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Localised Stocks of Cod (*Gadus morhua* L.) in the Northwest Atlantic: The Genetic Evidence And Otherwise.

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Abstract

If the critical assumption of panmixia in exploited cod *(Gadus morhua)* stocks is invalid at any management level, then management is functionally inconsistent with the principles of resource conservation and the maintenance of biodiversity. With this claim in mind we review some of our studies on the genetic structure of cod populations in the Northwest Atlantic and highlight aspects that may be of value to questions concerning localised cod populations in the Gulf of Maine. We employ microsatellite DNA polymorphism to resolve genetic differences among cod populations (under the null hypothesis of no significant differences between or among populations) as microsatellites are considered the ideal genetic tool for studying closely related groups of animals (high level of co-dominant allelic variation, rapid divergence times, and relative ease of examination). We employ other biological and oceanographic "knowns" in our assessment of the genetic results. Three case studies illustrate genetic heterogeneity among cod populations: 1) at the small-scale of oceanographic features; 2) at the meso-scale of coastal embayments; and 3) at the large-scale of offshore banks. Our review leads us to one specific hypothesis regarding the existence of at least one localised population in the Gulf of Maine having a specific behavioural cycle, and one general hypothesis that *structure in localised coastal cod populations in the NW Atlantic is likely to be revealed at scales of 60 to 100 nautical miles.*

Introduction

Determining the extent of gene flow among stock components in a marine fishery, and whether gene flow is sufficiently high to warrant managing a stock complex as a panmictic unit, or low enough to warrant management of the components within a complex, is difficult

to estimate. However, some measure of genetic structure, even as a first approximation given the associated management risks, is essential for the conservation of genetic resources and the appropriate analysis of population dynamics. This is especially important when the populations are subjected to intensive exploitation (Angel *et al.* 1994). Exploitation that incorrectly assumes panmixia (due to political or administrative constraints) can lead to the erosion of genetic resources via depletion of spawning components within a complex and is detrimental to the stock and to the species as a whole because it results in the selective removal of genetic resources (diversity) that may be, in part, responsible for resiliency. If the critical assumption of panmixia is invalid at any management level, then the management scheme is not only functionally inconsistent with the principles of resource conservation and the maintenance of biodiversity, it may actually be destructive. For cod (*Gadus morhua* L.), the importance of resolving cod stock structure is summarised by Rice (1997, p.2):

> "Clarifying the relationships among cod stock components . . . . is fundamental to sound and scientific assessment and management of cod, and conservation of biodiversity".

Atlantic cod are distributed in the western Atlantic from Labrador in the north (~63 ^{o}N) to Cape Hatteras in the south (~35 ^{o}N). Across this 3,000 km range their distribution is essentially contiguous on the continental shelf. However, different regions (e.g. NE Newfoundland Shelf, Gulf of St Lawrence etc.) encompass stocks or stock complexes that are explicitly recognised as management units. Spawning areas within the management units are relatively discrete, temporally stable and distant from other spawning areas on the shelf and elsewhere (Frank *et al.* 1994; Nakken 1994; Serchuck *et al.* 1994; Shopka 1994; Taggart *et al.* 1994). There are also differences in productive capacity among 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 stocks and their components constitute genetically distinct populations.

Different genetic markers vary in their ability to resolve genetic differences among cod populations. Analyses based on allozyme loci tend to show significant differences among 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 among populations throughout most of the species' range (Smith *et al.* 1989; Carr and Marshall 1991a; Árnason and Rand 1992; but see Dahle 1991), or within smaller geographic areas (e.g. Iceland; Árnason *et al.* 1992, Árnason and Palsson 1996), or among 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 among major management divisions in the NW Atlantic (e.g. Bentzen *et al.* 1996). Furthermore, studies using the microsatellite technique have revealed temporally stable population structure between inshore and offshore overwintering cod aggregations in

the Newfoundland region (Ruzzante *et al.* 1996a, 1997). This latter technique is important as, in general, Nielsen *et al.* (1997 and several references therein) consider microsatellites the best available genetic tool for studies on closely related groups of animals, partly because of their demonstrated high level of co-dominant allelic variation in repeat number (VNTR's: variable number of tandem repeats of di-nucleotides), their rapid divergence times that provide polymorphisms not available with other markers, and their relative ease of examination. The attributes of microsatellites as genetic markers and their application to fisheries have been reviewed elsewhere (Park and Moran 1994; Ward and Grewe 1994, Wright and Bentzen 1994; O'Reilly and Wright 1995; O'Connell and Wright 1997).

In this paper we discuss some of our recent and ongoing studies on the genetic structure of Atlantic cod populations in the Northwest Atlantic. Our goal is to highlight aspects of the our research approach and the results we have obtained that may be of significance in addressing questions related to localised stock structure in the Gulf of Maine. In all of our studies we have employed microsatellite DNA polymorphism to resolve genetic differences among cod populations and have generally done so under the null hypothesis of no significant differences between or among populations. However, in each case we also employ other biological and oceanographic "knowns" in our assessment of the genetic results - something we suggest is demonstrably, and perhaps critically, limited in most fishery related population genetic studies. We focus on three case studies, each of which illustrates genetic heterogeneity among cod populations at a variety of spatial scales, and some of which are consistent with the notion of localised stocks.

First, we examine small-scale processes by summarising evidence consistent with the existence of genetically defined larval cod cohorts within a well defined gyre-like feature on Western Bank of the Scotian Shelf. We illustrate how such features may play a critical role in stock structuring (e.g. Iles and Sinclair 1982). We also consider how the results link Cushing's (1972) match-mismatch hypothesis to Hedgecock's (1994) "sweepstakes" selection hypothesis. Next, we review the evidence for meso-scale population structure, genetic and otherwise, between inshore and offshore cod aggregations within the northern cod complex off Newfoundland. Subsequently, we examine emerging evidence for genetic differentiation among cod aggregations on the periphery of the Gulf of Maine (Georges Bank, Browns Bank, Bay of Fundy). Finally, we present suggestions for testing the working hypothesis of localised cod populations in the Gulf of Maine and examine some data that are consistent with this working hypothesis.

Sample and Tissue Collections, Genetic Techniques and Analyses

All of the genetic data presented here are derived from a subset of over 9,000 individual cod tissue samples collected between 1992 and 1997 in the Northwest Atlantic (Fig. 1) and elsewhere. Details of specific sample collections are provided in Ruzzante *et al.* (1996b) for the study on the genetic heterogeneity of a larval aggregation; in Ruzzante *et al.* (1996a, 1997) for the inshore-offshore genetic differentiation; and in Ruzzante *et al.* (in press) for those samples involving cod from Georges Bank, Browns Bank and the Bay of Fundy.

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.* (1996a). 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 behaviour (Goddard *et al.* 1994; Ruzzante *et al.* 1996a). In adult cod, high antifreeze activity (>0.2 ^{o}C thermal hysteresis) is indicative of overwintering in cold (<0 ^{o}C) water - generally inshore, while low or non-existent Winter antifreeze levels (<0.09 ^{o}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). Polymerase chain reaction (PCR) amplification was carried out as described by Brooker *et al.* (1994) using five dinucleotide microsatellite primers, *Gmo2*, *Gmo132*, and *Gmo145* (Brooker *et al.* 1994), *Gmo4* (Wright 1993), and *Gmo120* (Ruzzante *et al.* 1996a). A sixth locus (*Gmo141*) was used in the study on the genetic composition of a larval aggregation (Ruzzante *et al.* 1996b). *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 and alleles were sized relative to a sequence ladder generated from M13mp18 (Yanisch-Perron *et al.* 1985).

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). 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 (i.e. R_{ST} and F_{ST}) was estimated by bootstrapping (i.e. resampling with replacement; 1000 iterations) genotypes across individuals and populations and for each locus separately. Multilocus estimates of F_{ST} and R_{ST} were calculated by first averaging the numerators and denominators across loci and then calculating ratios as suggested by Weir and Cockerham (1984) and Slatkin (1995). Pairwise genetic distances among populations based on the Stepwise Mutational Model (SMM) were estimated using D_{SW} (Shriver *et al.* 1995). For comparative purposes we also estimated D_A (Nei *et al.* 1983), a non-SMM estimate of genetic distance with low variance relative to other non-SMM measures (Takezaki and Nei 1996), and $\delta\mu^2$ (Goldstein *et al.* 1995). Significance for distance measures was estimated by bootstrapping genotypes (1000 resampling trials with replacement) across individuals and populations for each

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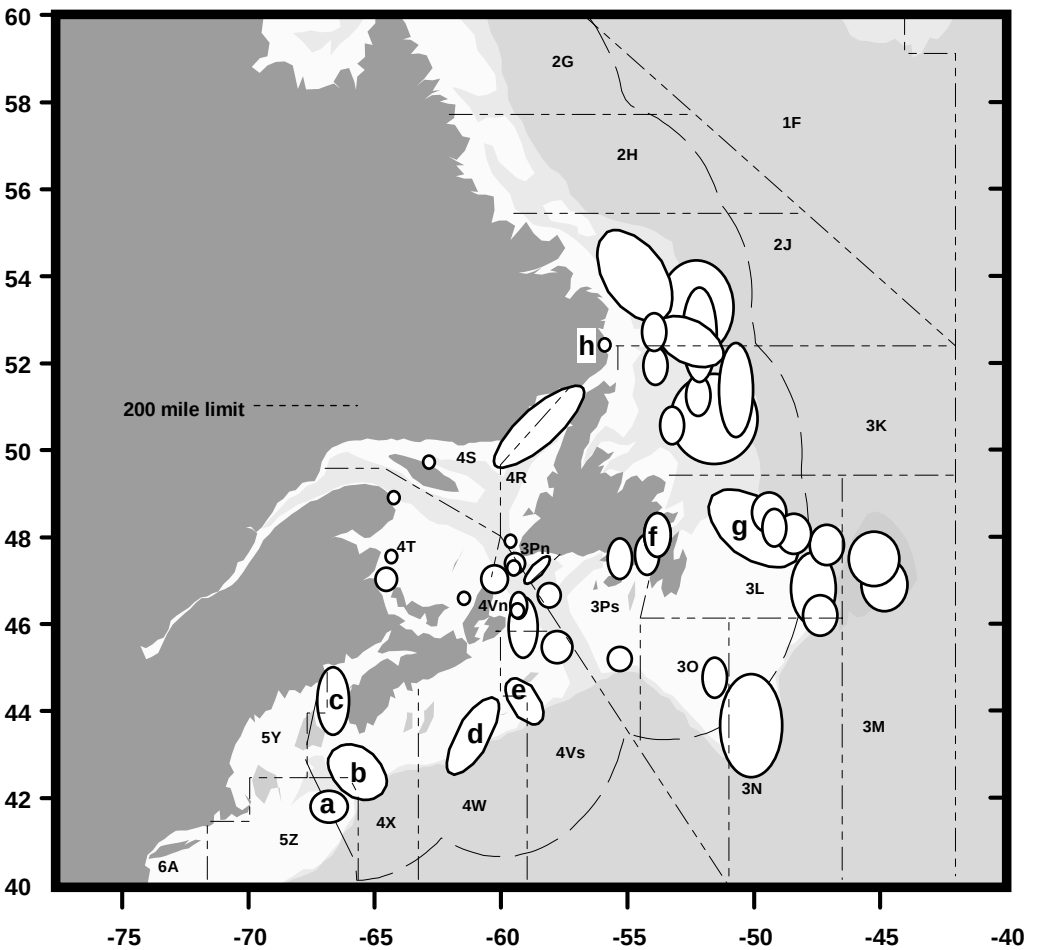

Figure 1. Bathymetric chart (1000 and 200 m isobath limits and landmass) of the NW Atlantic showing the North Atlantic Fisheries Organisation (NAFO) statistical and management divisions (e.g. 2J), the Canadian exclusive economic zone (200 mile limit), and sample population locations (geographic range for each collection defined by ellipse) from which a total of >9,000 individual cod tissues for use in genetic analyses have been collected over the period 1991-1997, including dried tissues taken from historical otolith collections dating from the 1960's, 70's and 80's. Locations for specific samples referred to in the text are from Georges Bank (a), Browns Bank (b), Bay of Fundy (c), Western Bank (d), Banquereau Bank (e), Trinity Bay (f), northern Grand Bank (g), and Gilbert Bay, Labrador (h).

locus separately. We also conducted tests of Hardy-Weinberg Equilibrium (HWE) and heterozygote deficiency (D) and estimated levels of significance by the bootstrap method. Bootstrapping in these two cases was conducted by resampling (with replacement) alleles across individuals and populations. All statistical tests and analyses of genetic distances and population structure were conducted using Splus© standard code or functions written by DER.

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 Western Bank of the Scotian Shelf (Fig. 1) 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.* 1996b) but not for a subset of the larvae that formed a single cohort on the basis of age-at-length (Table 1).

Table 1. 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 the entire larval cod aggregation (pool), those within the gyre-like crest water mass (CW) and the age-at-length cohort within the CW on Western Bank of the Scotian Shelf. Significant heterozygote deficiencies are highlighted in bold face.

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

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 subdivided, 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 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.* 1996b). 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 among subsets (Ruzzante *et al.* 1996b). 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 northeast, on Banquereau Bank (Fig. 1), indicated that no matter how measured, using either R_{ST} and F_{ST} (as in Ruzzante *et al.* 1996b) or using D_A , D_{SW} , and $\delta\mu^2$ (Table 2) 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 (at least two years) and are consistent with the notion of the existence of localised stock structure in cod at bank-scales (order 100 km).

Table 2. Measures of genetic distance (D_A , D_{SW} , and $\delta\mu^2$) and their respective levels of significance (below) between the larval cod cohort on Western Bank of the Scotian Shelf in November 1992 and adult cod sampled on Western Bank and Banquereau Banks two years later. Distances considered significant are highlighted in bold face.

Comparative groups	D_A	D_{SW}	$\delta\mu^2$
Larval cohort (n=316) vs Western Bank adults (n=48)	0.073	0.018	2.25
	0.137	0.180	0.359
Larval cohort (n=316) vs Banquereau Bank adults (n=48)	0.076	**0.067**	**5.89**
	0.054	**0.003**	**0.041**

The large larval aggregation described in this example was confined within a relatively small area of coastal ocean involving an anticyclonic eddy(order 20 km diameter) 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 (Wahlund effect; heterozygote deficiencies, Table 1). However, a subset (the common age-at-length 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 among 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 among cohorts in their contribution to recruitment. Such a process may explain part

of the relationship between observed and effective population sizes (Hedgecock 1994). There are other implications for these findings aside from those related to the "sweepstakes" selection hypothesis (Hedgecock 1994) and its ecological predecessor, the "match-mismatch" hypothesis (Cushing 1972). Finding a large larval cod aggregation within a spatially well defined (kilometre-scale) and temporally stable (at the scale of days to weeks) gyre-like system is consistent with the hypothesis that spawning products can be retained within well defined geographic areas on the Scotian Shelf. Thus, cod spawning aggregations from neighbouring banks may experience more or less independent dynamics.

Bay-Scale Inshore and Offshore Genetic Structure and Stability 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 degree of temporally stable fidelity to offshore spawning banks is sufficiently high for Lear (1984, p.157) 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".

The quantitative degree of that fidelity is readily apparent in Taggart (1997). 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 (1962, p.108) 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".

The regular occurrence of juvenile and adult cod overwintering in cold (<0 ^{o}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).

A pool of cod samples collected inshore in the area of Trinity Bay (Fig. 1), Newfoundland between 1992 and 1994 was genetically distinguishable from cod collected offshore on the northern Grand Bank (Fig. 1) 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 3). In all cases the genetic differences are small.

Table 3. Different measures of genetic distance (D_A , D_{SW}) and structure (R_{ST} and F_{ST}) and their respective levels of significance (below) among pools of various sizes (n_1 , n_2) of adult cod sampled offshore during Winter on the northern Grand Bank and inshore during late Winter in Trinity Bay. Inshore samples are further classified according to high or low levels of blood plasma antifreeze (AF) levels in the individual cod. Genetic distances and degrees of structure that can be considered significant are highlighted in bold face.

Comparative groups	n_1	n_2	D_A	D_{SW}	R_{ST}	F_{ST}
Inshore (n_1) vs.	308	140	**0.036**	**0.018**	**0.0062**	0.0008
Offshore (n_2)			**0.004**	**0.016**	**0.014**	0.084
Inshore high-AF (n_1) vs.	123	124	**0.048**	**0.024**	**0.0087**	0.0002
Offshore (n_2)			**0.007**	**0.015**	**0.014**	0.349
Inshore low-AF (n_1) vs.	58	124	0.061	-0.0003	-0.0010	0.0003
Offshore (n_2)			0.204	NA	NA	0.385
Inshore high-AF (n_1) vs.	123	58	0.047	-0.0073	-0.0003	-0.0012
Inshore low-AF (n_2)			0.796	NA	NA	NA

However, 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 ^oC) 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 previous set of comparisons which did not account for antifreeze content (Table 3). F_{ST} 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 3). Finally, inshore cod with high antifreeze content were not genetically distinguishable from inshore cod with low antifreeze content (Table 3). 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 4).

Table 4. R_{ST} and F_{ST} measures of genetic structure and significance (below) between inshore (n_1) and offshore (n_2) overwintering cod for each of 5 microsatellite loci and over all loci. Degrees of structure that can be considered significant are highlighted in bold face.

Structure measure	n_1	n_2	*Gmo2*	*Gmo4*	*Gmo120*	*Gmo132*	*Gmo145*	Overall
R_{ST}	308	140	-0.003	-0.002	**0.015**	0.005	**0.016**	**0.0062**
			NA	NA	**0.022**	0.102	**0.006**	**0.014**
F_{ST}	308	140	-	0.0004	**0.0016**	0.003	<0.0001	0.0008
			0.0008	0.277	**0.050**	0.070	0.420	0.084
			NA					

Collectively, the above results suggest that cod collected inshore, and in particular those exhibiting high antifreeze content (i.e. overwintering inshore) are genetically distinguishable from offshore overwintering cod. The pool of cod collected inshore and exhibiting low antifreeze content is hypothesised to represent a mixture of recently arrived offshore migrants and inshore overwintering cod, the latter having lost their blood antifreeze due either to movement into, and/or prolonged residence in, pockets of relatively warm water inshore (Ruzzante *et al.* 1996a).

The genetic differentiation between inshore overwintering cod from Trinity Bay and offshore overwintering cod appears to be stable over the period 1992 to 1995 as shown using a test set of samples. A new and different 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 cod collected inshore (and analysed above) during the period 1992 to 1994 (n=308; Table 5, line 1) 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, the 1995 inshore cod were genetically similar to the inshore cod in the 1992-94 period with high and low antifreeze, though more similar to the latter (Table 5, lines 2, 3). Despite evidence of genetic heterogeneity among 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 5, line 4).

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, are collectively, genetically indistinguishable from cod populations overwintering inshore in the same

general area during 1992-1994 and are genetically distinguishable from cod populations overwintering offshore along the edge of the continental shelf on the Grand Bank during 1992-94. 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 are now 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.

Table 5. Different measures of genetic distance (D_A , D_{SW}) and structure (R_{ST} and F_{ST}) and their respective levels of significance among pools of various sizes (n_1 , n_2) of adult cod sampled offshore during Winter on the northern Grand Bank during the period 1992-94 and inshore in Trinity Bay in 1992-94 and in 1995. Inshore samples are further classified according to high or low levels of blood plasma antifreeze (AF) levels in the individual cod. Genetic distances and degrees of structure that can be considered significant are highlighted in bold face.

Comparative groups	n_1	n_2	D_A	D_{SW}	***R_{ST}	F_{ST}
inshore 95 (n_1) vs all inshore 92-94 (n_2)	150	308	**0.039** **<0.001**	0.003 0.235	0.002 0.142	-0.0001 NA
****inshore 95 vs (n_1) vs inshore 92-94 high-AF (n_2)	150	123	**0.054** **<0.001**	0.004 0.270	0.005 0.051	-0.0001 NA
inshore 95 (n_1) vs inshore 92-94 low-AF (n_2)	150	58	0.062 0.161	-0.011 NA	-0.002 NA	0.0006 0.297
inshore 95 (n_1) vs offshore 92-94 (n_2)	150	140	**0.047** **<0.001**	**0.019** **0.021**	**0.010** **<0.001**	0.0014 0.057

***R_{ST} values here differ from those in Ruzzante *et al*. (1997) where the method of Slatkin (1995) was used. Here we use the derivation of Goodman (1997) but the inferences remain the same.

****R_{ST} and F_{ST} values for this comparative group are correct here and although they were reported incorrectly in Ruzzante *et al*. (1997), the inferences remain the same.

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 95 pool vs offshore 1992-94 pool showed that the genetic distance between the inshore and offshore pools remained virtually unchanged and significant at 2.10 and 2.17 respectively (Ruzzante *et al.* 1997).

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Additional evidence for bay-scale population structure is found in very recent and preliminary results based on microsatellite DNA analyses (unpublished data) of overwintering cod (N=11) collected from Gilbert Bay (Fig. 1), inshore Labrador (Wroblewski 1997; Wroblewski 1998 this volume). Cod from Gilbert Bay show allele distributions (loci *Gmo151* and *Gmo4*) that are strikingly different from offshore collections of northern cod and we are confident that these cod are neither *Boreogadus saida*, nor *Gadus ogac* (Fig. 2).

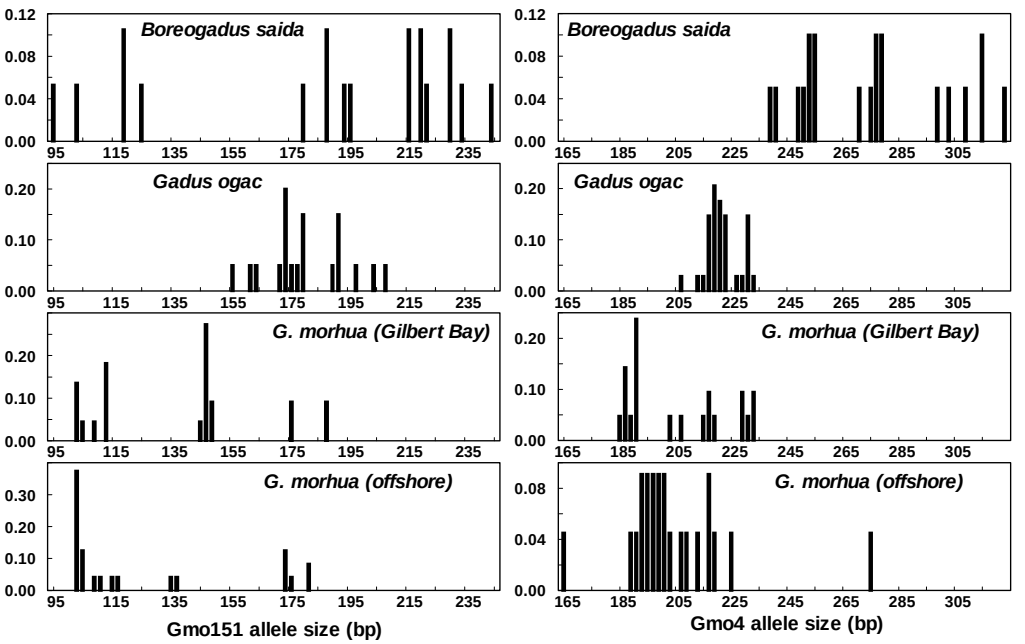

Figure 2. Proportional allele size (base-pair) frequency distributions for *Gmo151* and *Gmo4* microsatellite loci in samples of *B. saida, G. ogac*, and *G. morhua* where the latter were collected from a pool of overwintering cod in Gilbert Bay (inshore Labrador) and a pool of offshore northern cod.

Bank-scale Genetic Structure in Browns Bank, Georges Bank and Bay of Fundy Cod

Our last example involves a comparative examination of cod from three neighbouring regions on the periphery of the Gulf of Maine: Browns Bank (western Scotian Shelf), the Bay of Fundy, and Georges Bank (Fig. 1, 3).

Although all three areas are geographically very close to each other, they are characterised by distinct oceanographic regimes (systems, features, etc.). Georges Bank and Browns Bank are bathymetrically separated by the Fundian Channel and each is characterised 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), but not absolutely so (Townsend and Pettigrew 1996). The evidence thus suggests that retention mechanisms resulting from topographically induced eddies act to minimise dispersal of ichthyoplankton among these regions (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.

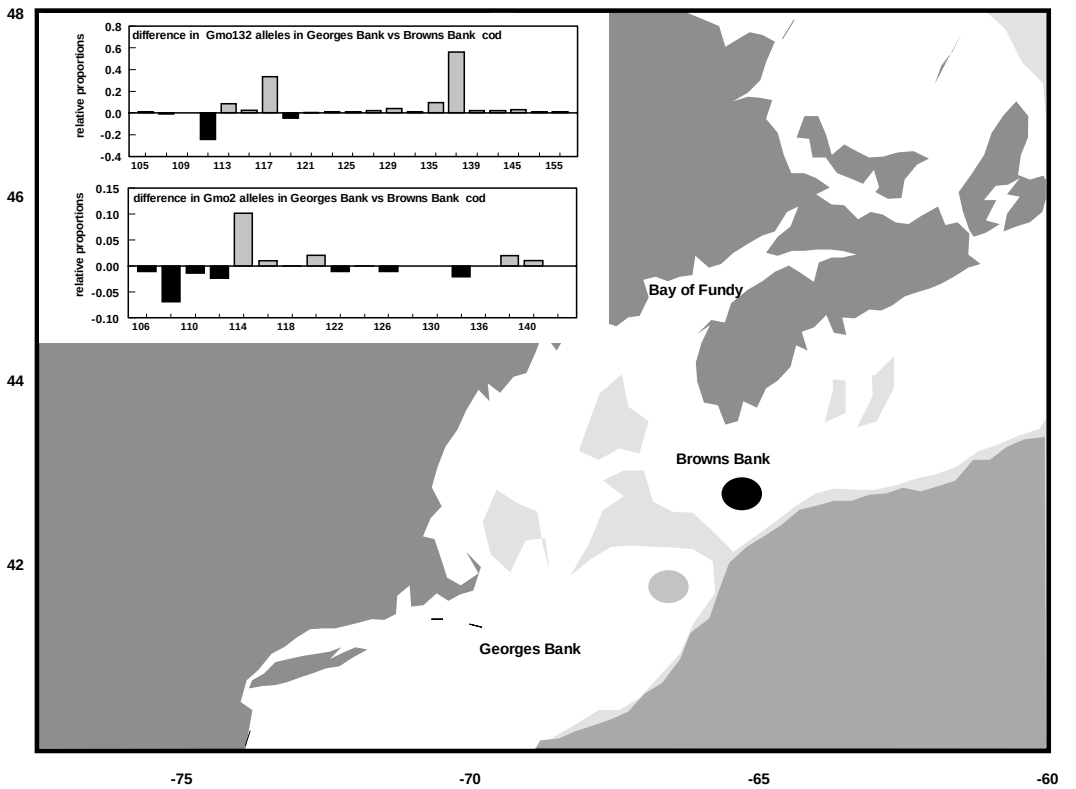

Figure 3. Bathymetric chart (1000 and 200 m isobath limits and landmass) of the Gulf of Maine region showing the sample locations for adult cod on either side of the Fundian Channel on Georges and Browns Banks and the relative proportions between the two populations for the *Gmo132* and *Gmo2* microsatellite alleles.

Despite their geographic proximity, cod collected from Georges and Browns Banks and the Bay of Fundy (Fig. 1) are genetically distinguishable from each other when examined with any one of four different genetic measures of distance and population structure (Tables 6 and 7). The genetic differences among these populations appear to be primarily related to the *Gmo132* and *Gmo145* loci, and to a lesser extent *Gmo4* (Table 7, Fig. 3).

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. This conclusion is supported by similar findings among fourteen different cod populations spanning the range of cod from Labrador to Georges Bank (Ruzzante *et al*. In Press). Maximum spawning time differs

among 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).

Table 6. D_A and D_{SW} measures of genetic distance (above the diagonal) and their degree of significance (below the diagonal) among adult cod collected from Browns and Georges Bank and the Bay of Fundy. Differences that can be considered significant are highlighted in bold face.

D_A	Browns Bank	Georges Bank	Bay of Fundy
Browns Bank (N=48)	--	0.118	**0.136**
Georges Bank (N=48)	0.066	--	0.116
Bay of Fundy (N=48)	**0.001**	0.085	--

D_{SW}	Browns Bank	Georges Bank	Bay of Fundy
Browns Bank (N=48)	--	**0.139**	**0.175**
Georges Bank (N=48)	**0.001**	--	0.036
Bay of Fundy (N=48)	**<0.001**	0.098	--

Table 7. R_{ST} and F_{ST} measures of genetic structure and significance (below) among adult cod collected from Browns and Georges Banks and the Bay of Fundy for each of 5 microsatellite loci and over all loci. Differences that can be considered significant are highlighted in bold face.

	Gmo2	*Gmo4*	*Gmo120*	*Gmo132*	*Gmo145*	Overall
R_{ST}	-0.005	0.014	-0.006	**0.091**	**0.030**	**0.025**
	NA	0.160	NA	**<0.001**	**0.027**	**<0.001**
F_{ST}	-0.002	0.003	-0.001	**0.030**	**0.005**	**0.007**
	NA	0.101	NA	**<0.001**	**0.050**	**<0.001**

Localised Population Structure in the Gulf of Maine: Evidence For A Testable Hypothesis

The case studies reviewed above provide sufficient evidence that the null hypothesis of "*no measurably significant genetically-based population structure in Atlantic cod"* can be rejected in some regions of the NW Atlantic at the scale of oceanographically determined water masses, and at bay-scales and bank-scales. Furthermore, spawning distributions, bathymetric and oceanographic structure, physiological adaptations, and tagging studies are consistent with the genetic evidence. Some of the tagging studies referred to above show perhaps the most consistent evidence of population-based spawning ground fidelity. The most important

studies are those that focussed on tag return distributions in time and space for fish that were tagged on or near their spawning grounds while in pre- or post-spawning aggregations (e.g. Lear 1984, Taggart 1997), and not while in a migratory phase and/or on the feeding grounds. Spawning ground fidelity is necessary (though not necessarily sufficient) for the establishment and maintenance of discrete populations. We have provided evidence above that there is bank-scale population structure on the periphery of the Gulf of Maine, - but what of the Gulf of Maine itself?

Perkins *et al.* (1997) provide some intriguing data based on cod tagging studies conducted on the spawning grounds in the Sheepscot Bay region in the Gulf of Maine during the period 1978 to 1983. We reanalysed their data in a different manner and focussed on the spatial and seasonal pattern in the tag recapture reporting rates (again excluding those reported within the first year subsequent to tagging) relative to the spawning (tagging) region (Fig. 4). From a spatial perspective, an objectively determined contour of the tag reporting rate shows that >70% of the reported returns are from the immediate vicinity (within 30 nautical miles; nm) of the original tagging region and <20% are reported at ranges exceeding 60 nm from the tagging region. From a temporal perspective it is clear that the highest proportion (>60%) of tag returns are reported from the region where they were originally tagged at or just prior to spawning, and consistently so during subsequent spawning periods (May-July, Fig. 4; and see Perkins *et al.* 1997). There is also evidence of overwintering fidelity to the spawning region as illustrated by the 60% reporting rate from the tagging region during January. It is reasonable to integrate these spatial and temporal patterns in tag reporting to hypothesise (as in Perkins *et al.* 1997) the existence of a localised spawning population in the Sheepscot Bay region with the following behavioural cycle: a) an overwintering fidelity period in the spawning region; followed by b) an along-coast feeding dispersal (see Perkins *et al.* for detailed distribution of tag reporting); followed by c) regional spawning fidelity; followed by d) post-spawning dispersal until Winter. Genetic analyses similar to those outlined above would represent one testable component of the above hypothesis, especially if the analyses were conducted in comparison to spawning cod from either the Georges Bank or the Bay of Fundy region.

Finally, we ask if is it reasonable to expect that the tagging-based evidence of localised populations in the Gulf of Maine can be supported by genetic evidence? Our speculative answer is yes, at least when based on a comparison with tagging studies conducted in the Trinity Bay region of Newfoundland for which we have shown above there is considerable genetic evidence for a localised population. Thus, we reanalysed the tagging data from Taggart *et al.* (1995) for the Trinity Bay region (see also Wroblewski *et al.* 1996) in a manner similar to our reanalyses of the Perkins *et al.* (1997) data for the Gulf of Main illustrated in Figure 4. Again we focussed on the spatial and seasonal pattern in the tag reporting rates (excluding those reported within the first six months subsequent to tagging) relative to the overwintering (tagging) region (Fig. 5).

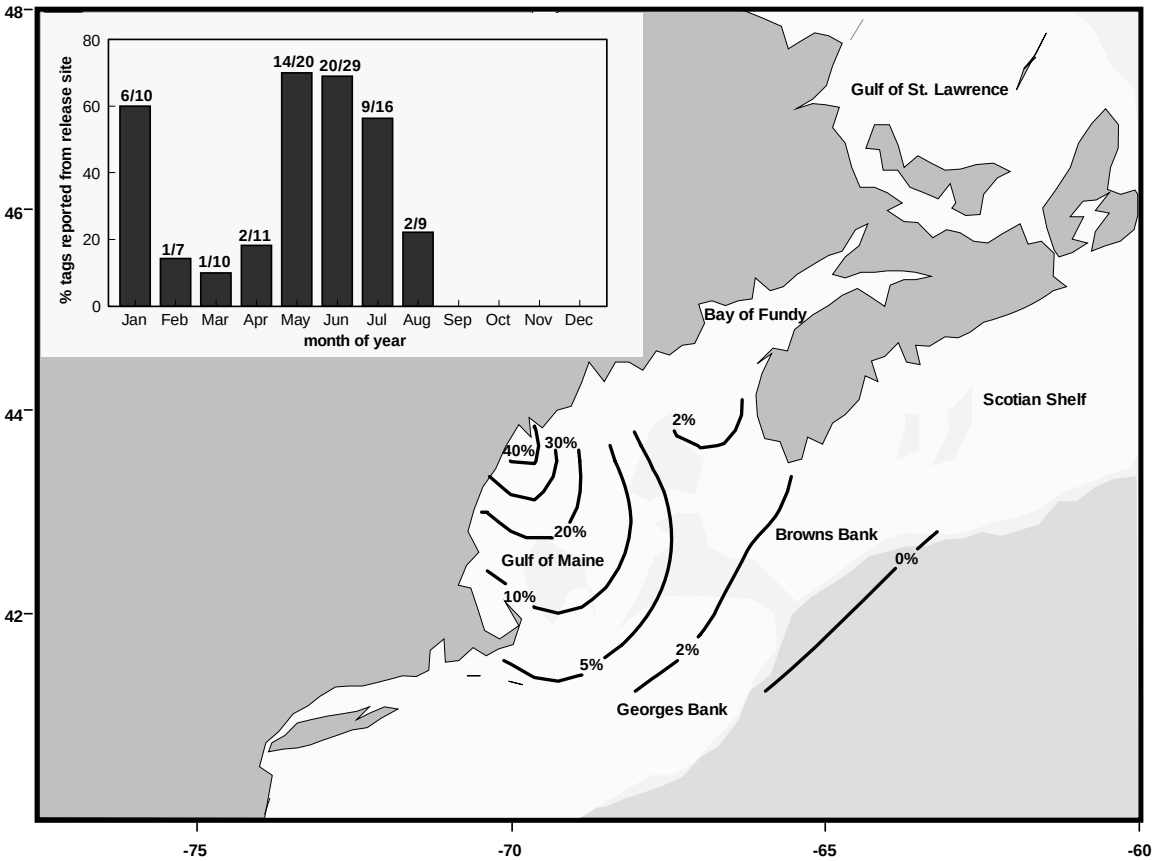

Figure 4. Bathymetric chart (1000 and 200 m isobath limits and land mass) of the Gulf of Maine showing objective contour isopleths of the percent tag reporting rate for the period 1978-85 for cod originally tagged and released during the spawning period in the Sheepscot Bay region and an inset showing the percent seasonal tag reporting rate from the original release region as derived from data in Perkins *et al.* (1997).

As in the Gulf of Maine, an objectively determined contour of the tag reporting rate shows that >70% of the reported returns are from the immediate vicinity (within 30 nm) of the original tagging region and <20% are reported at ranges exceeding 60 nm from the tagging region. Again, as in the Gulf of Maine, it is clear that the highest proportion (>60%) of tag returns are reported from the region where they were originally tagged prior to the spawning period, and consistently so during the Summer inshore spawning period (see Smedbol and Wroblewski 1997). There is also evidence of overwintering fidelity to the spawning region during Winter (e.g. December period, Fig. 5). Furthermore, the behavioural cycle hypothesised above for the putative Sheepscot Bay cod population in the Gulf of Maine could equally apply here, though the seasonal pattern in tag reporting rates suggest a higher degree of regional fidelity.

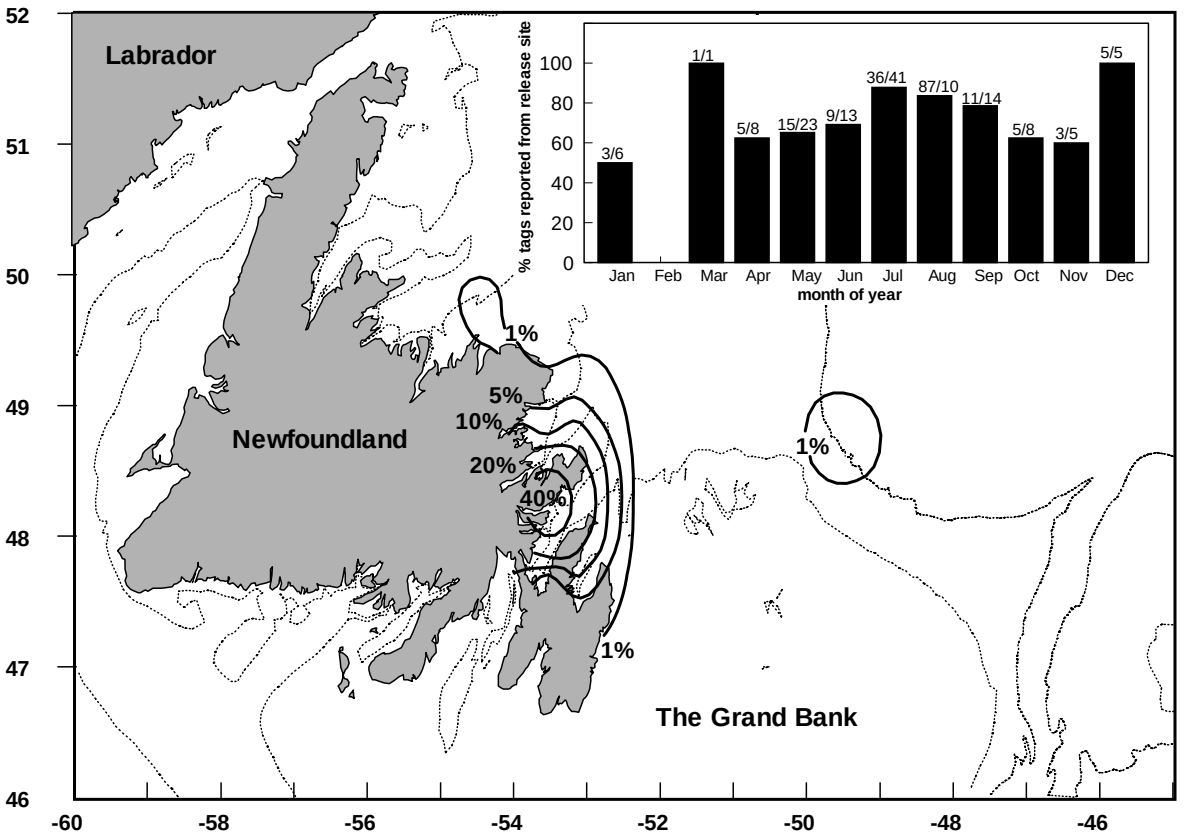

Figure 5. Bathymetric chart (1000 and 200 m isobaths) of the Newfoundland region showing objective contour isopleths of the percent tag reporting rate for the period 1988-95 for cod originally tagged and released during the pre-spawning overwintering period in the Random Island region of Trinity Bay during 1988, 1990, and 1991, and an inset showing the percent seasonal tag reporting rate from the original release region as derived from data in Taggart *et al.* (1995).

The above comparison leads us to a testable hypothesis: *measurably significant structure in localised coastal cod populations in the NW Atlantic is likely to be revealed at scales of 60 to 100 nm when focussed on spawning aggregations using a combination of nuclear DNA microsatellites and conventional tagging studies.* Contemporary concerns about cod stock structure, health, and the new management strategies that are evolving could be well served by a rigorous test of this hypothesis.

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