thompson, 1995). Limited data suggest that potential retention times for pelagic eggs or larvae in large coastal embayments may be of the order 20–40 days (de Young & Sanderson, 1995; Bradbury *et al*., 2000). This survey allowed the degree to which the fjord provides habitat suitable for marine species to be evaluated. Second, genetic structure of three species of marine fish: *G. morhua*, white hake *Urophycis tenuis* (Mitchill) and rainbow smelt *Osmerus mordax* (Mitchill), both within and outside Holyrood Pond, was examined using neutral genetic markers as well as one putatively non-neutral genetic marker. The approach was to use both multilocus trends and an examination of locus-specific spatial patterns because some of these loci have been reported to display elevated spatial structure (Nielsen *et al*., 2006). This combination of molecular and hydrographic measurements provides a thorough evaluation of fjord connectivity to other Newfoundland embayments. Genetic samples of each species from the north-east coast were included to place the magnitude of fjord-associated divergence in a broader spatial context. Moreover, to test the hypothesis that observed differences among species are the result of differences in heterozygosity, heterozygosity was corrected for following Hedrick (2005) and compared among the three species.

## MATERIALS AND METHODS

### FISH COLLECTIONS

Adult fishes were collected during May to July 2006 (also June 2005 for *O. mordax*) in Holyrood Pond and from several locations in coastal Newfoundland adjacent to and distant from the fjord (Fig. 1). *Osmerus mordax* were sampled in June at night using dip-nets in local spawning streams in Holyrood Pond, adjacent St Mary's Bay (Salmonier River) and Bonavista Bay (Traverse Pond), on the north-east coast of Newfoundland [Fig. 1(b)]. *Urophycis tenuis* were sampled using a combination of hand lines for adults in Holyrood Pond and beach seining for juveniles in St Mary's Bay and Bonavista Bay [Fig. 1(b)]. *Gadus morhua* were sampled using hand lines in Holyrood Pond, St Mary's Bay and Conception Bay [Fig. 1(b)].

### HYDROGRAPHIC SURVEY

Few oceanographic data exist for Holyrood Pond and, in light of the ambiguity in earlier reports on fjord topography and the absence of bathymetric contours, cross-channel and along-channel transects were conducted to determine bottom depths. Data from these transects were then contoured to produce a map of the depths in the fjord [Fig. 2(a)]. The CTD profiles were conducted at 14 stations within the fjord on 12 April 2006 using a SeaBird SBE-25 CTD (www.seabird.com) equipped with standard temperature, conductivity, pressure sensors, and a fluorometer and transmissometer. This time period was chosen to characterize spring conditions before the fjord was opened to the ocean. Bathymetry and environmental data were contoured and plotted using Matlab version 6.0 (www.mathworks.com).

### GENETIC ANALYSIS AND DIFFERENTIATION STATISTICS

Fin clips were placed in 95% ethanol. 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; www.perkinelmer.com). Individuals were genotyped using polymerase chain reaction (PCR) conditions of 5 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; www.neb.com), 0·3 μM each primer (forward primers were end-labelled

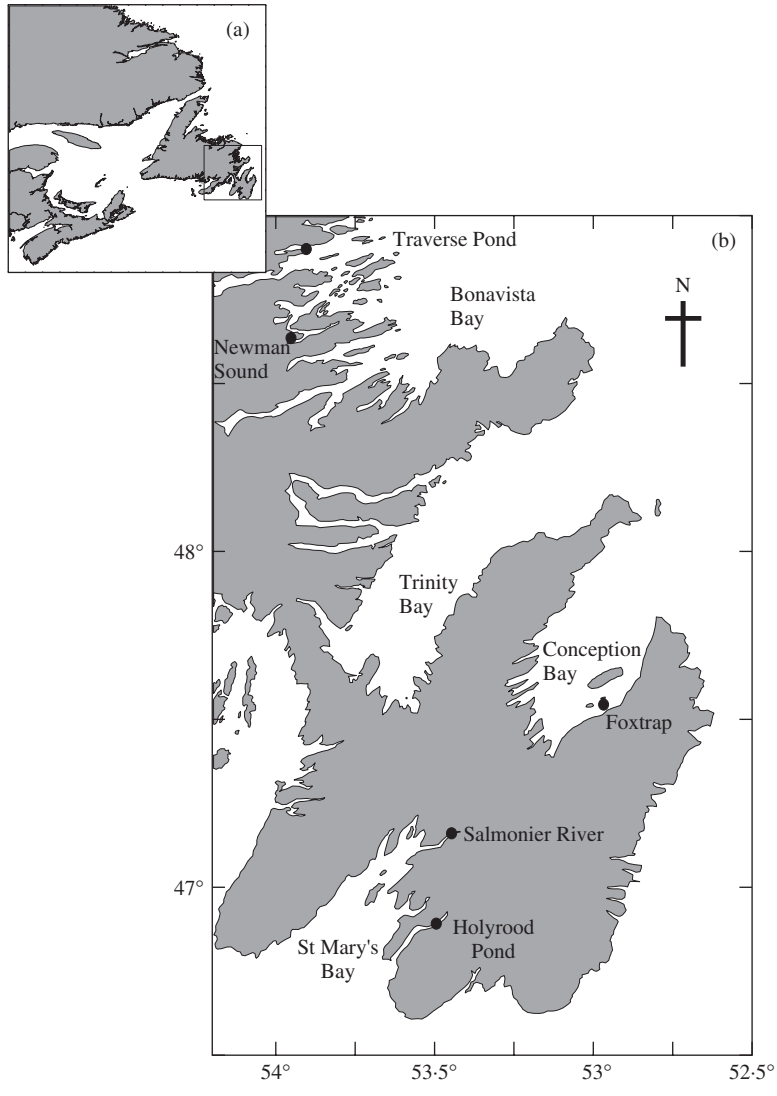


FIG. 1. Sample locations in coastal Newfoundland for *Gadus morhua*, *Osmerus mordax* and *Urophycis tenuis*. (a) Inset shows location of (b) region with respect to eastern Canada. North-east coast samples span several locations including Foxtrap (*G. morhua*), Newman Sound (*U. tenuis*) and Traverse Pond (*O. mordax*).

with HEX, or ROX dye) and 10× PCR buffer (10 mM Tris-HCl, pH 8·3; 50 mM KCl). PCR conditions were as follows: 94° C for 2 min, followed by four to five cycles of 94° C for 30 s, locus-specific annealing temperatures, 72° C for 30 s, followed by 25–26 cycles where the annealing temperature ($T_a$) 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 (Eppendorf; www.eppendorf.com) and imaged on an FMBioII system (Hitachi Genetic Systems; www.miraibo.com). For *O. mordax*, nine microsatellite loci were used (*Omo*1, *Omo*2, *Omo*3, *Omo*4, *Omo*5, *Omo*9, *Omo*11, *Omo*15 and *Omo*16) following Coulson *et al*. (2006). For *G. morhua*, 10 loci were used and were as follows: *Gmo*2 and *Gmo*132 (Brooker *et al*., 1994), *Gmo*34, *Gmo*8, *Gmo*35, *Gmo*37 and *Gmo*4 (Miller *et al*., 2000), and *Tch*11,

 

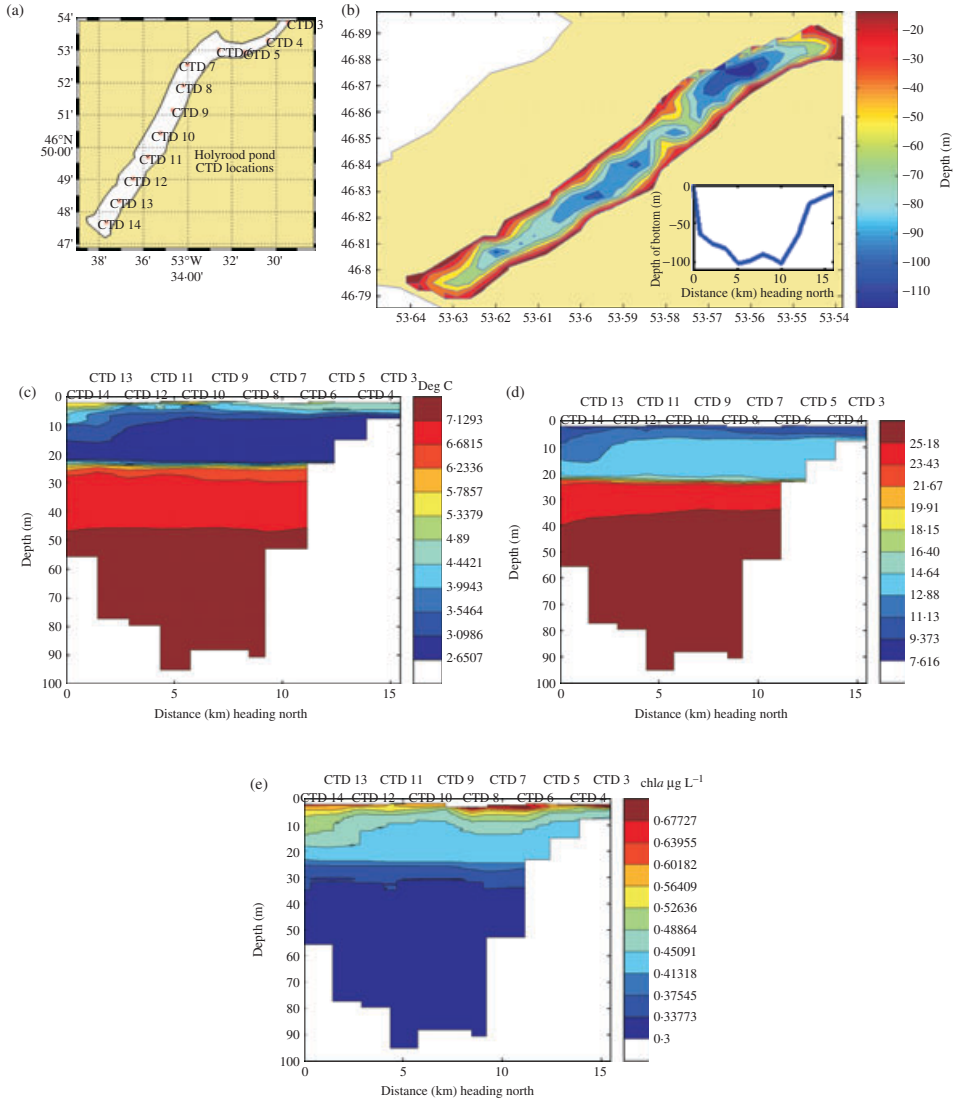


FIG. 2. Hydrographic characteristics of Holyrood Pond, Newfoundland, based on a survey (CTD, conductivity–temperature–depth) of (a) 12 stations. (b) Depth contours and hydrographic contours of (c) temperature, (d) salinity and (e) fluorescence. (b)–(d) were measured on 12 April 2006.

*Tch*19 and *Tch*5 (O'Reilly *et al*., 2004). For *U. tenuis*, seven tetranucleotide loci were chosen (Seibert & Ruzzante, 2006) as follows: *Ute*1, *Ute*12, *Ute*19, *Ute*22, *Ute*27, *Ute*34 and *Ute*35.

In addition to microsatellite loci, a nuclear protein-coding locus, *Pan*I was examined for polymorphism in *G. morhua*. Primers from Fevolden & Pogson (1997) were used to amplify a region of the *Pan*I gene (*via* PCR) that contained the polymorphic *Dra*I site. Following PCR with primer annealing at 55° C, the amplification products were digested with *Dra*I for 45 min at 37° C. The digested products were visualized in 1% agarose gels stained with ethidium bromide.

 

Data were examined for scoring errors and null alleles using Micro-Checker (van Oosterhout *et al*., 2004). Genetic polymorphism was quantified by examining 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 calculated in Genetix and FSTAT (version 2.9.3.3, Goudet, 1995). *F*-statistics and significance were calculated using FSAT and Arlequin (Schneider *et al*., 2000). Global and single-locus estimates of $G_{ST}$ were corrected for differences in variation among species and loci using $G'_{ST}$ following Hedrick (2005) as calculated in microsatellite analyser (MSA) (Dieringer & Schlötterer, 2003). Bayesian assignment testing was conducted in GeneClass 2.0 (Piry *et al*., 2004), using the assignment method of Rannala & Mountain (1997). A conservative level of assignment stringency was used (0·5), such that assignments with a probability below this level were excluded from analysis as they may represent immigrants from unsampled localities.

## RESULTS

### HYDROGRAPHIC SURVEY

The maximum depth of Holyrood Pond is $\leq$100 m, and there are two separate basins in the main arm of the fjord [Fig. 2(b)]. Surface temperature was 5–6° C [Fig. 2(c)]. Below the surface there was a cold, intermediate layer assumed to be a remnant of winter cooling, and below 50 m there was warmer water nearing 7° C. There was a well-defined and abrupt thermocline at *c*. 22 m depth [Fig. 2(c)]. Surface salinity was <10, whereas bottom salinity approached 24 [Fig. 2(d)]. Fluorescence data indicated elevated chlorophyll *a* concentrations in the surface waters but very low chlorophyll *a* below depths of 10 m [Fig. 2(e)]. Transmissometer measurements of particles indicated that there was an aggregation of particles at the pycnocline with very low concentrations and high light transmissivity in the deep water. This pattern suggests that particles from land runoff were trapped at the pycnocline.

### GENETIC DIFFERENTIATION

Genetic diversity and spatial structure varied among the species examined. *Osmerus mordax* were characterized by heterozygosities ranging from 0·71 to 0·67 at the nine microsatellite loci examined (Tables I and II). No evidence of linkage was observed, and deviations from HWE were rare and displayed no clear pattern. Tests for the presence of null alleles and scoring errors carried out in Micro-Checker suggest that such occurrences were rare and primarily associated with *Omo*3 and *Omo*16. Given that the removal of either locus had no significant effect on observed trends, they were included in further analyses. The estimate of global $F_{ST}$ was 0·08, which is indicative of significant structuring. Pair-wise estimates of $F_{ST}$ were all significant and ranged from 0·046 to 0·17 (Table III).

In comparison with *O. mordax*, *U. tenuis* and *G. morhua* displayed significantly lower genetic differentiation overall. In both species, the microsatellite loci revealed weak evidence of structure using $F_{ST}$, and average heterozygosities ranged from 0·30 to 0·97 (Tables I and II). Tests for the presence of null alleles and scoring errors in Micro-Checker suggested that departures from HWE were primarily associated with *Ute*35 and probably resulted from large allele dropout. Because removal of this locus had no significant effect on observed trends, it was included in further

 

TABLE I. Sample sizes and molecular summary statistics for all three species (see Fig. 1 for sample locations)

| Species | Site | *n* | Number of loci | $H_o$ | Mean number of alleles |
|---|---|---|---|---|---|
| *Osmerus mordax* | Holyrood Pond | 80 | 9 | 0·71 | 10·4 |
| | Salmonier, St Mary's Bay | 94 | 9 | 0·71 | 9·89 |
| | Traverse Pond, Bonavista Bay | 94 | 9 | 0·67 | 10·9 |
| *Urophycis tenuis* | Holyrood Pond | 80 | 7 | 0·81 | 17·7 |
| | St Mary's Bay | 50 | 7 | 0·76 | 15·6 |
| | Newman Sound, Bonavista Bay | 78 | 7 | 0·80 | 17·7 |
| *Gadus morhua* | Holyrood Pond | 58 | 10 | 0·69 | 12·2 |
| | St Mary's Bay | 93 | 10 | 0·70 | 15·0 |
| | Foxtrap, Conception Bay | 47 | 10 | 0·70 | 12·1 |

n, sample size.

TABLE II. Microsatellite locus-specific diversity measurements for three species of marine fishes sampled in coastal Newfoundland

| Species | | $N_a$ | $H_o$ | $H_e$ | $F_{is}$ |
|---|---|---|---|---|---|
| *Urophycis tenuis* | *Ute*1 | 10·0 | 0·750 | 0·782 | 0·040 |
| | *Ute*12 | 13·3 | 0·846 | 0·890 | 0·049 |
| | *Ute*19 | 7·3 | 0·667 | 0·735 | 0·088 |
| | *Ute*22 | 24·6 | 0·916 | 0·922 | 0·006 |
| | *Ute*27 | 16·0 | 0·807 | 0·798 | −0·012 |
| | *Ute*34 | 13·0 | 0·778 | 0·800 | 0·028 |
| | *Ute*35 | 35·0 | 0·759 | 0·947 | 0·198* |
| *Gadus morhua* | *Gmo*34 | 6·0 | 0·430 | 0·469 | 0·082 |
| | *Gmo*8 | 17·0 | 0·837 | 0·910 | 0·081 |
| | *Gmo*19 | 19·7 | 0·915 | 0·918 | 0·006 |
| | *Gmo*35 | 9·0 | 0·778 | 0·816 | 0·047 |
| | *Gmo*37 | 15·7 | 0·844 | 0·880 | 0·047 |
| | *Gmo*2 | 10·3 | 0·679 | 0·789 | 0·139 |
| | *Tch*11 | 21·0 | 0·830 | 0·925 | 0·102 |
| | *Tch*19 | 2·7 | 0·303 | 0·300 | −0·010 |
| | *Tch*5 | 22·0 | 0·865 | 0·926 | 0·065 |
| | *Tch*14 | 20·7 | 0·858 | 0·914 | 0·061 |
| *Osmerus mordax* | *Omo*1 | 6·0 | 0·502 | 0·481 | −0·043 |
| | *Omo*2 | 7·0 | 0·637 | 0·653 | 0·024 |
| | *Omo*3 | 15·3 | 0·811 | 0·860 | 0·057 |
| | *Omo*4 | 11·3 | 0·826 | 0·788 | −0·048 |
| | *Omo*5 | 12·7 | 0·826 | 0·839 | 0·014* |
| | *Omo*9 | 8·0 | 0·608 | 0·657 | 0·075 |
| | *Omo*11 | 8·0 | 0·512 | 0·524 | 0·022 |
| | *Omo*15 | 10·7 | 0·789 | 0·811 | 0·027 |
| | *Omo*16 | 14·7 | 0·762 | 0·854 | 0·106 |

*, significant test after Bonferroni correction; $N_a$, average number of alleles; $H_o$, observed heterozygosity; $H_e$, expected heterozygosity.

TABLE III. Pair-wise $F_{ST}$ (and significance) based on microsatellite loci and *Pan* I (see Fig. 1 for sample locations)

| | Pair-wise comparisons | | |
|---|---|---|---|
| Species | HRP × SMB | HRP × NEC | SMB × NEC |
| *Osmerus mordax* | 0·0468 (<0·001) | 0·1245 (<0·001) | 0·1684 (<0·001) |
| *Urophycis tenuis* | 0·0052 (<0·05) | 0·0028 (>0·05) | 0·0023 (>0·05) |
| *Gadus morhua* | 0·0054 (<0·01) | 0·0039 (>0·05) | 0·0061 (<0·001) |
| *Pan* I (*G. morhua*) | 0·0218 (>0·05) | 0·0121 (>0·05) | −0·0102 (>0·05) |

HRP, Holyrood Pond; SMB, St Mary's Bay; NEC, north-east coast.

analyses. Estimates of global $F_{ST}$ were 0·002 and 0·005 for *U. tenuis* and *G. morhua*, respectively (Table III). In contrast, elevated differentiation was observed at the *Pan*I locus (*G. morhua*) but only in comparisons involving Holyrood Pond (Table III).

Estimates of $G_{ST}$ suggest that *O. mordax* populations were the most highly structured (Fig. 3). Moreover, comparisons between St Mary's Bay and Holyrood Pond were usually associated with less divergence than other pair-wise comparisons. These trends were consistent after correction for differences in heterozygosity (Fig. 3). In each of the three species, associations were observed between locus-specific genetic divergence (*i.e.* $G_{ST}$) and expected heterozygosity ($H_e$) (Fig. 4). This association was negative in *O. mordax*, which was in contrast with a positive association in *G. morhua* and *U. tenuis*. Once the locus-specific variation in heterozygosity was taken into account, however, the negative relationship observed in *O. mordax* reversed and became positive (Fig. 4).

Despite the low differentiation in *G. morhua* and *U. tenuis*, high rates of Bayesian self-assignment were observed in all species and are consistent with discrete spatial structuring. For *O. mordax*, rates of self-assignment varied from 94 to 99%, with an average assignment score of 0·96 (Fig. 5). In *U. tenuis*, rates of self-assignment varied from 75 to 89%, with an average assignment score of 0·85 (Fig. 5). Estimates of self-assignment in *G. morhua* varied from 85 to 92%, with an average assignment score of 0·88 (Fig. 5).

## DISCUSSION

Spatial heterogeneity in marine species is influenced both by dispersal probabilities of organisms and by habitat structure or structural connectivity (DiBacco *et al*., 2006; Taylor *et al*., 2006). In marine species, the role of structural connectivity has often been neglected because broad-scale dispersal has been traditionally thought to be the predominant structuring agent (DiBacco *et al*., 2006, Levin, 2006). Evidence of population structuring in multiple coastal Newfoundland locations for three marine fish species with contrasting life histories is presented. Despite a pelagic larval stage in all three species, some structure was observed; however, structure was not elevated in the fjord location in comparison with adjacent bay contrasts. This observation suggests that despite complete isolation within the fjord for most of the year, short windows of connection are sufficient to prevent strong divergence such as those

 

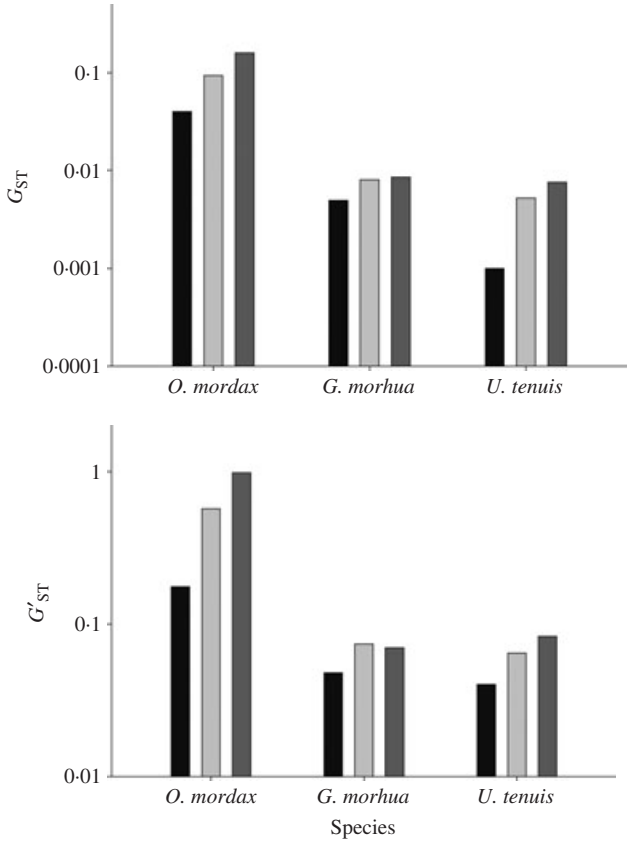


FIG. 3. Pair-wise comparisons of (a) $G_{ST}$ and (b) $G'_{ST}$ (Hedrick 2005) for three sample sets of *Osmerus mordax*, *Gadus morhua* and *Urophycis tenuis* from coastal Newfoundland [Holyrood Pond (HRP) and St Mary's Bay (SMB) (■), HRP and north-east coast (NEC) (▩) and SMB and NEC (□)].

observed in isolated populations, for example Gilbert Bay (Ruzzante *et al*., 2000). This result is consistent with previous data and anecdotal evidence suggesting that there is substantial movement of *G. morhua* and other species in and out of the fjord during periods when the fjord is open to the ocean (M. F. O'Connell, C. W. Andrews, J. P. O'Brian & E. G. Dawe, unpubl. data).

The structural connectivity of coastal fjords depends on local hydrography and geology, which dictate the environment (Asplin *et al*., 1999; Knutsen *et al*., 2007) and the degree of isolation experienced by various life-history stages of marine fishes (Morris & Green, 2002; Hardie *et al*., 2006; Wroblewski *et al*., 2007). The hydrographic survey of Holyrood Pond demonstrated strong stratification associated with spring runoff. Such stratification limits phytoplankton production and particles associated with land runoff and inflow to the upper layers (<20 m) and may, in turn, limit available production for *G. morhua* and *U. tenuis* in comparison with nearby coastal habitats. How this stratification is affected by the opening of the fjord is difficult to predict in the absence of detailed data; however, it is likely that the large outflow of low salinity surface water associated with opening the fjord

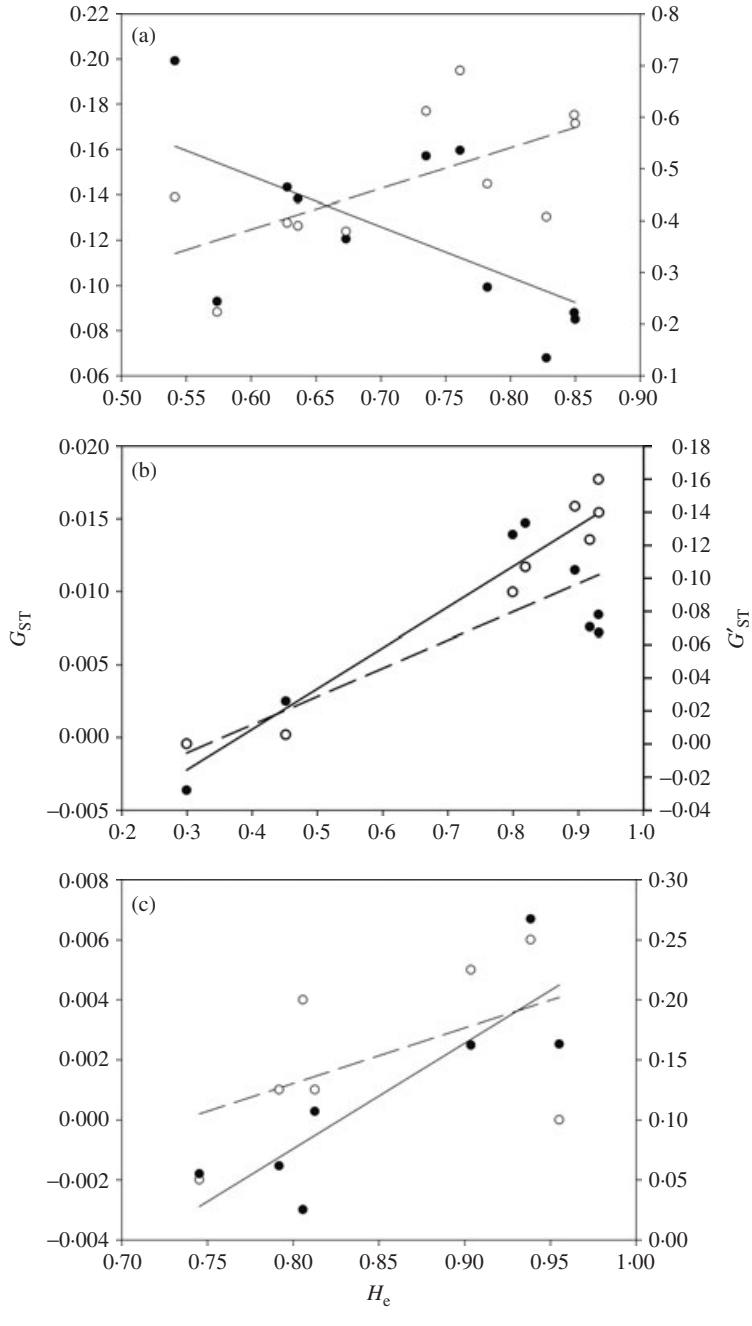


FIG. 4. Relationship between locus-specific differentiation ($G_{ST}$)(●) and $G'_{ST}$ (○) (Hedrick 2005) heterozygosity for three species of marine fishes (a) *Osmerus mordax*, (b) *Urophycis tenuis* and (c) *Gadus morhua* sampled at three locations in coastal Newfoundland. The curves were fitted by: (a) $G_{ST}y = -0{\cdot}22x + 0{\cdot}28$ ($r^2 = 0{\cdot}41$, $P < 0{\cdot}05$) and $G'_{ST}$ $y = 0{\cdot}79x - 0{\cdot}08$ ($r^2 = 0{\cdot}38$, $P < 0{\cdot}05$), (b) $G_{ST}y = 0{\cdot}02x - 0{\cdot}01$ ($r^2 = 0{\cdot}94$, $P < 0{\cdot}001$) and $G'_{ST}$ $y = 0{\cdot}25x - 0{\cdot}09$ ($r^2 = 0{\cdot}60$, $P = 0{\cdot}01$) and (c) $G_{ST}y = 0{\cdot}88x - 0{\cdot}63$ ($r^2 = 0{\cdot}72$, $P = 0{\cdot}01$) and $G'_{ST}$ non-significant ($r^2 = 0{\cdot}27$, $P > 0{\cdot}05$).

 

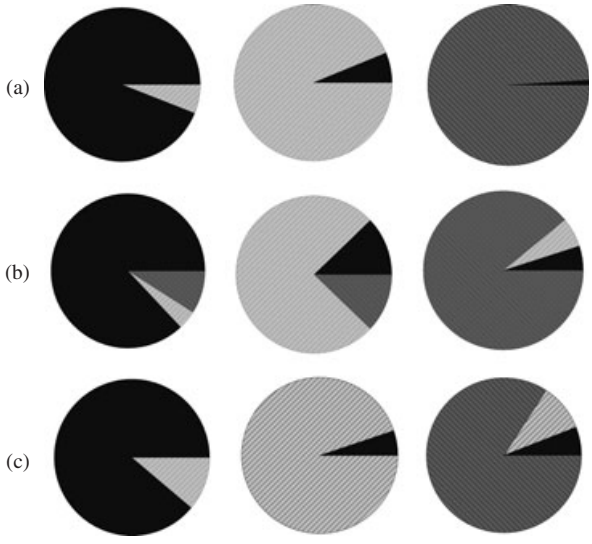


FIG. 5. Self-assignment of multilocus genetic samples for three species of marine fish sampled in coastal Newfoundland: (a) *Osmerus mordax*, (b) *Urophycis tenuis* and (c) *Gadus morhua*. Assignments based on the method of Rannala & Mountain (1997) as implemented in GeneClass 2·0 (Piry *et al*., 2004) (■, Holyrood Pond; ▨, St Mary's Bay; ▧, north-east coast).

would reduce stratification, resulting in higher export and lower residency times (Kaartvedt, 1989; Asplin *et al*., 1999). Studies elsewhere, in systems where isolation is incomplete, have suggested associations between freshwater inflow and isolation of marine fish populations (Morris & Green, 2002; Wroblewski *et al*., 2007). These types of conditions often occur where shallow sills are present (Knutsen *et al*., 2007); *G. morhua* separated from the offshore by a shallow sill are thought to have limited egg and larval dispersal as a result of a stable freshwater layer that extends to the depth of the sill and restricts eggs to the lower layer (Morris & Green, 2002; Knutsen *et al*., 2007; Wroblewski *et al*., 2007). Similarly, the large freshwater layer that occurs in Holyrood Pond may also play a role in retaining planktonic eggs and larvae during periods when the fjord is open to the sea.

Despite the apparent low structural connectivity, limited genetic differentiation of the fishes that inhabit Holyrood Pond on a scale similar to levels of divergence estimated for other locations in coastal Newfoundland was observed, though other locations were not as isolated as Holyrood Pond (Beacham *et al*., 2002; this study). High microsatellite differentiation was observed primarily in *O. mordax*, which is characterized by highly restricted dispersal among estuaries (Bradbury *et al*., 2006*a*, *b*). Nonetheless, levels of self-assignment proportions were significantly higher than random expectations in all three species. Previous examples of self-assignment testing with *G. morhua* have yielded rates of self-assignment between 33% (O'Leary *et al*., 2007) and 60% along the Skagerrak coast of Norway (Knutsen *et al*., 2003, 2004). The present estimates of self-assignment are higher than those obtained in previous studies, and the small number of populations examined may contribute to this difference (O'Leary *et al*., 2007). The exclusion of individuals

with low assignment probabilities, however, should prevent significant contributions from unsampled adjacent populations. Furthermore, estimates of self-assignment are comparable with direct estimates of adult straying in *O. mordax* (Bradbury *et al*., 2008*b*) and *G. morhua* (Robichaud & Rose, 2004; Wright *et al*., 2006). Nonetheless, it is unclear why the estimate of $F_{ST}$ for the Holyrood Pond and St Mary's Bay comparison was significant and the Holyrood Pond and north-east coast comparison was not, and this result requires further examination.

These findings support previous suggestions that multilocus assignment tests may be a more sensitive test of population structure than traditional $F_{ST}$-based methods (Castric & Bernatchez, 2004; Waples & Gaggiotti, 2006). These tests utilize multilocus genotypes to assign individuals to a population of origin and have the potential to accurately identify levels of straying and mixing among populations, even when dispersal is high (Hauser *et al*., 2006). Admittedly, the inability to sample spawning fishes in the case of *G. morhua* and *U. tenuis*, and the sampling of a different life-history stage in the case of *U. tenuis*, may have biased these results if either sample represented a mixture of groups of fishes. In the case of *U. tenuis*, the adults sampled may also have contributed to the juvenile cohort. This sampling bias, however, should reduce rather than increase population structure, and the evidence of structure in the assignment tests, despite this source of bias, supports its validity.

Previous authors have suggested that the ability of microsatellite loci to resolve spatial structure depends on locus-specific mutation rates and the associated levels of heterozygosity (O'Reilly *et al*., 2004), such that high mutation rates and homoplasy may prevent the resolution of spatial structure. In *O. mordax*, an inverse relationship between $F_{ST}$ and heterozygosity was observed, which is consistent with a reduction in observed structure with increased mutation rate. In contrast, this association was positive within *G. morhua* and *U. tenuis*, suggesting increased population resolution associated with increased mutation rate. A reasonable hypothesis is that the relative influence of gene flow and mutation rate will be expected to change with spatial scale, so that at spatial scales that are large relative to dispersal (mutation greater than dispersal) homoplasy should result in a negative association between differentiation and heterozygosity (O'Reilly *et al*., 2004). At smaller spatial scales (mutation less than dispersal), gene flow will overwhelm the influence of homoplasy, and a positive association is expected between differentiation and heterozygosity. This hypothesis is supported by the observed reversal of the negative $F_{ST}$ and $H_e$ relationship when divergence is corrected for $H_e$ ($G'_{ST}$) in *O. mordax*, a species that is characterized by low gene flow and limited dispersal.

Unlike microsatellite loci, the Pantophysin (*Pan*I) locus (Pogson, 2001; Pogson & Mesa, 2004, Westgaard & Fevolden, 2007) displayed elevated divergence among *G. morhua* samples. Pantophysin is an important membrane protein and functions in a variety of endocrine and endocytotic pathways (Haass *et al*., 1996) and may therefore be subject to intense contemporary environmental selection (Westgaard & Fevolden, 2007), although the direct nature of selection is not always clear (Pogson, 2001). Several studies have noted significant structure in population studies, including *G. morhua*, using *Pan*I in situations where microsatellite structure was weak or non-existent (Canino *et al*., 2005, Westgaard & Fevolden, 2007). Studies have documented a significant trend in *Pan*I polymorphism in *G. morhua* with a transition from inland fjords to the offshore in the north-east Arctic (Sarvas & Fevolden, 2005) or with depth (Pampoulie *et al*., 2006). One hypothesis as to why this pattern was

not observed within Holyrood Pond is that there may be considerably more gene flow than is observed elsewhere. Alternately, the selective gradient may be weaker in Holyrood Pond. Overall, the *Pan*I locus represents a potentially important tool for discriminating coastal *G. morhua* populations in Newfoundland waters as noted by Beacham *et al*. (2002). Although the divergence observed both in the microsatellites and in *Pan*I for Holyrood Pond and elsewhere is significantly less than differences observed in the eastern Atlantic, the reason remains unclear.

The differences in life-history strategies represented by these three species provide an interesting contrast. *Osmerus mordax* are largely restricted to estuarine waters throughout their entire life history (Bradbury, 2007), and rarely disperse between neighbouring estuaries; they also displayed the highest differentiation of the three species examined. *Urophycis tenuis* is a demersal, continental shelf species, which occurs primarily at depths of 200–1000 m. Although it exhibits limited large-scale movement, there is consistent offshore migration in autumn (Scott & Scott, 1988). *Gadus morhua* inhabit inshore regions, extending to the edge of the continental shelf and to maximum depths of 457 m. The level of divergence observed within *U. tenuis* and *G. morhua* is comparable and is consistent with extensive movements and higher rates of gene flow relative to those observed in *O. mordax*. Previous work on adult *G. morhua* annual spawning site fidelity suggests that homing rates may be as high as 50% in Newfoundland waters (Bradbury *et al*., 2008*c*) and 90% elsewhere (Wright *et al*., 2006), suggesting that homing probably plays a large role in the observed structure.

From a broader science perspective, the spatial ecology of wild populations is a critical consideration in the development of effective management and conservation strategies (Sale *et al*., 2005). Management failures and the depletion of unknown stock components may both result from a lack of spatial differentiation knowledge (R. L. Stephenson & E. Kenchington, unpubl. data). The ability to manage marine species successfully therefore depends on knowledge of both structural and functional connectivity, which have been notoriously difficult to measure in marine systems. Despite recent interest in localized inshore fish populations (Smedbol & Wroblewski, 1997; Ames, 2004), there have been few studies of connectivity of marine fishes in Newfoundland waters (Bradbury *et al*., 2000, 2001; Laurel *et al*., 2003, 2004). Although the present study suggests that the low structural connectivity of Holyrood Pond did not result in low dispersal, there may nonetheless be life-history stages (*e.g.* larvae and juveniles) where structural connectivity directly influences survival and predation rates. Further work is needed to address the role of small-scale structural connectivity on ecological dynamics of coastal *G. morhua* populations. The stability and longevity of these inshore populations will depend on local connectivity and their ability to persist despite disturbances. This work supports the presence of spatial genetic structure in coastal Newfoundland across a broad range of life histories, which is consistent with higher levels of biocomplexity and genetic diversity than past work has suggested.

The evidence of genetic structuring was observed in three species of marine fishes that inhabit coastal Newfoundland; however, isolation associated with regular closing of the fjord has apparently not translated into differentiation beyond that observed among adjacent bays. Thus, at the scales examined (10s–100s of km), genetic connectivity in these species may be primarily determined by dispersal probability (*i.e.* functional connectivity) even in a situation of repeated, protracted and

predictable isolation. This work supports the use of multilocus assignment testing and outlier loci in the identification of spatial structure in marine fish species over small scales such as adjacent bays in coastal Newfoundland, highlighting the inability of traditional $F_{ST}$-based approaches to capture spatial heterogeneity.

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