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A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence

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Abstract

We review our recent studies on the genetic structure of Atlantic cod (*Gadus morhua*) populations in the NW Atlantic. Our conclusions are based on knowledge of polymorphism at microsatellite DNA loci combined with known aspects of cod biology and ecology and with known oceanographic features in the NW Atlantic. Three case studies illustrate genetic heterogeneity between cod populations at the meso- and large-scales of coastal embayments and offshore banks and at the small-scale of oceanographic features. Our results generally highlight the importance of combining genetic with physiological, ecological, and oceanographic information, when assessing the genetic structure of highly abundant, widely distributed, and high gene-flow marine fish species. We highlight the role that oceanographic features (e.g., gyre-like systems) and known spatio-temporal differences in spawning time may play as barriers to gene-flow between and among neighboring and often contiguous cod populations in the NW Atlantic. We suggest that bathymetric and hydrodynamic/oceanographic structure represents a rational starting point for developing hypotheses aimed at assessing the genetic structure of high gene-flow marine fish species.

Keywords: *Gadus morhua*; Cod; Microsatellites; Genetic structure; Larvae; Bank-scale population structure

1. Introduction

Gene-flow between populations in highly abundant and widely distributed marine fish species is often relatively high (Ward et al., 1994; Waples, 1998). For such species determining whether the extent of dispersal and gene-flow between components of a stock complex is low enough for the complex not to be managed as a single panmictic unit is often difficult. However, the converse, i.e., determining whether migration between components of a stock complex is high enough to warrant management of the complex as a panmictic unit is essentially impossible using genetic data alone (Waples, 1998). Despite the inherent difficulties in estimation, some measure of genetic structure, however approximate, is often essential for

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the conservation of genetic resources and for the appropriate analysis of population dynamics of marine species especially when the populations are subjected to intensive exploitation. Failure to recognise the existence of population structure in exploited species can lead to overfishing and depletion of less productive populations (Larkin, 1977; Iles and Sinclair, 1982; Clark, 1990). Similarly for populations under recovery; differential recovery between (unidentified) components can lead to an inability to anticipate future patterns of recruitment that are necessary to define conservation and management policy.

The potential for either of the above outcomes is particularly increased in exploited and (or) depleted marine fish which, for political or administrative convenience, are managed under the assumption that the stock is comprised of a single, large and homogeneous breeding population. If this critical assumption is violated then the smaller or least productive stock components or those most readily captured are also those most readily eliminated (Larkin, 1977; Clark, 1990; Policansky and Magnuson, 1998). From a recovery perspective, population or stock components with the greatest recruitment potential (age/size structure, maturity, condition etc.) can be expected to recover most rapidly. Indifferent exploitation among putative stock components is detrimental to the stock and to the species as a whole because it results in the selective removal of genetic diversity.

Atlantic cod are distributed in the Western Atlantic from Labrador (∼63°N) to Cape Hatteras (∼35°N). Across this 3000 km range their distribution is essentially contiguous with different regions encompassing stock complexes that are recognised as units within explicit management divisions that are generally defined by latitude and bathymetry. Spawning areas within management Divisions are relatively discrete and temporally stable (Frank et al., 1994; Nakken, 1994; Serchuk et al., 1994; Shopka, 1994; Taggart et al., 1994). There are also differences in productive capacity between as well as within these stock complexes — a capacity that is partly non-heritable and partly heritable (Brander, 1994). Nevertheless, it is uncertain at what level these stock complexes and their components constitute genetically distinct populations. For cod (*Gadus morhua* L.), the importance of resolving cod stock structure is summarised by Rice: "*Clarifying the relationships among cod stock components ..... is fundamental to sound and scientific assessment and management of cod, and conservation of biodiversity*" (Rice, 1997, p. 2).

Different genetic markers vary in their ability to resolve genetic differences between cod populations. Analyses based on allozyme loci tend to show significant differences between populations when a limited number of specific loci are examined (Møller, 1968; Jamieson and Otterlind, 1971; Jamieson, 1975; Cross and Payne, 1978; Dahle and Jørstad, 1993). However, such differences are not apparent when a larger number of conventional loci are examined (Mork et al., 1982, 1985). Studies based on mitochondrial DNA variation show limited or no differentiation between populations throughout most of the species' range (Smith et al., 1989; Carr and Marshall, 1991a; Árnason and Rand, 1992), or within smaller geographic areas (e.g., Iceland; Árnason et al., 1992; Árnason and Pálsson, 1996), or between management divisions within the range of northern cod off Newfoundland (Carr and Marshall, 1991a, b; Pepin and Carr, 1993; Carr et al., 1995). Recent studies using nuclear DNA restriction fragment length polymorphism (RFLP) loci (Pogson et al., 1995), and nuclear DNA microsatellite loci (Bentzen et al., 1996) detect genetic population structure in cod at ocean basin scales as well as between major management divisions in the NW Atlantic. Furthermore, studies using the nuclear DNA microsatellite technique have revealed temporally stable population structure between inshore and offshore overwintering cod aggregations in the Newfoundland region (Ruzzante et al., 1996b, 1997). Microsatellite DNA loci are abundant and widely distributed throughout the eukaryotic genome and often exhibit high levels of allelic polymorphism. Microsatellite loci can be generated with relative ease by PCR amplification from minute quantities of fresh or preserved tissue. These qualities of microsatellites make them very useful and often the tool of choice (see Nielsen et al., 1997, and references therein) as genetic markers for their application to fisheries (Park and Moran, 1994; Ward and Grewe, 1994; Wright and Bentzen, 1994; O'Reilly and Wright, 1995).

We review our recent studies on the genetic structure of Atlantic cod populations in the NW Atlantic, and stress aspects of our research that are likely to be relevant to the identification of stock components in

high gene-flow marine fish species. Although our conclusions rely primarily on knowledge of nuclear, microsatellite DNA polymorphism, we complement this information with known aspects of cod biology and ecology and with known oceanographic features in the NW Atlantic. We focus on three case studies, each illustrating the existence of genetic heterogeneity between cod populations at a different spatial scale. First, we review the evidence for meso-scale population structure, between components of the northern cod complex off Newfoundland. We emphasize the evidence for genetic differentiation between inshore and offshore, as well as between offshore adult cod (i.e., demersal) aggregations within the complex. We then relate this information with that available on the population affinities of a number of larval (pelagic juveniles) aggregations recently collected on eastern Newfoundland waters and in the northern Gulf of St. Lawrence. Second, we examine small-scale processes by summarising evidence of genetically defined larval cod cohorts within a well defined gyre-like feature on the western bank on the Scotian Shelf. We discuss how such features may play a significant role in stock structuring (e.g., Iles and Sinclair, 1982) and relate our results to Cushing's match–mismatch hypothesis and Hedgecock's ''sweepstakes'' selection hypothesis (Cushing, 1972; Hedgecock, 1994). Third, we examine emerging evidence for genetic differentiation between neighboring cod aggregations on Browns and Georges Banks and in the Bay of Fundy.

2. Sample and tissue collections

All of the genetic data presented here are derived from a subset of over 9000 individual cod samples collected between 1992 and 1997 in the NW Atlantic (Fig. 1) and elsewhere. Details of specific sample collections are provided in Ruzzante et al. (1996a) for the study on the genetic heterogeneity within a large larval aggregation; in Ruzzante et al. (1996b, 1997) for the inshore–offshore genetic differentiation within northern cod; in Bentzen et al. (1996) and Ruzzante et al. (1998) for offshore samples from northern cod and those samples involving cod from Georges Bank, Browns Bank and the Bay of Fundy. We also describe genetic data on cod larvae collected during research surveys conducted between August and October 1994. Three of these larval collections are from the northern cod complex off eastern Newfoundland (NAFO Management Division 3K, $N = 44$; inshore Newfoundland near the Fogo Island region, $N = 23$; and NAFO Management Division 3NO, $N = 29$; Fig. 2). The fourth sample was collected in the northern Gulf of St. Lawrence region (Management Division 4RS; $N = 37$; Fig. 2).

3. Genetic techniques

Cod blood preserved in 95% ETOH was used as the primary source of nuclear DNA for genetic analysis. Soft muscle tissue taken from the posterior of the tongue and preserved in 95% ETOH was employed for DNA extraction when blood tissue was unavailable. Details of this procedure are available in Ruzzante et al. (1996b, 1997) and references therein. Data on antifreeze activity within blood plasma samples were obtained using the protocol described in Goddard et al. (1994) and outlined in Ruzzante et al. (1996b). In previous studies of adult cod, antifreeze levels have been used in conjunction with details of location and time of capture to provide evidence of overwintering behavior (Goddard et al., 1994; Ruzzante et al., 1996b). In adult cod, high antifreeze activity (>0.2°C thermal hysteresis) is indicative of overwintering in cold (<0°C) water — generally inshore, while low or non-existent winter antifreeze levels (<0.09°C) are indicative of overwintering in warm water — generally offshore.

DNA extraction of alcohol preserved tissue from cod samples is detailed elsewhere (Bentzen et al., 1996; Ruzzante et al., 1996a, b, 1997, 1998). PCR analysis was carried out as described by Brooker et al. (1994) using five radiolabelled dinucleotide microsatellite primers, *Gmo2*, *Gmo132*, and *Gmo145* (Brooker et al., 1994), *Gmo4* (Wright, 1993), and *Gmo120* (Ruzzante et al., 1996b). A sixth locus (*Gmo141*) was used in the study on the genetic composition of a larval aggregation (Ruzzante et al., 1996a) and in one of the studies on the genetic structure of northern cod (Bentzen et al., 1996). *Gmo2*, *Gmo4*, *Gmo120*, *Gmo132*, and *Gmo141* are perfect GT repeats, and *Gmo145* is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). PCR products were resolved on 6.5% denaturing polyacrylamide gels.

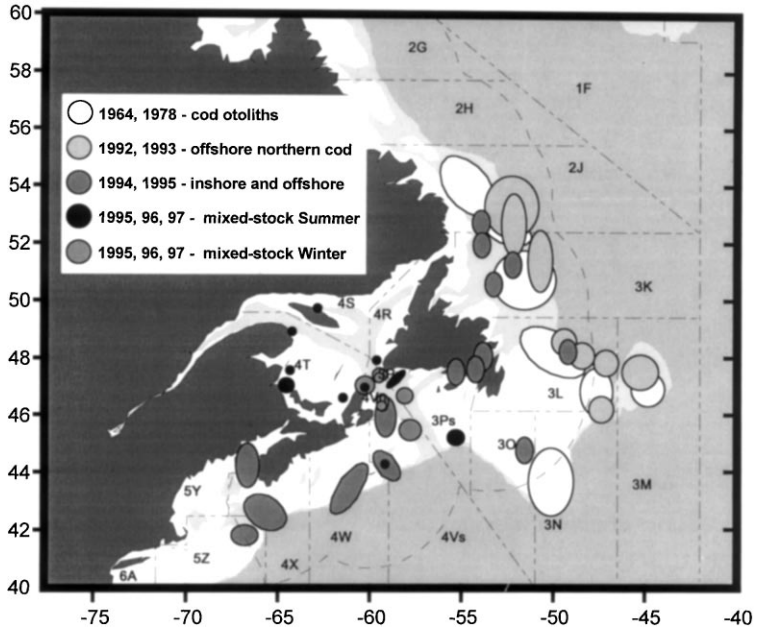

Fig. 1. Bathymetric chart (200 and 1000 m shaded isobaths) of the NW Atlantic showing the North Atlantic Fisheries Organization (NAFO) management divisions (dotted lines; e.g. 2J), the 200 mile exclusive economic zone (dashed line) and the locations and ranges (shaded ellipses) where over 9000 individual cod tissue samples have been collected for population structure analyses related to: historical populations (cod otoliths); offshore northern cod; inshore and offshore cod from Labrador to Georges Bank; and mixed stock (Winter) analyses related to spawning populations (Summer) in the Gulf of St. Lawrence and near the Cabot Strait entrance to the Gulf.

3.1. Allele scoring and null alleles

To standardize scoring and ensure consistency among gels all alleles were scored relative to two standards: a sequence ladder generated from M13mp18 (Yanisch-Perron et al., 1985) and a standard battery of individuals for which there is virtually unlimited supply of DNA. These individuals were run in each PCR reaction and on each gel. They were chosen to cover as much as possible of the expected range in allele size and were systematically run interspersed throughout the gel. The size of the standard individuals was established by repeated independent amplification and sizing to verify consistency in size of the amplification products. Loci used in our surveys were selected on the basis of production of a clear major band (yields of the stutter bands were significantly lower than those of major band(s)). Because of the differences in yield, alleles could easily be distinguished on the basis of product intensity even in cases of size overlap in heterozygous individuals. Samples were scored from lane to lane starting with a standard individual. Interspersed standard individuals were scored blindly and their scores had to agree with their known sizes. Each gel was also independently scored by two people. The extreme high heterozygosities and variability in size characteristic of all the cod microsatellite loci we have examined thus far would make null alleles readily detectable if they were present at any significant frequency. Also, the generally good agreement between observed and expected heterozygosities indicates that if null alleles are present they occur at a very low frequency and would thus not be expected to impact significantly on the comparative analyses.

4. Statistical analyses

Estimates of subpopulation structure were obtained using F_{ST} (Wright, 1951) and R_{ST} (Slatkin, 1995). F_{ST} was estimated following Weir and Cockerham (1984).

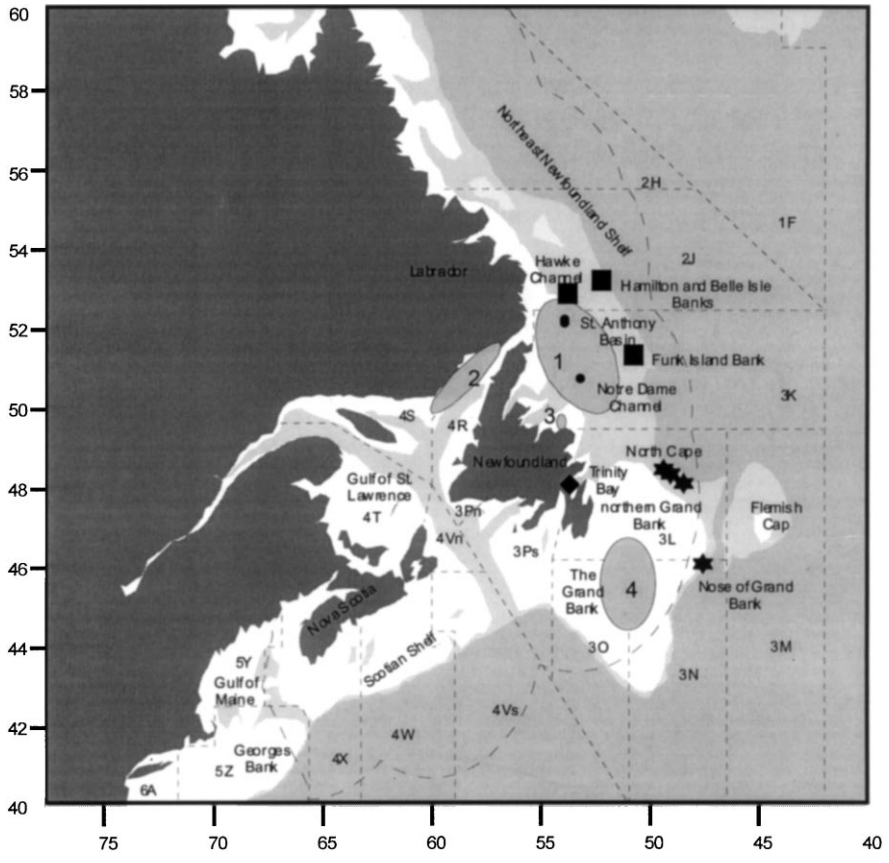

Fig. 2. Bathymetric chart (200 and 1000 m shaded isobaths) of the NW Atlantic showing the North Atlantic Fisheries Organization (NAFO) management divisions (dotted lines; e.g. 2J), the 200 mile exclusive economic zone (dashed line) and the locations where adult cod (solid symbols) were collected inshore (Trinity Bay, solid rhomb) and offshore [North (solid squares): Hamilton, Belle Isle and Funk Island Banks, and Hawke Channel, Sand (solid circles): St. Anthony Basin and Notre Dame Channel, and South (solid stars): North Cape, and nose of the Grand Bank], and ranges over which pelagic larval cod were collected (shaded ellipses) on the NE Newfoundland Shelf (1), the Strait of Belle Isle (2), the NE Newfoundland Coast (3) and the Grand Bank (4).

R_{ST} was calculated following Goodman (1997); see also Michalakis and Excoffier (1996) to minimise the variance due to differences in sample size (see Ruzzante, 1998) prior to standardisation of allele sizes. Significance for both structure measures was estimated by bootstrapping genotypes across individuals and populations and for each locus separately. Multilocus estimates of F_{ST} and R_{ST} were calculated by first summing numerators and denominators across loci and then calculating ratios as suggested by Weir and Cockerham (1984) and Slatkin (1995). Pairwise genetic distances between populations were estimated using D_{SW} (Shriver et al., 1995) a measure based on the stepwise mutational model (SMM), and also using D_A(Nei et al., 1983), a non-SMM measure of genetic distance with low variance relative to other non-SMM measures (Takezaki and Nei, 1996; see also Ruzzante, 1998). In some cases, for comparative purposes we also estimated $\delta\mu^2$ (Goldstein et al., 1995). Significance for distance measures was estimated by bootstrapping genotypes (1000 resampling trials with replacement) across individuals and populations for each locus separately. We also conducted tests of Hardy–Weinberg equilibrium (HWE) and heterozygote deficiency (D) and estimated probabilities by the bootstrap method. Bootstrapping in these two cases

was conducted by resampling (with replacement) alleles across individuals and populations. To avoid loss of information, we report original statistical significance values, i.e., uncorrected for multiple comparisons and where applicable in the tables, we specify which cases remain significant after Bonferroni corrections for K initial simultaneous tests (Rice, 1989). All statistical tests and analyses of genetic distances and population structure were conducted using Splus© standard code or functions written by DER.

5. Genetic structure in northern cod

Cod populations inhabiting the region off Labrador and Newfoundland, referred to as northern cod, exhibit an annual pattern of inshore–offshore migration (Lear, 1984, 1986) with most mature individuals overwintering on the continental shelf or along its margins. Within the northern cod region, spawning takes place over a three to four month period from late Winter through early Summer and generally begins earlier in the north than in the south (Myers et al., 1993). Following offshore spawning on the continental shelf, adult cod migrate inshore to the Summer feeding grounds. They then return offshore in late Autumn–early Winter (Templeman, 1966; reviewed in Lear and Green, 1984). The majority of the cod show the above pattern of migration, and the degree of temporally stable fidelity to offshore spawning banks inferred from tagging studies is sufficiently high for Lear to conclude that "*There is evidence of substantial inshore migration in Summer and subsequent homing to specific offshore overwintering and spawning areas, although with some straying*" (Lear, 1984, p. 157). The quantitative degree of that fidelity is readily apparent from the tagging studies conducted over three decades starting in the 1960's and recently compiled by Taggart (1997); see also below. Additional evidence that distinct offshore spawning components exist is based on the analysis of variation in vertebral complement (Templeman, 1981; Lear and Wells, 1984), and on the geographic distribution of spawning (Hutchings et al., 1993).

However, it has long been known that some fish remain inshore through the Winter (Fletcher et al., 1987). The extent to which fish overwintering inshore versus offshore constitute distinct populations remains uncertain (Lear, 1984; Hutchings et al., 1993; Angel et al., 1994) though Templeman thought that "*differences will be found to indicate a number of north–south and inshore–offshore sub-stocks of this Labrador–Newfoundland stock which either do not intermingle greatly or separate out at certain seasons*" (Templeman, 1962, p. 108). The regular occurrence of juvenile and adult cod overwintering in cold (<0°C), coastal waters (Fletcher et al., 1987; Valerio et al., 1992; Goddard et al., 1992, 1994; Wroblewski et al., 1994) coupled with evidence of inshore spawning (Smedbol and Wroblewski, 1997) suggest that inshore population(s), distinct from the offshore population(s) may exist (Templeman, 1966).

5.1. Genetic differences between inshore and offshore components

A pool of cod samples collected inshore in the area of Trinity Bay, Newfoundland between 1992 and 1994 (see Fig. 2) was genetically distinguishable from cod collected offshore on the northern Grand Bank when examined with D_A and D_{SW} measures of genetic distance and with the R_{ST} measure of population structure, and approached statistical significance when measured with F_{ST} (Table 1, see Ruzzante et al., 1996b). In all cases the genetic differences were small.

The cod collected inshore could be classified according to their blood thermal hysteresis. Thermal hysteresis is an index of the antifreeze content in the blood and it is a calibrated indicator of past residence in cold (<0°C) water, characteristic of coastal Newfoundland in Winter (Goddard et al., 1994). Both estimates of genetic distance (D_A and D_{SW}) and the R_{ST} measure of population structure increased with respect to the initial comparison, which did not account for antifreeze content (Table 1). F_{ST}, however, decreased in magnitude and significance. None of the measures indicated that inshore cod with low antifreeze content (i.e. not overwintering inshore) were genetically distinguishable from cod collected offshore (Table 1). Finally, inshore cod with high antifreeze content were not genetically distinguishable from inshore cod with low antifreeze content (Table 1), suggesting that cod in this latter group are a mixture of recently arrived migrants from offshore, and of inshore resident cod which may have lost their

Table 1
D_A and D_{SW} measures of genetic distance and F_{ST} and R_{ST} measures of population structure between pools of various sizes (N_1, N_2) of adult cod sampled offshore during Winters of 1992–1994 on the northern Grand Bank and inshore in Trinity Bay during late Winters of 1992–1994, and separately in the late Winter of 1995 (inshore samples are further classified according to high or low levels of blood plasma antifreeze (AF) levels in the individual cod)

Groups	N_1	N_2	D_A	D_{SW}	F_{ST}	R_{ST}
Inshore 92–94 (N_1) vs. Offshore 92–94 (N_2)	308	140	0.036***	0.018**	0.0008*	0.0062**
Inshore 92–94 high-AF (N_1) vs. Offshore 92–94 (N_2)	123	124[a]	0.048***	0.024***	0.0002	0.0087**
Inshore 92–94 low-AF (N_1) vs. Offshore 92–94 (N_2)	58	124[a]	0.061	−0.0003	0.0003	−0.0010
Inshore 92–94 high-AF (N_1) vs. Inshore 92–94 low-AF(N_2)	123	58	0.047	−0.0073	–0.0012	−0.0003
Inshore 95 (N_1) vs. All inshore 92–94 (N_2)	150	308	0.039****	0.003	–0.0001	0.002
Inshore 95 (N_1) vs. Inshore 92–94 high-AF(N_2)	150	123	0.054****	0.004	0.003	0.028*
Inshore 95 (N_1) vs. Inshore 92-94 low-AF (N_2)	150	58	0.062	−0.011	0.001	0.035
Inshore 95 (N_1) vs. Offshore 92–94 (N_2)	150	140	0.047****	0.019***	0.014**	0.001

* $P < 0.100$; ** $P < 0.050$; *** $P < 0.010$; **** $P < 0.001$.
[a] Individuals within the offshore 92–94 pool for which antifreeze information was available.

antifreeze because of prolonged residence in pockets of relatively warm water inshore (Ruzzante et al., 1996b). When measured with R_{ST} and F_{ST}, the genetic differentiation between inshore and offshore overwintering cod is primarily due to differences in allele frequencies at three loci: *Gmo120*, *Gmo132*, and *Gmo145* (Table 2).

Collectively, the above results suggest that cod collected inshore, and in particular those exhibiting high antifreeze content (a measure of inshore overwintering) are genetically distinguishable from offshore overwintering cod. We have also shown that this genetic differentiation was stable over the period 1992–1995. An independent pool of cod samples collected inshore during 1995 ($n = 150$) were collectively genetically indistinguishable (with three out of four measures; D_{SW}, R_{ST}, F_{ST}) from the cod collected inshore during the period 1992 to 1994 ($n = 308$; Table 1, line 5) despite the fact that both pools of inshore cod themselves showed some heterogeneity (Ruzzante et al., 1996b, 1997). When the 1995 samples were compared to the subsets of 1992–1994 inshore cod containing high ($n = 123$) or low ($n = 58$) antifreeze, all tests, except for one measure (D_A) in one of the comparisons showed that the 1995 inshore cod were genetically similar to the inshore cod in the 1992–94 period with high and low antifreeze (Table 1, lines 6, 7). Despite evidence of genetic heterogeneity between cod aggregations inshore, the cod collected inshore during 1995 were collectively genetically distinguishable from cod collected offshore over the period 1992–94 ($N = 140$, Table 1, line 8). In addition, a comparison of the $\delta\mu^2$ estimate for the inshore 1992–94 pool vs. the offshore 1992–94 pool and the inshore 1995 pool vs. offshore 1992–94 pool showed that the genetic distance between the inshore and offshore pools remained virtually unchanged and significant at $\delta\mu^2 = 2.10$ and 2.17, respectively (Ruzzante et al., 1997).

Table 2
R_{ST} and F_{ST} measures of genetic structure between inshore (N_1) and offshore (N_2) overwintering cod for each of five microsatellite loci and over all loci ($\alpha = 0.012$ after sequential Bonferroni correction for 4 (i.e., 4 loci) simultaneous tests (initial $K = 4$), thus, only locus *Gmo* 145 in the R_{ST} test remains significant after correction for multiple tests. No correction applies to the tests overall loci.)

Structure measure	N_1	N_2	*Gmo2*	*Gmo4*	*Gmo120*	*Gmo132*	*Gmo145*	Overall loci
F_{ST}	308	140	−0.0008	0.0004	0.0016**	0.003*	<0.0001	0.0008*
R_{ST}	308	140	−0.003	−0.002	0.015**	0.005	0.016***	0.0062***

* $P < 0.100$; ** $P < 0.050$; *** $P < 0.010$.

We have thus shown, on the basis of microsatellite DNA variation, that cod sampled inshore at various times and locations during 1995 within Trinity Bay, Newfoundland, were collectively, genetically indistinguishable from cod populations overwintering inshore in the same general area during 1992–1994 and were genetically distinguishable from cod populations overwintering offshore along the edge of the continental shelf on the Grand Bank. These results therefore provide evidence of temporal stability in the genetic differences between inshore and offshore overwintering cod populations in the region, at least at the scale of two to three years. Although these results provide evidence of temporal stability in the genetic structure within the northern cod complex overwintering inshore and offshore, they represent only a partial test of temporal stability as no wild cod samples from the northern Grand Bank region (i.e. offshore) were available in 1995. A more rigorous test of the temporal stability hypothesis would require the collection and processing of more or less contemporaneous inshore and offshore samples, but these were difficult to obtain. Nevertheless, a temporally stable genetic structure implies that existing, separate breeding components persist over time and are thus likely to experience independent population dynamics.

The case study reviewed above provides sufficient evidence that the null hypothesis of "*no significant genetic population structure in northern Atlantic cod*" can be rejected at least between inshore overwintering cod from Trinity Bay and offshore overwintering cod from the Grand Bank. Furthermore, spawning distributions, physiological adaptations, and tagging studies are all consistent with the genetic evidence. Tagging studies that focus on the temporal and spatial distribution of tag returns for fish that were tagged while overwintering in the Trinity Bay (pre-spawning aggregations; Lear, 1984; Taggart et al., 1995) show evidence of spawning ground fidelity that is consistent with the existence of population structure (Fig. 3). A seasonal analysis of tag report data from Taggart et al. (1995), excluding reports occurring within the first six months subsequent to tagging (see also Wroblewski et al., 1996) shows that >60% of the reported returns are from the immediate vicinity (within 30 nautical miles) of the original tagging region and <20% are reported at ranges exceeding 60 nm from the tagging region (Fig. 3).

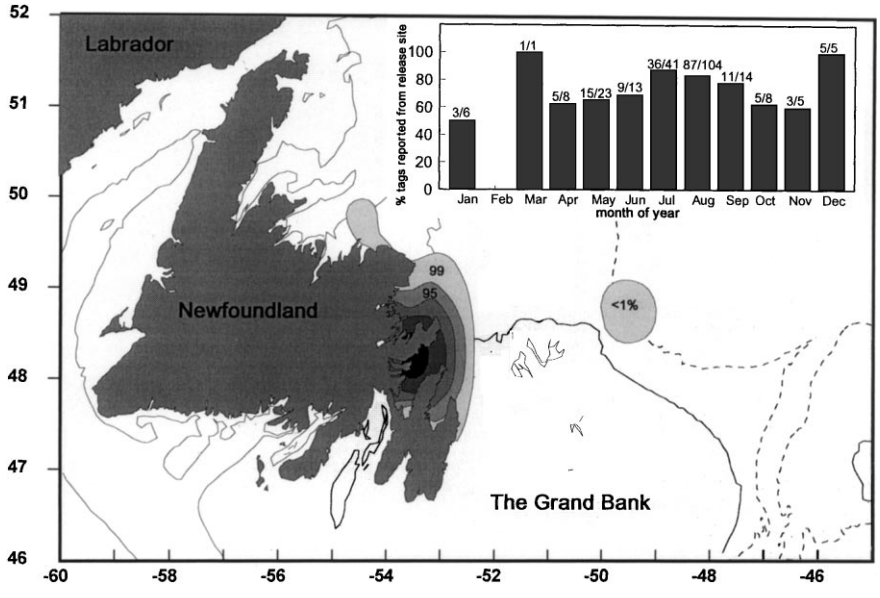

Fig. 3. Bathymetric chart (1000 and 200 m isobaths) of the Newfoundland region showing objective contour isopleths of the reporting rate (%) for the period 1988–1995 for cod originally tagged and released during the pre-spawning overwintering period in the Random Island region of Trinity Bay during 1988, 1990, and 1991. The inset shows the seasonal tag reporting rate (%) for tags reported captured in the original tag and release region. (after Taggart et al., 1998 and data in Taggart et al., 1995).

5.2. Genetic differences between offshore components

We have shown that inshore overwintering cod from Trinity Bay are genetically distinguishable from offshore overwintering cod from the Grand Bank region with which they intermingle in the Summer during the inshore feeding migration by offshore cod. Are there detectable genetic differences between other components of the northern cod complex? The answer appears to be yes. In a preliminary study Bentzen et al. (1996) described evidence consistent with the existence of small but significant genetic differences between northern and southern offshore components of the northern cod complex. More recently, Ruzzante et al. (1998) examined microsatellite DNA variation between cod populations spanning the latitudinal range of the species in the NW Atlantic from the Northeast Newfoundland shelf to Georges Bank and provided evidence of further genetic structure between adult cod aggregations from various offshore regions within the northern cod complex as well as between these and the inshore overwintering cod from Trinity Bay in Newfoundland (Fig. 2). For northern cod and neighboring regions an appropriate, yet perhaps conservative approximation to the stock structure should comprise (see Ruzzante et al., 1998): (1) a non-migratory Flemish Cap component; (2) two cross-shelf migrant components, one with spawning (Winter) fidelity to the various banks on the Norheast Newfoundland Shelf, and the other with spawning (Winter) fidelity to the northern Grand Bank region; and (3) an along-shelf migrant component with inshore or nearshore winter and spawning fidelity along coastal Newfoundland and Labrador. Taggart et al. (1998) and Ruzzante et al. (1998) also hypothesized, and recently confirmed with adult cod overwintering in Gilbert Bay, Labrador (Wroblewski, 1997) that there is significant genetic heterogeneity and structure at the scale of different bays (unpublished results and see also below, results concerning an inshore larval collection from the Fogo Island region of northeastern Newfoundland). Furthermore, tagging studies conducted over a period of three decades starting in the 1960's support the existence of two genetically distinguishable cross-shelf migrant components of northern cod (Taggart, 1997).

The average distribution of cod tagged in Winter in the vicinities of Hamilton Bank and Belle Isle Bank on the Northeast Newfoundland shelf overlap (Fig. 4) and they are not genetically distinguishable, while the average distribution of cod tagged in Winter in the North Cape region of the Grand Bank do not overlap with those to the north (Fig. 4), and they are genetically distinguishable.

The genetic differences between these stock components are small and standard population genetic models would indicate high levels of migration between them. We, however, stress two points: First, any interpretation of gene-flow estimates derived from F_{ST} and R_{ST} assumes that the populations are in equilibrium — likely an invalid assumption given the extreme fluctuations in population sizes that have occurred under intense exploitation over the last four decades. Second, assuming that violations of the assumptions are largely inconsequential, such high levels of gene-flow may be sufficient to prevent genetic differentiation of local populations in evolutionary timescales (see Slatkin, 1985, 1987; Lande and Barrowclough, 1987; Mills and Allendorf, 1996) but they are probably low in the context of population dynamics at time scales compatible with management issues (Ruzzante et al., 1998) because they are unlikely to prevent the different local populations from experiencing their own more or less independent recruitment dynamics (see Waples, 1998, for further discussion on this point).

5.3. Population affinities of larval cod aggregations

With the exception of one study that revealed small scale genetic heterogeneity within a large larval cod aggregation on the Scotian Shelf (reviewed below; see Ruzzante et al., 1996a), all our recent studies on the genetic structure of cod (Bentzen et al., 1996; Ruzzante et al., 1996b, 1997, 1998) are based on demersal, adult cod of a range of ages and sizes. Studies on the genetic composition of pelagic larval aggregations are expected to produce results broadly consistent with those obtained from adult cod collections. Alternatively, lack of consistency between studies involving adult and larval cod samples may reveal dynamic complexities in the system that are not apparent when only adult cod are examined. We examined the genetic composition of four larval cod aggregations, three of

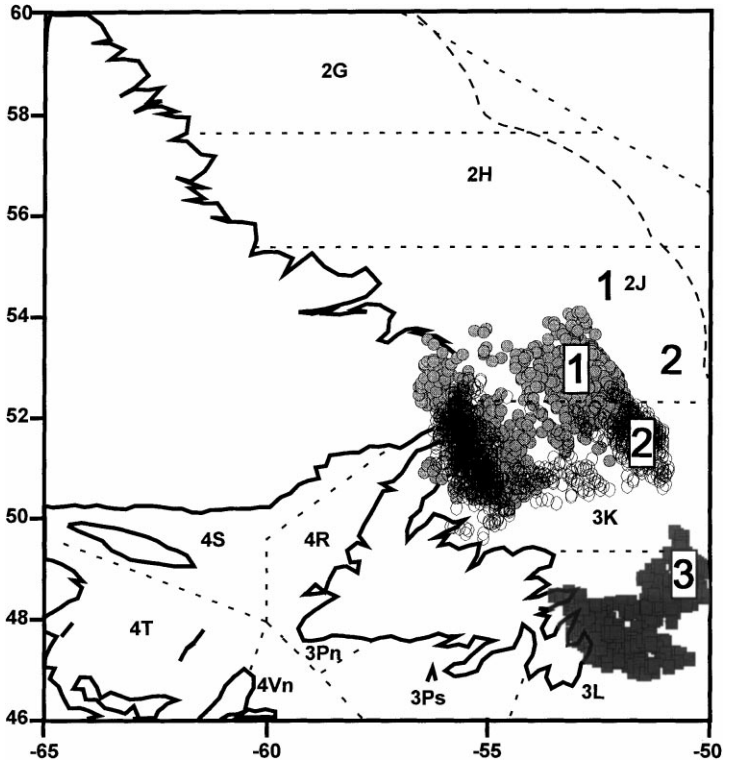

Fig. 4. Chart of the NW Atlantic region of Newfoundland showing the North Atlantic Fisheries Organization (NAFO) management divisions (dotted lines; e.g. 2J), the 200 mile exclusive economic zone (dashed line) and the combined average progressive vector geographic positions of reported cod-tag returns as a function of day of the year (aggregated offshore in Winter and inshore in Summer) from tagging experiments conducted on: (1) Hamilton Bank during the Winter periods of 1964, 1966, 1981, and 1982; (2) on Belle Isle Bank during the Winter periods of 1978 and 1983; and (3) near the North Cape region of the Grand Bank during the Winter periods of 1980, 1982, 1983, 1990, and 1991 (after Taggart, 1997 and data from Taggart et al., 1995).

them collected within the northern cod complex off eastern Newfoundland and the fourth collected in the northern Gulf of St. Lawrence region (see methods and Fig. 2).

Substantial genetic differences were revealed between the four larval cod aggregations with both measures of population structure, F_{ST} and R_{ST} (Table 3, line 1), and the differences remained statistically significant, though they decreased in magnitude, when only the three larval groups collected on the Northeast Newfoundland shelf and the Grand Bank region were included in the analysis (Table 3, line 2). The main conclusion of substantial and significant ($P < 0.05$) genetic differentiation remained essentially unchanged when the four adult cod collections from the northern cod complex were included in the analysis, regardless of whether or not the larvae collected on the northern Gulf of St. Lawrence were also included (Table 3, lines 3 and 4).

5.3.1. Genetic distance measures: $D_A$and D_{SW}

The two measures of genetic distance we employed, D_A and D_{SW}, provided highly concordant results. Examination of Table 4 reveals that (1) the cod larvae collected inshore near the Fogo Island region of Northeast Newfoundland and, (2) the larvae collected in the northern Gulf of St. Lawrence (region 4RS) were genetically distiguishable from each other and from all other groups including larval and adult collections (Table 4). The larvae collected in the offshore 3K region of northern cod were genetically distinguishable from the larvae collected inshore and from the

Table 3
Estimates of population structure: F_{ST} and R_{ST} among three larval cod collections from three areas (3*K*, $N = 44$; nearshore $N = 23$, and 3NO $N = 29$) within the northern cod region and one larval cod collection from the northern Gulf of St. Lawrence region (4RS $N = 37$) as well as among these and four adult northern cod collections (North $N = 174$; Sand $N = 96$; South = 249; Trinity $N = 303$) (see Fig. 2). Estimates involving the northern Gulf of St. Lawrence larval collection are based on four microsatellite loci (i.e., *Gmo2, Gmo4, Gmo120, and Gmo145*) ($\alpha = 0.010$ after Bonferoni correction for 5 (i.e., 5 loci) simultaneous tests (initial $K = 5$) thus cases with at least *** remain significant. No correction applies to tests overall loci.)

Groups	*Gmo2*	*Gmo4*	*Gmo120*	*Gmo132*	*Gmo145*	Overall loci
F_{ST}						
Northern cod and Gulf larvae (4 groups)	0.062^{****}	0.015^{****}	0.025^{****}	NA	0.004^{*}	0.025^{****}
Northern cod larvae (3 groups)	0.022^{***}	−0.000	0.003	0.047^{****}	0.005	0.014^{****}
Northern cod and Gulf larvae and Northern cod adults (8 groups)	0.012^{****}	0.004^{****}	0.006^{****}	NA	0.002^{***}	0.005^{****}
Northern cod larvae and adults (7 groups)	0.002^{*}	0.001	0.001^{*}	0.007^{****}	0.002^{***}	0.002^{****}
R_{ST}						
Northern cod and Gulf larvae (4 groups)	0.089^{****}	0.205^{****}	0.352^{****}	NA	–0.0010	0.171^{****}
Northern cod larvae (3 groups)	−0.002	0.071^{***}	−0.020	0.098^{***}	0.008	0.031^{**}
Northern cod and Gulf larvae and Northern cod adults (8 groups)	0.016^{****}	0.040^{****}	0.114^{****}	NA	0.007^{****}	0.051^{****}
Northern cod larvae and adults (7 groups)	−0.004	0.010^{****}	−0.010	0.009^{****}	0.008^{****}	0.002^{****}

* $P < 0.100$; ** $P < 0.050$; *** $P < 0.010$; **** $P < 0.001$.

larvae collected in the Gulf of St. Lawrence but not from the larvae collected on the Grand Bank or from any of the adult groups. The genetic affinities of the adult cod groups presented here (Table 4) are broadly consistent with those described elsewhere (Ruzzante et al., 1998) based on polymorphism at five loci. The group of cod collected between 1992 and 1994 offshore on the Northeast Newfoundland shelf and referred to as North ($N = 174$) were distinguishable from inshore overwintering cod fromTrinity Bay (Trinity, $N = 303$), from cod collected on the North Cape region of the Grand Bank (South, $N = 249$), and with one measure (D_{SW}) from cod collected in the St. Athony Basin and Notre Dame Channel areas (Sand,

Table 4
D_A (above diagonal) and D_{SW} (below diagonal) between larval aggregations in the northern cod and Gulf of St. Lawrence regions and adult northern cod from offshore and inshore areas. Estimates are based on four loci: *Gmo2, Gmo4, Gmo120, and Gmo145*. Note: Estimates between the adult populations differ slightly from those published in Ruzzante et al. (1998) because of the difference in the number of loci involved (Those in Ruzzante et al. (1998) are based on 5 loci) ($\alpha = 0.002$ after sequential Bonferroni correction for initial $K = 28$ (Rice, 1989), thus cases with **** remain significant after correction for multiple tests.)

		Larvae				Adults			
		3K	Inshore	3NO	4RS	North	Sand	South	Trinity
Larvae	3 *K* ($N = 44$)	–	0.291^{****}	0.133	0.380^{****}	0.103^{*}	0.098	0.104^{**}	0.096^{*}
	Inshore ($N = 23$)	0.191^{***}	–	0.286^{***}	0.349^{****}	0.230^{****}	0.228^{****}	0.217^{****}	0.235^{****}
	3NO ($N = 29$)	0.061	0.467^{****}	–	0.427^{****}	0.113	0.113	0.109	0.099
	4RS ($N = 37$)	2.212^{****}	1.462^{****}	2.72^{***}	–	0.413^{****}	0.405^{****}	0.367^{****}	0.360^{****}
AdultS	North ($N = 174$)	0.071	0.402^{****}	0.005	2.933^{****}	–	0.059	0.044^{***}	0.043^{****}
	Sand ($N = 96$)	0.036	0.303^{****}	0.014	2.458^{****}	0.064^{****}	–	0.048	0.055^{*}
	South ($N = 249$)	–0.006	0.215^{****}	0.078	2.479^{****}	0.06^{****}	0.045^{***}	–	0.037^{****}
	Trinity (303)	0.019	0.285^{****}	0.011	2.475^{****}	0.038^{****}	−0.002	0.02^{***}	–

* $P < 0.100$; ** $P < 0.050$; *** $P < 0.010$; **** $P < 0.001$.

$N = 96$). As described in more detail above, the cod collected offshore in the North Cape region of the Grand Bank (South) was genetically distinguishable from inshore overwintering cod from Trinity Bay (Trinity).

Some of the results concerning the genetic population affinities of the larval collections from the NE Newfoundland shelf were not entirely consistent with our expectations based on knowledge of the genetic composition of the adult samples. For example the larvae collected in the 3K region of the NE Newfoundland shelf ($N = 44$) and those collected on the Grand Bank (3NO, $N = 29$) were genetically indistinguishable from each other and from any of the adult cod samples from northern cod. In general, however the genetic results involving the larval collections from the NE Newfoundland shelf and the northern Gulf of St. Lawrence should be interpreted with caution because of limited sample sizes (see Ruzzante, 1998). Sampling size considerations aside these results suggest cod larvae originated on the NE Newfoundland shelf may disperse throughout the region and eventually reach the Grand Bank area. Existence of a statistically and biologically significant degree of population structure (Ruzzante et al., 1998) despite potentially extensive dispersal at the larval stage can in principle be explained if (1) the dispersed larvae do not recruit successfully to any adult population, or (2) they recruit successfully but eventually migrate back to spawn in their general natal area, a behavior that may be facilitated by topographically induced gyre-like circulations or other hydrodynamic features that can act as retention mechanisms for eggs and larvae. Alternatively, the dispersed larvae may generally recruit and reproduce successfully anywhere within the northern cod complex, as suggested by the relatively low (but significant) magnitude of F_{ST} and/or R_{ST} estimates (see Ruzzante et al., 1998) but they are simply not numerous enough compared to larvae that do not disperse, to eliminate the observed genetic structure and result in panmixia. Some further insights to address these and related issues may be gained by next focusing on a group of larvae found in a gyre-like feature on the Scotian shelf.

6. Small-scale genetic heterogeneity in a larval cod aggregation: genetics and ecology

During November and December 1992 a large aggregation of larval cod was tracked offshore on the Scotian Shelf and sampled repeatedly over a period of three weeks (Taggart et al., 1996). On the basis of polymorphism at six microsatellite DNA loci among 1337 cod larvae we found strong evidence of heterozygote deficiency and departure from Hardy–Weinberg expectations for the larval aggregation as a whole and for a subset found within a single water mass (i.e. the crestwater, CW; see Ruzzante et al., 1996a) but not for a subset of the larvae that formed a single cohort on the basis of age-at-length (Table 5).

HWE is characteristic of large, randomly mating populations. A population is in HWE for a given locus if the probability of observing a given genotype is equal to the product of the probabilities of observing each of the alleles (i.e. the allele frequencies) for a homozygote, or twice this product for a heterozygous genotype. If a population is sub-divided, and there are two or more groups that differ in allele frequencies at a given locus, then analysis of the entire population without considering its structure will show a deficiency of heterozygotes. This is essentially what our analysis described for the entire larval aggregation

Table 5
Genetic variation at six microsatellite loci described by samples per locus, number of alleles, average observed (H_{obs}) and expected (H_{exp}) heterozygosity and heterozygote deficiency (D) and its significance (P) among all larvae in the entire larval cod aggregation (pool), among larvae within the gyre-like crest water mass (CW) and the age-at-size cohort found within the CW on Western Bank of the Scotian Shelf

Larval Group	Mean sample size per locus	Total alleles	Mean H_{obs}	Mean H_{exp}	Mean D	P
pool	1226.8	276	0.895	0.902	−0.009	0.015
CW	705.7	256	0.891	0.901	−0.013	0.008
Cohort	283.3	212	0.897	0.898	−0.004	0.263

and the subset of all larvae sampled in the CW water mass — they appear to have originated from several spawning groups among which there were marked differences in allele frequencies (see also Herbinger et al., 1997). In contrast, there was neither evidence for heterozygote deficiency nor strong evidence for departures from HWE among the cod larvae that formed a single cohort within the aggregation in the CW water mass (Ruzzante et al., 1996a). The power to detect small deviations from HWE decreases with sample size, thus, the detection of departures from HWE in the entire larval aggregation but not in the cohort subset may be related to the difference in sample size. We believe, however, that this is not the case in this particular instance for two reasons: first, sample sizes are still relatively large even in the cohort subset, and H_{obs} and H_{exp} are closer in the cohort sample than in either aggregated sample. Departures from HWE can also result from factors other than population subdivision, such as selection, inbreeding, phenotypic assortative mating, and/or the presence of null alleles (Devlin et al., 1990; Chakraborty and Jin, 1992), but population subdivision is thought to be the most important of these factors for microsatellite loci (Lander, 1989). Thus we concluded that the entire aggregation originated from several distinct spawning events involving spawners with heterogeneous allelic compositions. However, the larvae forming the cohort originated from a single spawning event.

There was no evidence that the entire larval aggregation originated from different populations as measured by $\delta\mu^2$ distance, R_{ST}, and F_{ST} estimates between subsets (Ruzzante et al., 1996a). On the other hand, comparison of the cohort larvae to adult cod sampled two years later on Western Bank and approximately 200 km to the NE, on Banquereau Bank, indicated that no matter how measured, using either R_{ST} and F_{ST} (as in Ruzzante et al., 1996a) or using D_A, D_{SW}, and $\delta\mu^2$ (Table 6) the cohort larvae were consistently more similar to adult cod collected locally on Western Bank than to adult cod from Banquereau Bank. These analyses clearly suggest that the genetic composition of cod on Western Bank remains stable over time and are consistent with the notion of the existence of localized stock structure in cod at bank-scales (order 100 km).

The large larval aggregation described in this example was confined within a relatively small area (order 100 km^2) of coastal ocean involving a rotating slab of well mixed water overlying the crest of a spawning bank (Sanderson, 1995) and contained larvae spanning a range of sizes and ages (Taggart et al., 1996; Lochmann et al., 1997). Considered as a whole, the aggregation was genetically heterogeneous. However, a subset (the common age-at-size cohort) was genetically homogeneous. The evidence indicates that the larval aggregation as a whole resulted from several spawning groups whose offspring form genetically defined larval cohorts upon which temporally and (or) spatially varying biotic and oceanographic processes can act to effect differential reproductive success between spawning groups (see Lambert, 1984). If this interpretation is correct, then moderate or minor differences in the timing of spawning or differences in the availability of resources to the offspring (i.e. match–mismatch; Cushing, 1972; see also Levitan and Petersen, 1995) appear to be the mechanisms that lead to high variance between cohorts in their contribution to recruitment. These findings therefore, have implications in the context of the ''match–mismatch'' hypothesis (Cushing, 1972) and its genetic counterpart, the ''sweepstakes'' selection hypothesis (Hedgecock, 1994). The ''sweepstakes'' hypothesis attributes the discrepancies between effective and actual population sizes characteristic of highly abundant and widely distributed marine species to the fact

Table 6
Genetic distance estimates (D_A, D_{SW}, and $\delta\mu^2$) between the larval cod cohort collected on Western Bank of the Scotian Shelf in November 1992 and adult cod sampled on Western Bank and Banquereau Banks two years later

Groups	D_A	D_{SW}	$\delta\mu^2$
Larval cohort ($N = 316$) vs Western Bank adults ($N = 48$)	0.073	0.018	2.25
Larval cohort ($N = 316$) vs Banquereau Bank adults ($N = 48$)	0.076*	0.067**	5.89**

* $P < 0.100$; ** $P < 0.050$.

that in each generation a small minority of individuals can replace the entire population by a ''sweepstakes-chance matching of reproductive activity with oceanographic conditions conducive to ... successful recruitment'' (Hedgecock, 1994). Also, finding a large larval cod aggregation within a spatially well defined (kilometre-scale) and temporally stable (at the scale of days) gyre-like water circulation system provides support to the hypothesis that spawning products can be retained within well defined geographic areas on the Scotian Shelf suggesting that cod spawning aggregations from neighboring banks may experience more or less independent dynamics and may exhibit further evidence of genetic structure.

7. Bank-scale genetic structure in Browns Bank, Georges Bank and Bay of Fundy cod

Our last example involves an examination of the genetic composition of cod from three neighbouring regions on Browns Bank (western Scotian Shelf), the Bay of Fundy, and Georges Bank (Fig. 5). Although all three areas are geographically very close to each other, they are characterized by distinct oceanographic regimes. Georges Bank and Browns Bank are bathymetrically separated by the Fundian Channel and each is characterized by distinct and relatively persistent gyre-like circulations. Juvenile and adult cod migration as well as mixing of spawning products between these banks are thought to be low or negligible (Campana et al., 1989; Suthers and Frank, 1989). The evidence thus suggests that retention mechanisms resulting from topographically induced eddies act to minimise dispersal of ichthyoplankton between these banks (Smith, 1983, 1989a, b; Loder et al., 1988, for Browns Bank; Hopkins and Garfield, 1981; Smith and Morse, 1985; Loder et al., 1988; Werner et al., 1993 for Georges Bank; Iles and Sinclair, 1982; O'Boyle et al., 1984; review in Frank et al., 1994) and/or to allow for sufficient imprinting necessary for subsequent spawning on natal banks.

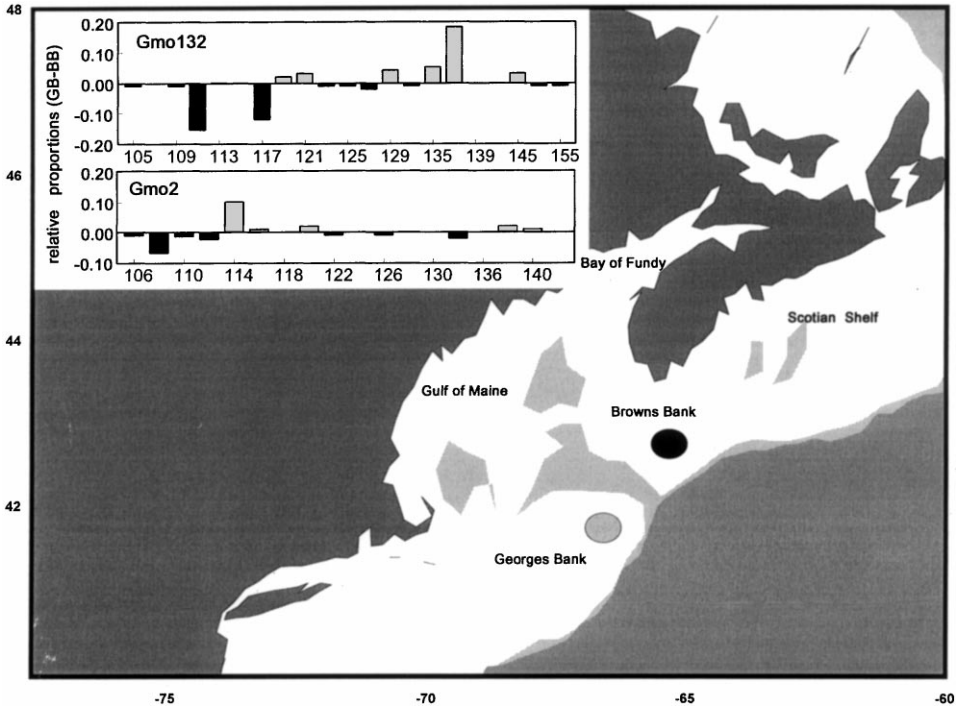

Fig. 5. Bathymetric chart (1000 and 200 m isobaths) of the Scotia–Fundy region showing sample locations for Georges Bank (GB) and Browns Bank (BB) cod. The inset shows the between-population relative proportions (proportion of GB cod with allele minus proportion of BB cod with the same allele) of different alleles (base-pairs) for the *Gmo132* and *Gmo2* microsatellite loci.

Table 7
F_{ST} and R_{ST} measures of genetic structure between adult cod collected from Browns and Georges Bank and the Bay of Fundy for each of 5 microsatellite loci and over all loci ($\alpha = 0.012$ after Bonferroni correction for 4 (i.e., 4 loci) simultaneous tests (initial $K = 4$), thus, cases with**** remain significant after correction. No correction applies to the overall loci test.)

	Gmo2	*Gmo4*	*Gmo120*	*Gmo132*	*Gmo145*	Overall loci
F_{ST}	_0.002	0.003*	−0.001	0.030****	0.005**	0.007****
R_{ST}	–0.005	0.014*	−0.006	0.091****	0.030**	0.025****

* $P < 0.180$; ** $P < 0.050$; **** $P < 0.001$.

Table 8
D_A (above diagonal) and D_{SW} (below diagonal) measures of genetic distance between cod collected on Browns and Georges Bank and in the Bay of Fundy ($\alpha = 0.050/3 = 0.017$ after sequential Bonferroni correction for three simultaneous tests, thus cases with at least*** remain significant after correction)

Groups	Browns Bank	Georges Bank	Bay of Fundy
Browns Bank ($N = 48$)	–	0.118*	0.136***
Georges Bank ($N = 48$)	0.139****	–	0.116*
Bay of Fundy ($N = 48$)	0.175****	0.036	-

* $P < 0.100$; *** $P < 0.010$; $^{****}P < 0.001$.

Despite their geographic proximity, cod collected at these three locations are genetically distinguishable from each other when examined with any one of four different genetic measures of distance and population structure (Tables 7 and 8). The genetic differences between these populations appear to be primarily related to the *Gmo132* and *Gmo145* loci, and to a lesser extent *Gmo4* (Table 7, Fig. 5).

The bathymetry and oceanography of the region from which these cod were collected as well as the spatio-temporal distribution of spawning are all consistent with cod from these locations experiencing independent population dynamics. Maximum spawning time differs between these regions (Colton et al., 1979; Sherman et al., 1984; Hurley and Campana, 1989) with egg concentrations appearing first on Georges Bank (January–February), and then on Browns Bank (March–April).

8. Conclusion

Our main goal in reviewing these three case studies was to show that using a combination of genetic, physiological and ecological, as well as oceanographic information, biologically significant differences in genetic composition can be detected between cod populations at a variety of geographic scales. We stressed those aspects of our research that in our view are likely to provide significant insights into issues related to the identification of stocks and stock components in widely distributed, highly abundant and high gene-flow marine fish species. For example, our results suggest the existence of significant genetic differences between cod populations at bank- and bay-scales and imply that oceanographic features (e.g., recirculation systems) and known spatio-temporal differences in spawning time may constitute important barriers to gene-flow between and among neighboring cod aggregations in the NW Atlantic. Topographically (bank) induced gyre-like circulations (eddies), which in some regions have been shown to act as retention mechanisms for cod eggs, larvae, and juveniles may facilitate post-dispersal spawning fidelity to natal areas (i.e., homing) and thus, may help explain some of the detected genetic differences between neighbouring cod aggregations. We suggest that bathymetric and hydrodynamic/oceanographic structure represents a rational starting point for developing hypotheses aimed at assessing the genetic structure of high gene-flow marine fish species.

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