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Population structure of Atlantic cod (*Gadus morhua*) in the Newfoundland and Labrador area determined from genetic variation

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Abstract

The purpose of this study was to describe population structure and to determine the potential for genetic stock identification of Atlantic cod (*Gadus morhua*) in Newfoundland and Labrador using microsatellite loci, synaptophysin (SypI) locus, and a major histocompatibility complex (Mhc) locus. Variation at seven microsatellite loci (*Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, and *Gmo*37) and SypI was surveyed in approximately 5,050 cod from 19 putative populations. Variation at a class I Mhc locus was surveyed in 2,000 fish from the 19 populations. Ten populations were sampled over two or more years, and variation among populations was on average about 18 times greater than annual variation within populations. Regional structuring of the populations was apparent with inshore and offshore spawning populations forming distinct groups. The Flemish Cap population was the most distinctive of the offshore group, and the Gilbert's Bay population in Labrador was the most distinctive of the inshore group. In Divisions 2J3KL, no significant genetic differentiation was observed among inshore cod sampling sites in Notre Dame Bay and Bonavista Bay. Some differentiation was observed between sites in Conception Bay and Trinity Bay, and also with other inshore sites, providing some evidence of distinct "bay" stocks of cod along the northeast coast of Newfoundland. All inshore cod samples were genetically distinct from all offshore samples of northern cod. The offshore samples were more heterogeneous, and there may be at least three distinct offshore spawning populations of northern cod. In Subdiv. 3Ps, genetic differentiation was observed between the inshore Placentia Bay and Fortune Bay samples, and the Placentia Bay sample was distinct from offshore samples of northern cod. Simulated mixed-stock fishery samples of northern cod suggested that variation at the seven microsatellite loci, the synaptophysin locus, and Mhc locus C should provide reasonably accurate estimates of stock composition (inshore vs. offshore) when the inshore component comprises at least 50% of the mixture. In Subdiv. 3Ps, bias of estimated stock compositions was marginal when offshore populations (Burgeo Bank, Halibut Channel) comprised the majority of the sample. However, bias in the estimated stock compositions increased when inshore populations comprised the majority of the sample. Increased baseline population sample sizes or additional discriminating markers are likely required to decrease the degree of bias in the estimated stock composition in this application.

Résumé

La présente étude avait pour objectif de décrire la structure des populations et de déterminer la possibilité d'identifier génétiquement les stocks de morue de l'Atlantique (Gadus morhua) de Terre-Neuve et du Labrador en utilisant les locus de microsatellites, le locus de la synaptophysine (SypI) et le locus d'un important complexe d'histocompatibilité (Mhc). La variation à sept locus de microsatellites (Gmo3, Gmo8, Gmo19, Gmo34, Gmo35, Gmo36 et Gmo37) et à celui de la SypI a été déterminée chez environ 5 050 morues provenant de 19 populations présumées. La variation à un locus Mhc de classe I a été étudiée chez 2 000 poissons de 19 populations. Dix populations ont fait l'objet de prélèvements au cours d'une période d'au moins deux années et la variation entre les populations était en moyenne 18 fois supérieure à celle notée au sein des populations. Une structuration régionale des populations était apparente, les populations de géniteurs côtiers et hauturiers formant des groupes distincts. La population du Bonnet Flamand était la plus distincte du groupe hauturier et celle de Gilbert's Bay, au Labrador, la plus distincte du groupe côtier. Aucune différenciation génétique significative n'a été observée entre les sites d'échantillonnage de morues côtières des baies Notre-Dame et Bonavista, dans les divisions 2J3KL. Une certaine différenciation a été notée entre les sites de Conception Bay et Trinity Bay, et à d'autres sites côtiers, ce qui constitue un indice de l'existence de stocks de morue de « baie » distincts le long de la côte nord-est de Terre-Neuve. Tous les échantillons de morue côtière différaient génétiquement de tous les échantillons de morue du Nord hauturière. Les échantillons de morue hauturière étaient plus hétérogènes et il pourrait exister au moins trois populations de géniteurs hauturiers distinctes de morue

du Nord. Dans la sous-division 3Ps, une différenciation génétique a été notée entre les échantillons côtiers de Placentia Bay et Fortune Bay et l'échantillon de Placentia Bay différait des échantillons hauturiers de morue du Nord. Des échantillons d'une pêche simulée d'un stock mixte de morue du Nord indiquaient que la variation aux sept locus de microsatellites, au locus de la synaptophysine et au locus C du Mhc devraient donner des estimations raisonnablement exactes de la composition des stocks (côtiers ou hauturiers) lorsque la composante côtière représente au moins 50 % du mélange. Dans la sous-division 3Ps, le biais de la composition estimée du stock était marginal lorsque les populations hauturières (Burgeo Bank et Halibut Channel) constituaient la majorité de l'échantillon. Mais le biais augmentait lorsque les populations côtières représentaient la plus grande partie de l'échantillon. Il sera sans doute nécessaire de disposer d'effectifs d'échantillons de base plus importants ou de marqueurs discriminateurs supplémentaires pour réduire le biais de l'estimation de la composition des stocks dans cette application.

Introduction

Atlantic cod (*Gadus morhua*) historically supported substantial fisheries in the northwest Atlantic Ocean (Halliday and Pinhorn 1996). However, declining abundance in the 1980s and early 1990s, attributed largely to overexploitation (Myers et al. 1997) and with some effect of environmental changes (Rose et al. 1994), led to closure of Canadian fisheries beginning in 1992. The decline of greatest magnitude occurred in the northern cod stock complex off Labrador, the East coast of Newfoundland and the northern edge of the Grand Bank (NAFO Divisions 2J3KL). Some limited fisheries have since reopened, largely along the southern coast of Newfoundland (NAFO subdivision 3Ps). There are aggregations of adult cod in some inshore areas off northeastern Newfoundland (Brattey 1996), but there has been little recovery in offshore sites formerly occupied by the northern cod stock complex (Rice 1997; Lilly et al. 1998). An issue of current concern is whether exploitation of inshore populations will inhibit rebuilding of offshore populations. If inshore and offshore spawning groups of northern cod are components of a single stock, then it is possible that inshore locales could contribute recruits to offshore sites. If inshore and offshore populations constitute separate stocks, with independent population dynamics, then there is unlikely to be recruitment to offshore spawning areas by inshore cod, and limited exploitation of inshore populations would not influence recovery of offshore populations.

Stock structure of cod adjacent to Newfoundland and Labrador has been investigated with a variety of techniques. Early work (reviewed by Halliday and Pinhorn (1990)) centered on age, growth, sexual maturity (Fleming 1960), tagging, parasites, and vertebral counts (Templeman 1974; Templeman et al. 1976; Templeman 1981). These characters, influenced by both environmental and genetic factors, were used to delineate the major northern cod stock complex, and other stocks located further south on the Grand Bank, at Flemish Cap, and in locations along the southern and western shores of Newfoundland. Tagging has indicated that the northern cod stock may have a number of partially isolated subcomponents (Lear 1984), even possibly at the geographic scale of coastal bays (Taggart et al. 1995). The level of reproductive isolation, if any, among these subcomponents or local populations, is uncertain. Delineation of population structure is fundamental to the assessment, conservation, and management of Atlantic cod. Genetic differentiation at neutral genetic loci among spawning groups, indicative of restricted gene flow and independent population dynamics among the groups, is a good indicator of population structure. Moreover, if sufficient genetic differentiation is observed among populations, the genetic markers can be used to provide estimates of population or stock composition in areas of population mixing. This enables determination of catch by population with subsequent estimation of exploitation rates, allowing managers to protect less productive populations from overexploitation in regions of mixing.

Fidelity of spawning individuals to specific areas, with little exchange of spawners among areas, is a basic requirement in the designation of a "stock". The restriction of gene flow among spawning groups that results from this fidelity enables the development over time of genetic differentiation. For a marine fish such as Atlantic cod, a stock may consist of a single large, randomly-breeding aggregate, or may be subdivided into smaller groups within which mating is random, but among which there is more limited exchange of individuals. These local populations within a stock are more similar to each other than to populations in another stock complex. Analysis of genetic variation in Atlantic cod has not revealed an entirely consistent pattern of stock structure in the northwest Atlantic Ocean. Given that there are aggregations of adult cod in some inshore areas of northeastern Newfoundland, with little recovery in offshore locations, the issue of inshore and offshore stock structure is of considerable practical significance in formulation of exploitation strategies. Surveys of variation at allozyme loci indicated the existence of three major cod stocks in North America (Cross and Payne 1978). Little variation was observed in North American cod in mitochondrial DNA (Carr and Marshall 1991; Pepin and Carr 1993) and the mitochondrial results have been used to suggest that inshore and offshore cod

populations constitute one stock (Carr et al. 1995). However, higher levels of genetic variation have been observed at microsatellite loci (Bentzen et al. 1996; Ruzzante et al. 1998) and a comparison of northern cod samples from inshore bays with those from offshore sites suggested that separate stocks exist (Ruzzante et al. 1996; 1997; Beacham et al. 1999). The genetic structure of cod spawning in inshore and offshore sites needs to be resolved.

The primary objective of this study was to use microsatellite variation to investigate population structure of cod around Newfoundland and Labrador and in particular to examine the degree of genetic differentiation between inshore-spawning populations and offshore-spawning populations to determine whether they constitute separate stocks. We also examined whether there are distinct "bay stocks" of cod, i.e., is there genetic differentiation among cod populations in neighboring bays along the northeastern coast and southern coasts of Newfoundland? Finally, we evaluated the utility of using genetic variation for estimation of stock composition in mixed-stock fisheries.

Materials and Methods

Collection of DNA samples and PCR

Blood, heart, or muscle samples were collected from pre-spawning, spawning, or post-spawning cod from approximately 5,050 fish in several locations around Newfoundland (Table 1, Fig.1). All inshore samples were collected during the spring spawning season, but most offshore northern cod samples were collected in the fall. For spring samples, the gonads of individual fish were examined to determine maturation stages and samples were only collected from fish with ripe, running, or partly spent gonads; immature and spent fish were not sampled. For fall samples, fish with ripening gonads were sampled. For the tissue samples, approximately 0.3 g of tissue was placed in each well of a 96-well plate containing 0.2 ml of 5% chelex in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA pH 8.0, 0.10 mg/ml proteinase K, and 0.1% SDS) and incubated for 15 min at 50°C, and then incubated for an additional 15 min at 95°C. The supernatant from each well was collected and placed in a fresh 96-well plate and stored at -20°C. About 1 µl of this extract was required for each amplification of the sample by the polymerase chain reaction (PCR).

Primers for microsatellite loci developed at the Pacific Biological Station were: *Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, and *Gmo*37 (Miller et al. 2000), as well as the synaptophysin locus (SypI) (Fevolden and Pogson 1997). Variation at the α2 exon of the class I major histocompatibility complex (Mhc) locus Mhc-*Gamo*-C (hereafter called locus C) was surveyed using denaturing gradient gel electrophoresis (DGGE) technology (Miller et al. 1999). For all microsatellite primer sets, PCR was conducted in 12.5-µl reactions containing 15 pmol (0.60 µM) each primer, 0.3 µL DNA polymerase, 80 µM each nucleotide, 20 mM Tris-pH 8.8, 2 mM MgSO4, 10 mM KCl, 0.1% Triton X-100, 10 mM (NH4)SO4, and 0.1 mg/ml nuclease-free Bovine Serum Albumin. All microsatellite PCR in this study was preceded by an initial denaturation step of three min at 94°C. All cycle extension steps (30 cycles for all loci) were for 60 sec at 72°C and all cycle denaturation steps were for 20 sec at 94°C. PCR amplification of loci *Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, *Gmo*37 and SypI was accomplished with annealing temperatures of 46°C, 50°C, 50°C, 50°C, 55°C, 50°C, 46°C, and 55°C respectively. Annealing times were 60 sec. for all loci. For *Gmo*19 and *Gmo*34 together, PCR amplification was conveniently multiplexed in the same 12.5-µl reactions. SypI PCR products were digested with DraI (New England Biolabs, Ontario, Canada) for 2 hours at 37°C. A nested PCR was used to amplify a specific region of the Mhc locus C using primers developed by Miller et al. (unpub.). The initial denaturation step was conducted for 60 sec at 94°C for both PCR, with a subsequent annealing temperature of either 50° or 52°C for 60 sec or 120 sec. All cycle extension steps (35 cycles for both PCR) were for 2 min at 72°C, with a final extension of 10 min at 72°C.

Gel electrophoresis and band analysis

Microsatellite PCR products were size fractionated on 16 cm by 17 cm non-denaturing polyacrylamide gels and visualized by staining with 0.5 mg/ml ethidium bromide in water and ultraviolet light illumination. Nelson et al. (1998) provide a complete description of gel electrophoretic conditions. All microsatellite gels were run for 14-18 hr at 65-70 V, using 8% acrylamide for analysis of *Gmo*3, *Gmo*36, and *Gmo*37, and 10% acrylamide for analysis of *Gmo*8, *Gmo*19, *Gmo*34, and *Gmo*35. Twenty-nine lanes per gel were loaded, with one outside lane containing one-kb ladder (Gibco BRL), three lanes containing 20-bp ladder (Gensura Labs Inc., Del Mar, CA) evenly spaced across the gel, one lane containing DNA from a standard fish to determine precision of estimation of allele size, and 24 lanes of DNA amplified from individual fish for analysis.

Gels were scanned at a 1024 x 1024 pixel density with a Kodak charge coupled device camera with low light capability and a yellow filter. Images were analyzed using BioImage Whole Band software (Millipore Corp. Imaging Systems, Ann Arbor, Michigan), with the size of the amplified microsatellite alleles reported to the nearest bp based upon the molecular size grid created with the 20-bp markers.

As some uncertainty occurred in estimation of microsatellite allele size from the 20-bp grid, we identified alleles on the basis of a binning procedure (Gill et al., 1990). Peaks in the estimated allele size frequency distribution by base pair were used to identify alleles empirically, and bin widths generally corresponding to a repeat unit were set with the peak occurring in the middle of the bin. Precision of estimation of allele size was evaluated with the standard fish analyzed for each locus.

Standards for the DGGE gels used in the Mhc analysis were comprised of observed alleles at the locus. These observed alleles were used in an analogous manner to the size standards in the microsatellite analysis, and alleles in the fish that were analyzed identified with a grid created with the standard alleles.

Data Analysis

Annual variation in allele frequencies within populations was tested with GENEPOP version 3.1 with the Markov-Chain approach using χ^2 probability values (Raymond and Rousset 1995). The dememorization number was set at 1,000 and 50 batches were run for each test with 1,000 iterations/batch. Each population in each year (31 total comparisons) at each locus was tested for departure from Hardy-Weinberg equilibrium using GENEPOP. Tests of genetic differentiation utilizing pairwise comparisons among the populations were also conducted using GENEPOP with the Markov-Chain approach using χ^2 probability values. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice, 1989). Cavalli-Sforza and Edwards (1967) chord distance was used to estimate distance among populations. An unrooted neighbor-joining dendrogram of northern cod samples was generated with PHYLIP (Felsenstein 1993). F_{ST} estimates for each locus were calculated with GENEPOP, as was an estimate of gene flow between inshore-spawning and offshore-spawning cod in Divs. 2J3KL. Estimation of variance components of stock differences and annual variation within stocks was determined with BIOSYS (Swofford and Selander, 1981).

Estimation of stock composition

The utility of variation at microsatellite loci for the practical assessment of stock composition in mixed-stock fisheries of Atlantic cod was evaluated from the accuracy and precision of stock composition estimates obtained for simulated fishery samples. The evaluation was conducted by determining allele frequencies for each locus in each stock, and using the model of Fournier et al. (1984) to estimate stock composition by the conditional maximum likelihood method. Eleven alleles were scored at *Gmo*3 but were subsequently condensed by binning into 4 alleles (providing 10 genotypes). The 24 alleles scored at each of *Gmo*8 and *Gmo*19 were condensed to 7 alleles (28 genotypes at each locus) by binning adjacent alleles. There was no binning of the 9 alleles scored at *Gmo*34 (45 genotypes) or 13

alleles at *Gmo*35 (91 genotypes). The 13 alleles detected at *Gmo*36 were condensed to 9 alleles (45 genotypes) and the 15 alleles at *Gmo*37 were condensed to 7 alleles (28 genotypes). No binning of the two alleles was done at *Syp*I, but 38 alleles scored at Mhc locus C were condensed to 23 alleles (276 genotypes) for estimation of stock composition. Combining low frequency adjacent alleles reduced the number of genotypic frequencies to be estimated using the available samples, with little or no loss in the ability to discriminate among samples. For the microsatellite loci, baseline genotypic frequencies for each of the stocks were calculated from the observed allele frequencies under the assumption of Hardy-Weinberg equilibrium. Observed genotypic frequencies were used for the SypI locus and Mhc locus C as genotypic frequencies in each of these loci were not those expected under Hardy-Weinberg equilibrium. Each baseline stock was resampled with replacement in order to simulate the random variation involved in the collection of the baseline samples during the estimation of stock composition of each mixture. Hypothetical fishery samples of 200 fish with fixed stock composition were generated by randomly resampling with replacement the baseline stocks, and adding the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then determined, with the whole process repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates.

Results

Precision of estimation of allele size

Standard deviations of the estimate of allele size for the heterozygous standard fish analyzed at each locus ranged from 0.43 to 0.92 bp, with the larger alleles estimated with the least precision (Table 2). For the trinucleotide-repeat *Gmo*35 locus, all estimated sizes of a particular standard allele were within a three-base pair (bp) interval. For the trinucleotide repeat *Gmo*36 locus, all estimated sizes of the smaller (185 bp) allele were within a three-bp interval, and 97% (91/94) of the estimated sizes of the larger allele were within a three-bp interval. Estimated sizes for the alleles of the standard fish at the tetranucleotide-repeat *Gmo*3, *Gmo*8, *Gmo*19, and *Gmo*34 loci were all within a four-bp interval. At the *Gmo*37 locus, estimated allele sizes for the 237 bp allele were within a four-bp interval for 99% (95/96) of occurrences, and those of the 285 bp allele were within a four-bp interval for 98% (94/96) of occurrences. Precision of estimation of allele size was well within the range required for consistent determination of size. There was no size variation in the Mhc alleles, only sequence variation, and alleles at Mhc locus C were scored directly from the standards containing all known alleles.

Variation within populations

Observed heterozygosities of the loci examined over all populations were as follows: *Gmo*3 0.31 (population range 0.23-0.44), *Gmo*8 0.84 (0.73-1.00), *Gmo*19 0.87 (0.60-1.00), *Gmo*34 0.45 (0.00-0.48), *Gmo*35 0.73 (0.56-0.82), *Gmo*36 0.57 (0.38-0.73), *Gmo*37 0.79 (0.45-0.82), SypI 0.53 (0.18-0.87), and Mhc locus C 0.49 (0.40-0.74). Genotypic frequencies observed at the seven microsatellite loci surveyed in our study were those expected for populations in Hardy-Weinberg equilibrium with a few exceptions. Of 31 tests conducted at each locus (217 total tests for microsatellites), three populations were not in Hardy-Weinberg equilibrium at *Gmo3,* seven populations at Gmo8, four populations at Gmo19, one population each at Gmo34 and Gmo35, and five populations each for Gmo36 and Gmo37. There was no evidence of a consistent departure of genotypic frequencies from Hardy-Weinberg distribution at any locus, indicating that null alleles were not in significant frequency. Over all microsatellite loci, the Burgeo Bank population samples accounted for four of the 26 tests that were significant, as did the Div. 3O population sample. Nonconformance to Hardy-Weinberg equilibrium was due to an excess of homozygous fish at the loci concerned. This indicates that these samples may have contained fish from two separate spawning populations (homozygote excess due to the Wahlund effect).

At SypI, significant departures from the expected Hardy-Weinberg distribution of genotypic frequencies were observed in 11 of the 31 tests conducted. In each case, there was an excess of heterozygotes observed indicating that balancing selection favouring heterozygotes may operate at SypI in some populations. At Mhc locus C, all populations were not in Hardy-Weinberg equilibrium, with an excess of homozygotes observed in all instances, which suggests that a null (non-amplifying or not expressed) allele or haplotype diversity may be present at the locus.

Ten populations were sampled in multiple years. In six populations, significant annual variation in allele frequencies was observed for at least one locus (Table 3). We then compared the relative levels of temporal variation in allele frequencies within putative populations with differentiation among all putative populations. Differentiation among populations exceeded annual variation within populations at all loci, with an average over all seven microsatellite loci and the synaptophysin locus surveyed of about 18 times (Table 4). The Mhc locus C was not included in the overall analysis as only four populations were surveyed in more than one year. The results indicate a relative stability of the genetic characteristics surveyed. This supports the general designation of sampling sites as populations in this study, although additional sampling of offshore populations in Div. 2J and Divs. 3KL would be prudent.

Variation among populations

Genetic differentiation at all loci was observed among the putative cod populations surveyed in our study. For example, the frequency of *Gmo*3^{186} in offshore populations (Flemish Cap 0.59, 2J3KL <0.80) was generally less than that observed in other populations (>0.75), with the Gilbert's Bay population having the highest observed frequency (0.88). The Gilbert's Bay sample was similarly distinctive at *Gmo*8, with a frequency of *Gmo*8^{144} (0.49) substantially higher than that of other populations (<0.27). The cod population in Division 2G had higher frequencies of *Gmo*19^{144} (0.20) than did other populations (all other populations < 0.10). Offshore populations in Divs. 2J3KL and Flemish Cap had higher frequencies of *Gmo*34^{98} (e.g. Hawke Channel 0.79, Funk Island Bank 0.80, Flemish Cap 0.91) than did inshore populations or those in 3Ps (all < 0.75), with the Gilbert's Bay population having the lowest observed frequency (0.38). Differentiation between inshore and offshore populations in 2J3KL was also observed at *Gmo*35 and *Gmo*36. The Hawke Channel population tended to have higher frequencies of *Gmo*37^{256} (0.22) than did other populations (usually < 0.15). At SypI, offshore populations of northern cod had higher frequencies of the allele possessing the DraI restriction site than did inshore populations. Considerable variation among populations was also observed at locus C.

Population differentiation was examined by comparing allele frequencies of cod in different areas. Cod from offshore banks were compared to those from Flemish Cap, with the expectation that genetic differentiation would be observed, as Flemish Cap is considered to be a distinct stock based upon tagging studies and other biological characters. There was significant genetic differentiation in allele frequencies between the Flemish Cap population and each of four offshore bank populations (Hawke Channel, Funk Island Bank, northern Grand Bank, southern Grand Bank) for at least five of the loci surveyed in each comparison. This is strongly indicative of restricted gene flow between the Flemish Cap population and the offshore bank populations, and provides confirmation of the Flemish Cap population as a separate breeding stock (Table 5). Having demonstrated that the microsatellite loci, SypI, and Mhc locus C were capable of detecting significant population differentiation strongly supported by other types of data, we then compared allele frequencies of each of the offshore 2J3KL populations (Hawke Channel, Funk Island Bank, and northern Grand Bank) with each of the inshore 2J3KL populations (Gilbert's Bay, Notre Dame Bay, Bonavista Bay, Trinity Bay, and Conception Bay). Significant differentiation was observed between the Hawke Channel population and each of the five inshore populations, ranging from four significant differences in comparison with the Trinity Bay population to eight significant differences in comparison with the Gilbert's Bay population (Table 5). Similarly, differentiation was also observed between the Funk Island Bank population and all inshore 2J3KL populations, ranging from two significant differences in comparison with the Trinity Bay population to eight significant differences in comparison with the Gilbert's Bay population. In addition,

the northern Grand Bank population was also differentiated from the inshore populations, with one (Trinity Bay) to eight (Gilbert's Bay) significant differences in allele frequencies observed. These results indicate that inshore and offshore populations of the northern cod stock complex constitute separate breeding stocks. Do the offshore 2J3KL populations comprise a single breeding population? Pairwise population comparisons of the allele frequencies of the three offshore populations indicated that there were from one to three significant differences observed, which suggests that there may be at least three breeding populations or stocks in offshore 2J3KL (Table 5).

Differentiation was also observed among inshore 2J3KL populations. The Gilbert's Bay population was strongly differentiated from all other inshore populations, with significant differences observed in allele frequencies at seven or eight of the nine loci surveyed (Table 5). Along the northeast coast of Newfoundland, no genetic differentiation was observed between the Notre Dame Bay and Bonvista Bay populations, limited differentiation between the Notre Dame Bay and Trinity Bay populations (locus C), and greater differentiation between the Notre Dame Bay population and more distant populations (Table 5). The Bonavista Bay population was differentiated from those in Trinity Bay (two significant differences) and Conception Bay (three significant differences) (Table 5), with a limited amount of differentiation between the Trinity Bay and Conception Bay populations (locus C). There is thus some evidence for the existence of discrete "bay" stocks, but perhaps not in every bay.

We examined structure of the four samples collected within Subdivision 3Ps. These four samples were not drawn from a single breeding population, as there was significant differentiation in the population comparisons (Table 5). Genetic differentiation at four loci was observed between the two inshore samples (Fortune Bay and Placentia Bay), at one locus between the two offshore samples (Burgeo Bank, Halibut Channel), and at one to three loci between the inshore and offshore samples. There was a clear differentiation between the Fortune Bay and Placentia Bay populations, indicative of discrete "bay" populations. There is population structure within the breeding populations in Subdivision 3Ps. There was also a significant difference in allele frequencies at three loci between the Halibut Channel population and the southern Grand Bank (Div. 3O) population, suggestive of discrete breeding populations.

We compared allele frequencies between inshore populations along the northeast coast and those along the south coast of Newfoundland. The Placentia Bay population was differentiated from the four populations surveyed along the northeast coast. Significant differences in allele frequencies were observed at from one to three loci in the pairwise population comparisons (Table 5). The Fortune Bay population was also generally differentiated from all four populations, the only exception being the comparison between the Trinity Bay and Fortune Bay populations. Based upon an evaluation of the distance measure among populations, the Gilbert's Bay population was the most distinctive of all populations surveyed (Table 6).

Allele frequencies were compared between the Placentia Bay population and offshore spawning populations in 2J3KL and Flemish Cap. There was little similarity between the Placentia Bay and offshore cod populations, with significant genetic differentiation at three to six loci observed between the Placentia Bay population and all five of offshore samples examined (Table 3). There is thus no genetic evidence to indicate that there has been extensive movement of cod from offshore populations into the Placentia Bay population.

Population Structure

F_{ST} estimates by locus were: *Gmo*3 0.0068, *Gmo*8 0.0047, *Gmo*19 0.0027, *Gmo*34 0.0162, *Gmo*35 0.0028, *Gmo*36 0.0069, *Gmo*37 0.0038, SypI 0.0374, and locus C 0.0062, with the mean over all loci of 0.0080. Of all populations sampled in all areas, the Flemish Cap population and that from Div. 2G were the most differentiated of the offshore group and the Gilbert's Bay population the most differentiated of the inshore group. The Gilbert's Bay population and the Flemish Cap population were the most distinctive surveyed in our study (Fig. 2); the inshore and offshore components of the northern cod population group reasonably well into inshore and offshore components. Only the Conception Bay

(inshore) sample appears anomalous, being more associated with the offshore groups; However, allele frequencies of the Conception Bay population were significantly different from those of all offshore populations at two to four of the nine loci surveyed, indicative of genetic differentiation between inshore and offshore populations.

Estimation of stock composition

We tested the utility of the microsatellite loci, SypI, and locus C for estimation of stock composition in mixed-stock fisheries by estimating the accuracy and precision of stock composition estimates in simulated fishery samples. Simulated mixed stock samples were developed that spanned a range of potential inshore/offshore abundances of cod in fisheries in Divisions 2J3KL and Subdivision 3Ps. In simulated fishery samples from Divisions 2J3KL, estimated stock compositions of the inshore component were reasonably accurate when the inshore component ranged from 50-80% of the mixture, but the inshore component was overestimated by about 7% when it comprised only 20% of the mixture (Table 6). Estimated mean stock compositions of the individual offshore populations were generally within 4% or less of the true value over the range of values comprising the mixture. Given the genetic differentiation between some of the inshore Divs. 3KL populations, precision of estimated stock compositions of individual inshore populations was reasonable.

Evaluation of simulated mixed stock samples from Subdivision 3Ps indicated that the bias in estimated stock compositions was greater and the precision lower in comparison with simulated mixed-stock samples from Divs. 2J3KL (Table 8). Bias of estimated stock compositions was marginal when offshore populations (Burgeo Bank, Halibut Channel) comprised the majority of the sample. For example, bias was between 3-5% for samples when offshore populations comprised 60-80% of the samples. However, when inshore populations comprised 60% of the sample, estimated stock compositions of the samples underestimated the actual inshore component of the samples by 10% (Table 8).

Discussion

A requirement for development of genetic differentiation among putative cod stocks is fidelity to specific spawning locations, with a resulting restriction in gene flow among stocks. Collection of samples in the current study centred on the spring spawning season, when stocks should have returned to their spawning sites from overwintering locations. Single spawning populations appear to have been sampled, with the exception of the Burgeo Bank sample and the southern Grand Bank (Div. 3O) sample. One component of the Burgeo Bank sample was collected between April 6-7, 1998, and this may have contained an admixture of populations, prior to their return to separate spawning sites. Tagging of individuals captured on Burgeo Bank, conducted at the same time as DNA sample collection, indicated that some individuals subsequently moved northwest into the Gulf of St. Lawrence, whereas others moved northeast to the south coast of Newfoundland or eastward to the Grand Banks (Brattey et al. 1999).

Ten locations were sampled in at least two years, and the limited evaluation of annual variation in allele frequencies within sampling locations relative to differentiation among locations indicated that there was substantially greater genetic differentiation among putative populations than among sampling years within populations. Temporal stability of microsatellite variation in cod has also been observed by Ruzzante et al. (1997). In applications of genetic differentiation to estimation of stock composition in mixed-stock fishery analysis, it is highly desirable that the characters used in stock identification be stable over time, or that annual variation is much less than differentiation among stocks. The data on the relative levels of within- and among-population variation are preliminary but indicate that annual estimation of allele frequencies in baseline populations would not be required for practical applications, although some monitoring of allele frequencies over time would be prudent. Demonstration that

population differentiation is persistent over time increases the likelihood that the appropriate population structure has been elucidated (Waples 1998).

Are there genetically distinct "bay" stocks of cod in Divs. 3KL? Surveys of variation at seven microsatellite loci, SypI, and Mhc locus C did not reveal any significant differentiation among the cod populations in Notre Dame Bay and Bonavista Bay, but some differentiation of cod in Trinity Bay and Conception Bay compared with the other populations. A previous survey at five different microsatellite loci indicated that there was no differentiation among cod from Trinity Bay, St. Anthony Basin, and the Notre Dame Channel (Ruzzante et al. 1998), although sample sizes were small relative to the number of alleles present, decreasing the power of the tests to detect differentiation. The genetic surveys conducted to date provide some evidence to indicate that there may be separate bay stocks of cod along the northeast coast of Newfoundland. Levels of migration (resulting in gene flow) among cod spawning in the Notre Dame Bay and Bonavista Bay are apparently sufficiently high to preclude genetic differentiation. However, there was clear genetic differentiation between inshore cod populations along the northeast coast of Newfoundland and the Gilbert's Bay population from Labrador.

Are there genetically distinct "inshore" and "offshore" stocks of cod in Divs. 2J3KL? The microsatellite loci, SypI, and Mhc locus C surveyed in our study clearly indicate that inshore and offshore populations comprise genetically distinct stocks. Genetic differentiation at microsatellite loci between inshore and offshore cod in Divs. 2J3KL was previously observed by Ruzzante et al. (1996) at one of five microsatellite loci surveyed. In our survey of nine different loci, significant differences were observed at an average of five loci between Hawke Channel population and the four inshore 3KL populations (Notre Dame Bay, Bonavista Bay, Trinity Bay, and Conception Bay), at an average of four loci between the Funk Island Bank population and the inshore populations, and at an average of two loci between the northern Grand Bank population and the inshore populations. Greater differentiation was generally observed between more northern offshore populations and the inshore populations along the northeast coast of Newfoundland. However, the level of differentiation observed convincingly demonstrates significant genetic differentiation between inshore- and offshore-spawning populations. The microsatellite loci, the synaptophysin locus, and the Mhc locus C were able to detect genetic differentiation on a finer geographic scale than was apparent in a survey of mitochondrial DNA variation (Pepin and Carr 1993; Carr et al. 1995). Tagging data indicate that there is substantial fidelity of offshore cod to specific spawning areas (Lear 1984; Taggart et al. 1995; Taggart 1997). Finally, there are aggregations of adult cod spawning in parts of the inshore of the northeast coast of Newfoundland, but seven years after the moratorium on cod exploitation was introduced, there is still no significant abundance of cod in offshore spawning areas. The observed genetic differentiation, fidelity to spawning areas, and different responses in abundance with respect to the moratorium are all consistent with the concept that cod in inshore and offshore spawning areas constitute separate stocks. Furthermore, the microsatellite data in our current study and those of Bentzen et al. (1996) and Ruzzante et al. (1998) as well as the tagging data of Lear (1984) all suggest that there is more than one offshore spawning stock. Although our data indicate that there could be at least three spawning stocks of cod in offshore Divs. 2J3KL, the samples we analysed were collected during fall and their exact spawning locations within the offshore are not known. More intensive sampling of spawning cod in these locations is required before any definitive conclusions can be drawn.

Will the inshore spawning stock contribute to rebuilding of the offshore spawning cod stock? Unless the patterns of migration between the inshore and offshore locations have changed greatly in recent years, the genetic data indicate that it is unlikely the inshore-spawning stock will contribute significantly to recovery in abundance of the offshore-spawning stocks. Similarly, it is unlikely that the Flemish Cap population would contribute significantly to recovery of the other offshore populations, given its degree of genetic differentiation. For the microsatellite loci, such differentiation indicates a significant restriction of gene flow between cod of the two areas. At the SypI locus and perhaps the Mhc locus C, selection may have contributed to the observed differences in allelic frequencies, providing an example of the adaptive differentiation that can occur between stocks once gene flow between them is

reduced. Tagging data also indicate that there is little exchange between the Flemish Cap and offshore populations (Taggart et al. 1995).

Although the genetic differentiation between the inshore and offshore populations in Divs. 2J3KL was not as large as between the Flemish Cap population and the offshore populations, it was substantial. The greater genetic similarity of the inshore samples to one another and of the offshore samples to one another in this region indicates that the observed differentiation among populations is not a result of random fragmentation of a single large stock into many small units during the period of population decline. Instead, it suggests that the inshore-offshore differentiation existed prior to declines in abundance. Moreover, the differentiation would not be observed if substantial numbers of offshore fish had recently migrated to spawn inshore or vice-versa. Thus, even if abundance of inshore populations increases, it seems unlikely that they will provide significant numbers of reproductively successful migrants to the offshore populations.

Analysis of genetic variation of cod in Subdivision 3Ps revealed a less well-defined distinction between inshore and offshore samples. There was also significant differentiation between inshore spawning cod in Fortune Bay and Placentia Bay at four of nine loci surveyed, indicative of some degree of restricted gene flow between these two populations. All populations sampled in Subdiv. 3Ps were differentiated from one another to some degree. However, the degree of differentiation among the putative populations in this region should be confirmed by sampling in subsequent years.

Is the Placentia Bay population distinct from offshore-spawning populations? Significant genetic differentiation between the Placentia Bay population and the offshore populations was observed at from three to six loci in comparisons with five offshore populations surveyed. These data suggest that the increased abundance of cod in Placentia Bay is not the result of offshore-spawning cod from the Newfoundland Shelf migrating to the inshore and subsequently breeding and remaining in Placentia Bay. Increased abundance of cod in Placentia Bay is likely the result of local recruitment.

The simulated mixtures evaluated for cod in Divs. 2J3KL indicated that the genetic variation surveyed in our study could be used to provide accurate and reasonably precise estimates of inshore- and offshore-spawning components when the inshore-spawning component comprised at least 50% of the sample. When the inshore-spawning component comprised only 20% of the sample, an average bias of about 7% in estimated stock compositions was observed. In applications where the inshore-spawning component is expected to be present but only comprise a small (<30%) portion of the sample, two approaches are possible. Additional characters can be incorporated that aid in discrimination between inshore and offshore populations and thus reduce bias in the estimated stock compositions. Alternately, simulations can be conducted to quantify the level of bias expected at specific stock compositions, and estimates of stock composition could be corrected to account for the known bias in the estimation procedure.

Simulated mixtures evaluated for cod in Subdiv. 3Ps indicated that the accuracy and precision of the estimated stock compositions were lower than those observed in simulated mixtures of northern cod. This lower level of accuracy and precision reflects the lower level of genetic differentiation observed among putative populations in Subdiv. 3Ps in comparison with inshore and offshore stocks of northern cod. Increased baseline population sample sizes or additional discriminating markers are likely required to decrease the degree of bias in the estimated stock composition in this application.

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Table 1. Samples collected and analyzed from cod populations in the Newfoundland region. N is sample size in each year. Locations sampled during 1999 are shown in Fig 1.

Area	(Ref. #) Location	Local area	Date	Year sampled	Latitude (dec.)	Longitude (dec.)	N	Total N
2G offshore	(1) Offshore	-	Oct. 6	1996	60.25	61.25	20	20
2J offshore	(2) Hamilton Bank	-	November 9-13	1999	-[1]		16	16
	(3) Hawke Channel	-	June 13-16	1998	52.75	53.00	236	236
	(4) Belle Isle Bank	-	November 17-25	1999	-[2]		10	10
inshore	(5) Gilbert's Bay	-	May 29	1997	52.58	55.83	86	132
			May 25	1998	52.58	55.83	46	
3K offshore	(6) Funk Island Bank	-	November 11-21	1996	-[3]	-	97	188
			Nov 23- Dec 10	1999	-[4]	-	91	
inshore	(7) Notre Dame Bay	Miles Cove	June 18-July 3	1997	49.54	55.77	126	
		Fogo	June 18	1998	49.65	54.05	213	
		Jacksons Cove	June 22-24	1999	49.70	55.98	200	739
		Fogo	June 1	1999	49.73	54.25	200	
3K/3L offshore	(8) Northern Grand Bank	-	June 25-27	1998	-[5]	-	113	
		-	June 13-20	1999	[6]	-	50	218
			Nov. 23- Dec. 10	1999	[7]	-	55	
inshore	(9) Bonavista Bay	Open Hall	May 29-June 11	1997	48.51	53.51	147	
		Plate Cove	June 4	1998	48.52	53.52	162	
		Sandy Cove	June 10	1999	48.64	53.71	5	
		Rachet Cove	June 10	1999	48.61	53.73	12	
		Shag Islands	June 12	1999	48.71	53.63	32	
		Rachet Cove	June 13	1999	48.60	53.77	24	
		Swale Island	June 13	1999	48.62	53.69	19	
		Plate Cove	June 28	1999	48.52	53.52	18	
		Plate Cove	July 7	1999	48.51	53.51	51	503
	(10) Trinity Bay	West Trinity Bay	April 23-26	1997	48.18	53.61	155	355
		Petley	May 28, June 23	1999	48.18	53.79	200	
	(11) Conception Bay	Brigus	June 1- July 7	1999	47.56	53.18	200	200
3M	(12) Flemish Cap	-	Sept 25 – Oct 12	1996	-[8]	-	103	103
3N	(13) Southern Grand Bank	-	May 30	1999	42.89	49.95	13	113
3O	(14) Southern Grand Bank	-	May 11-14	1999	-[9]	-	100	
3Pn	(15) Rose Blanche Bank	-	April 18	1999	47.55	58.25	17	17
3Ps offshore	(16) Halibut Channel	-	April 2-4	1998	45.10	55.20	214	413
			April 1-3	1999	45.30	55.40	199	
3Ps	(17) Burgeo Bank	-	April 6-7	1998	46.75	57.65	250	500

			April 4-18	1999	46.80	57.65	250	
inshore	(18) Placentia Bay	Bar Haven,	April 22-26	1998	47.73	54.19	223	
		Warehams Rock	May 1-3	1998	47.70	54.15	205	
		Paradise Sound	April 27-29	1998	47.51	54.49	95	
		Head of Placentia Bay	April 30- May 3	1999	47.70	54.20	200	723
	(19) Fortune Bay	Pools Cove	May 21-28	1998	47.70	55.38	264	
			May 18-20	1999	47.70	55.38	106	565
		Pass Island	April 8	1999	47.40	56.20	195	

[1] collected at various locations on or adjacent to Hamilton Bank during Teleost trip 86.
[2] collected at various locations on or adjacent to Belle Isle Bank during Teleost trip 86 and 87.
[3] collected at various locations on or adjacent to Funk Island Bank during Teleost trip 40 and Wilfred Templeman trip 198.
[4] collected at various locations on or adjacent to Funk Island Bank during Teleost trip 87 and 88.
[5] collected at various offshore locations in 3L north of 47.6°N during Wilfred Templeman trip 198.
[6] collected at various offshore locations in 3L north of 46.0°N during Wilfred Templeman trips 240 and 241.
[7] collected at various offshore locations in southern 3K and 3L north of 46.0°N during Wilfred Templeman trips 247 and 248.
[8] collected at various locations on Flemish Cap between 44.0°W - 46.0°W and 46.0°N - 48.0°N during Wilfred Templeman trip 195 and part of 196.
[9] collected at various locations in 3O south of 45.9°N during Wilfred Templeman trip 238.

Table 2. Precision of estimates of allele size (in basepairs) at each microsatellite locus for standard fish run only once per electrophoretic gel. N is the number of gels on which allele sizes for a standard fish were estimated. Standard deviation is in parentheses.

Locus	N	Allele Size	Range	Allele Size	Range
*Gmo*3	90	180.0 (0.69)	179-181	187.4 (0.83)	186-189
*Gmo*8	83	126.4 (0.54)	125-128	164.9 (0.73)	163-166
	25	126.2 (0.50)	125-127	164.5 (0.59)	163-165
*Gmo*19	37	148.6 (0.65)	147-150	183.2 (0.60)	182-184
	32	148.5 (0.57)	148-150	183.1 (0.75)	182-185
*Gmo*34	75	91.7 (0.50)	91-92	107.0 (0.50)	106-108
*Gmo*35	101	120.2 (0.43)	120-121	134.7 (0.55)	134-136
*Gmo*36	95	185.0 (0.67)	184-186	202.4 (0.61)	201-204
*Gmo*37	96	237.3 (0.84)	235-239	284.9 (0.92)	283-287

Table 3. Probability of homogeneity of annual allele frequencies estimated from probability tests derived from GENEPOP version 3.1 with the Markov-Chain approach using χ^2 probability values (Raymond and Rousset 1995). Values considered statistically significant are in bold type.

Population	*Gmo*3	*Gmo*8	*Gmo*19	*Gmo*34	*Gmo*35	*Gmo*36	*Gmo*37	SypI	Locus C
Inshore populations									
Gilbert's	0.8279	0.4188	0.8302	0.8035	0.0902	0.1816	**0.0000**	1.0000	-
Notre Dame Bay	0.0268	0.0296	0.5787	0.4031	0.0813	0.9877	**0.0000**	0.1082	0.0223
Bonavista Bay	0.7559	0.1392	0.2644	0.8293	0.3369	0.2889	0.6942	0.6471	0.2037
Trinity Bay	**0.0000**	0.5937	0.2434	0.1621	0.2256	0.8690	0.9902	0.0442	-
Placentia Bay	0.0132	0.3904	0.2081	0.0598	0.1374	**0.0000**	0.2262	0.4172	-
Fortune Bay	0.0544	0.7398	0.5278	0.2892	0.0594	0.3015	0.0535	**0.0000**	-
Offshore populations									
Funk Island Bank	0.0666	0.2901	0.8001	0.8537	0.0939	0.2581	0.0760	0.0613	0.0110
Northern Grand Bank	0.0195	0.2945	0.7415	0.2470	0.0629	0.8962	0.7029	0.3999	0.0067
Burgeo Bank	**0.0000**	0.6142	0.2621	0.0855	0.1430	0.0245	0.2663	**0.0003**	-
Halibut Channel	0.9554	0.2378	0.2621	0.0895	0.8370	0.6998	0.0179	0.6388	-

Table 4. Hierarchical gene-diversity analysis of 19 putative populations of Atlantic cod for seven microsatellite loci and the SypI locus. The relative diversity owing to sampling years within populations and among populations are indicated, as well as the ratio of among population versus among years within population diversity.

	Absolute diversity		Relative diversity			
Locus	Total	Within Populations	Within Populations	Among years within pops	Among pops	Pops/ Years
*Gmo*3	0.3453	0.3413	0.9884	0.0051	0.0065	1.3
*Gmo*8	0.9166	0.9020	0.9840	0.0000	0.0160	798.0
*Gmo*19	0.9355	0.9237	0.9874	0.0000	0.0126	-
*Gmo*34	0.4599	0.4405	0.9578	0.0000	0.0423	-
*Gmo*35	0.7716	0.7640	0.9936	0.0000	0.0064	-
Gmo36	0.5898	0.5830	0.9885	0.0000	0.0115	-
Gmo37	0.8409	0.8175	0.9722	0.0042	0.0235	5.6
SypI	0.4567	0.4171	0.9133	0.0027	0.0840	31.0
Locus C	0.9205	0.9032	0.9812	0.0020	0.0167	8.3
All[1]			0.9739	0.0013	0.0222	17.6

[1] All does not include Mhc locus C.

Table 5. Probability of homogeneity of allele frequencies estimated from pairwise probability tests derived from GENEPOP version 3.1 with the Markov-Chain approach using χ^2 probability values (Raymond and Rousset 1995). Values considered statistically significant are in bold type.

Comparison		*Gmo*3	*Gmo*8	*Gmo*19	*Gmo*34	*Gmo*35	*Gmo*36	*Gmo*37	SypI	Locus C
Flemish Cap	Funk Island	**0.0028**	**0.0075**	0.0717	**0.0000**	0.2380	**0.0003**	0.0316	**0.0000**	**0.0014**
	Hawke Channel	**0.0000**	**0.0014**	**0.0015**	**0.0000**	0.3309	**0.0000**	**0.0001**	**0.0000**	**0.0000**
	N. Grand Bank	**0.0000**	0.0316	0.0142	**0.0000**	0.1274	**0.0000**	0.2308	**0.0000**	**0.0032**
	S. Grand Bank	**0.0011**	0.4690	**0.0000**	**0.0000**	0.1534	**0.0000**	0.0246	**0.0000**	**0.0000**
Funk Island Bank	Gilbert's Bay	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.0271	**0.0000**	**0.0000**	**0.0000**
	Notre Dame	0.2046	**0.0015**	0.4281	**0.0046**	0.5535	**0.0004**	**0.0000**	**0.0000**	**0.0000**
	Bonavista	**0.0002**	0.0203	0.7194	**0.0000**	0.1966	0.1900	**0.0000**	**0.0000**	**0.0000**
	Trinity	0.6565	0.4943	0.6933	0.1405	0.8484	0.0284	**0.0000**	**0.0068**	0.0426
	Conception	0.1349	0.6128	0.3507	0.3270	0.0902	0.2216	**0.0001**	0.3408	**0.0000**
Hawke Channel	Gilbert's Bay	0.0352	**0.0000**	**0.0000**	**0.0000**	**0.0014**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
	Notre Dame	0.9852	0.0173	**0.0044**	**0.0005**	0.3059	**0.0000**	**0.0021**	0.**0000**	**0.0000**
	Bonavista	0.2835	0.8738	**0.0037**	**0.0000**	0.0917	**0.0000**	**0.0000**	**0.0000**	**0.0007**
	Trinity	0.7056	0.1113	0.0251	0.0888	0.3550	**0.0001**	**0.0001**	**0.0002**	**0.0000**
	Conception	0.3542	0.5966	**0.0001**	0.3605	0.2112	**0.0000**	**0.0000**	0.1037	**0.0000**
N. Grand Bank	Gilbert's Bay	0.0992	**0.0000**	**0.0000**	**0.0000**	**0.0008**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
	Notre Dame	0.4161	0.4046	0.6139	0.5941	0.0231	0.0219	0.0857	**0.0015**	**0.0003**
	Bonavista	0.3640	0.9480	0.0729	0.0721	**0.0062**	0.0207	0.1840	**0.0000**	**0.0053**
	Trinity	0.5986	0.6692	0.1439	0.9896	0.0191	0.1109	0.2648	0.3783	**0.0000**
	Conception	0.0897	0.9543	0.0515	0.9789	0.1107	**0.0008**	0.2174	0.6158	**0.0000**
Single offshore pop. 2J3KL?										
Hawke Ch.	Funk Island	0.4998	0.1546	0.4151	0.9187	0.1684	**0.0004**	**0.0000**	0.5676	**0.0000**
	Northern GB	0.8214	0.6057	0.5419	0.2454	0.3748	0.0840	**0.0004**	0.0118	0.0070
Funk Is.	Northern GB	0.0878	0.4595	0.5527	0.2906	0.0586	**0.0044**	**0.0000**	0.0923	**0.0000**
Inshore										

population structure										
Gilbert's Bay	Notre Dame	0.0451	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.0391	**0.0000**	**0.0000**	**0.0000**
	Bonavista	0.5171	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.0236	**0.0000**	**0.0000**	**0.0000**
	Trinity	**0.0066**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.0254	**0.0000**	**0.0000**	**0.0000**
	Conception	0.0400	**0.0000**	**0.0000**	**0.0000**	**0.0049**	0.0432	**0.0000**	**0.0000**	**0.0000**
	Placentia	0.0584	**0.0000**	**0.0000**	**0.0000**	**0.0041**	**0.0059**	**0.0000**	**0.0000**	**0.0000**
	Fortune	0.0387	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.0707	**0.0000**	**0.0000**	**0.0006**
Notre Dame	Bonavista	0.2171	0.1047	0.0900	0.8197	0.0240	0.0897	0.7210	0.3460	0.1440
	Trinity	0.5999	0.0173	0.6997	0.5830	0.1105	0.4481	0.0215	0.0413	**0.0000**
	Conception	0.1622	0.2169	0.2046	0.3688	0.3441	**0.0009**	0.0962	**0.0012**	**0.0027**
	Placentia	0.4157	0.0478	0.7155	0.9159	**0.0048**	**0.0003**	0.1873	0.6470	**0.0000**
	Fortune	0.1446	**0.0000**	0.0555	0.2126	0.2841	**0.0012**	0.7115	**0.0000**	0.0301
Bonavista	Trinity	0.0535	0.3677	0.0657	0.1455	0.2989	0.3739	0.4827	**0.0040**	**0.0010**
	Conception	0.2318	0.4261	0.2091	0.0862	**0.0009**	0.0353	0.6366	**0.0004**	**0.0021**
	Placentia	0.0448	0.7467	0.4244	0.7927	**0.0000**	0.2385	0.7896	0.1419	**0.0000**
	Fortune	0.0723	0.1137	0.0662	0.0525	0.2965	**0.0016**	0.3939	**0.0000**	0.5362
Trinity	Conception	0.5384	0.5671	0.5215	0.8177	0.0216	0.0300	0.2724	0.0145	**0.0000**
	Placentia	0.8233	0.2108	0.5574	0.3082	0.2811	0.0305	0.3305	0.0811	**0.0000**
	Fortune	0.8517	0.2726	0.4880	0.8535	0.2781	0.2344	0.1187	0.2856	0.0088
Conception	Placentia	0.4323	0.3246	0.2526	0.0949	0.0460	0.0681	0.0636	**0.0029**	**0.0000**
	Fortune	0.4288	0.0098	0.1326	0.8346	0.0148	**0.0015**	0.0775	0.5127	**0.0000**
Placentia	Fortune	0.2259	0.1483	0.1360	0.0669	**0.0004**	**0.0000**	0.1401	**0.0028**	**0.0010**
Subdivision 3Ps										
Burgeo Bank	Halibut Chan.	**0.0017**	0.0664	0.0614	0.7183	0.9706	0.0279	0.0172	0.3480	0.0068
	Fortune Bay	**0.0000**	**0.0035**	0.2385	0.9547	0.0490	**0.0036**	0.6359	0.8812	0.1318
	Placentia Bay	**0.0000**	0.2506	0.6312	0.0867	0.6157	0.0694	0.8848	**0.0015**	**0.0000**
Halibut Chan	Fortune Bay	0.2136	**0.0001**	0.0581	0.9859	0.0443	0.0147	0.0246	0.3904	0.0262
	Placentia Bay	0.5977	0.3919	0.0582	0.1288	0.3924	**0.0000**	0.1667	**0.0000**	**0.0000**
Fortune Bay	Placentia Bay	0.2259	0.1483	0.1360	0.0669	**0.0006**	**0.0000**	0.1401	**0.0028**	**0.0010**
Halibut Channel versus Div. 3O offshore										
Halibut Chan	Div. 3O	0.6626	0.1594	**0.0026**	0.1426	0.0282	**0.0000**	0.6377	0.0306	**0.0003**

Placentia Bay and offshore populations										
Placentia	Flemish Cap	**0.0000**	**0.0024**	0.0686	**0.0000**	0.3015	**0.0000**	0.2074	**0.0000**	**0.0028**
	Hawke Ch.	0.8178	0.3631	0.0138	**0.0000**	0.5821	**0.0000**	**0.0000**	**0.0000**	**0.0000**
	Funk Island	0.2257	0.1114	0.5677	**0.0048**	0.5435	0.0156	**0.0000**	**0.0000**	**0.0000**
	Northern GB	0.3109	0.8753	0.3339	0.2757	0.2211	**0.0000**	0.1099	**0.0033**	**0.0000**
	Div. 3O	0.9911	0.1773	**0.0000**	0.2104	0.0265	**0.0056**	0.1922	1.0000	**0.0000**

Table 6. Pairwise Cavalli-Sforza and Edwards (1967) chord distance among 15 populations of Atlantic cod derived from seven microsatellite loci, the synaptophysin locus, and Mhc locus C.

Population	Notre	Flemish	Trinity	Bonav	Gilbert	Burgeo	Halibut	Fortune	Placentia	Hawke	2G	N.Gr. Bk	3O	Concept'n
Funk Island	0.0054	0.0120	0.0040	0.0051	0.0379	0.0044	0.0050	0.0051	0.0061	0.0065	0.0150	0.0049	0.0095	0.0056
Notre Dame		0.0154	0.0035	0.0022	0.0312	0.0030	0.0032	0.0034	0.0041	0.0058	0.0165	0.0027	0.0066	0.0044
Flemish Cap			0.0151	0.0151	0.0513	0.0134	0.0134	0.0148	0.0152	0.0133	0.0226	0.0134	0.0212	0.0138
Trinity Bay				0.0038	0.0343	0.0038	0.0043	0.0038	0.0051	0.0056	0.0164	0.0043	0.0079	0.0057
Bonavista Bay					0.0323	0.0027	0.0034	0.0029	0.0045	0.0054	0.0149	0.0030	0.0071	0.0051
Gilbert's Bay						0.0341	0.0344	0.0341	0.0356	0.0356	0.0496	0.0326	0.0377	0.0377
Burgeo Bank							0.0027	0.0034	0.0035	0.0041	0.0146	0.0026	0.0066	0.0043
Halibut Channel								0.0042	0.0051	0.0051	0.0145	0.0026	0.0071	0.0047
Fortune Bay									0.0048	0.0049	0.0152	0.0035	0.0070	0.0059
Placentia Bay										0.0068	0.0172	0.0046	0.0093	0.0058
Hawke Channel											0.0142	0.0039	0.0086	0.0070
2G												0.0140	0.0178	0.0166
Northern GB													0.0071	0.0048
3O														0.0087

Table 7. Estimated percentage composition of three simulated mixtures of inshore and offshore NAFO Divisions 2J3KL Atlantic cod incorporating variation at seven microsatellite loci, SypI, and Mhc locus C. Each mixture of 200 fish was generated 100 times with replacement, and stock compositions of the mixtures estimated by resampling each baseline population with replacement to obtain a new distribution of allele frequencies (sample size was constant). The individual estimates for all populations with a group have been summed to provide regional estimates (Σ) of stock composition. Standard deviation is in parentheses.

	Mixture 1		Mixture 2		Mixture 3	
Population	Actual	Estimated	Actual	Estimated	Actual	Estimated
Gilbert's Bay	0	0.1 (0.5)	0	0.0 (0.3)	0	0.0 (0.2)
Notre Dame Bay	30	28.6 (7.8)	20	18.2 (6.1)	5	7.8 (3.7)
Bonavista Bay	20	19.6 (4.8)	10	13.3 (5.1)	5	7.8 (4.0)
Trinity Bay	20	16.3 (4.6)	10	9.6 (3.9)	5	6.6 (4.0)
Conception Bay	10	10.7 (4.2)	10	9.9 (3.9)	5	5.4 (3.4)
ΣInshore	**80**	**75.3 (4.6)**	**50**	**51.0 (6.3)**	**20**	**27.5 (5.7)**
Hawke Channel	10	8.9 (3.1)	25	19.4 (2.8)	30	26.1 (3.7)
Funk Island Bank	5	6.7 (3.1)	15	16.9 (5.2)	30	26.4 (5.7)
Northern Grand Bk	5	9.1 (3.7)	10	12.7 (6.9)	20	20.0 (5.2)
ΣOffshore	**20**	**24.7 (4.6)**	**50**	**49.0 (6.3)**	**80**	**72.5 (5.7)**

Table 8. Estimated percentage composition of three simulated mixtures of inshore and offshore NAFO Subdivision 3Ps Atlantic cod incorporating variation at seven microsatellite loci, SypI, and Mhc locus C. Each mixture of 200 fish was generated 100 times with replacement, and stock compositions of the mixtures estimated by resampling each baseline population with replacement to obtain a new distribution of allele frequencies (sample size was constant). The individual estimates for all populations with a group have been summed to provide regional estimates (Σ) of stock composition. Standard deviation is in parentheses.

	Mixture 1		Mixture 2		Mixture 3	
Population	Actual	Estimated	Actual	Estimated	Actual	Estimated
Fortune Bay	10	11.3 (4.9)	15	14.6 (4.7)	30	23.6 (5.2)
Placentia Bay	10	13.2 (4.9)	25	22.6 (5.2)	30	25.8 (5.3)
ΣInshore	**20**	**24.5 (5.7)**	**40**	**37.2 (6.5)**	**60**	**49.6 (6.2)**
Burgeo Bank	30	32.1 (6.8)	35	37.0 (6.1)	20	27.5 (5.7)
Halibut Channel	50	43.4 (5.6)	25	25.8 (5.7)	20	23.1 (4.7)
ΣOffshore	**80**	**75.5 (5.7)**	**60**	**62.8 (6.5)**	**40**	**50.6 (6.2)**

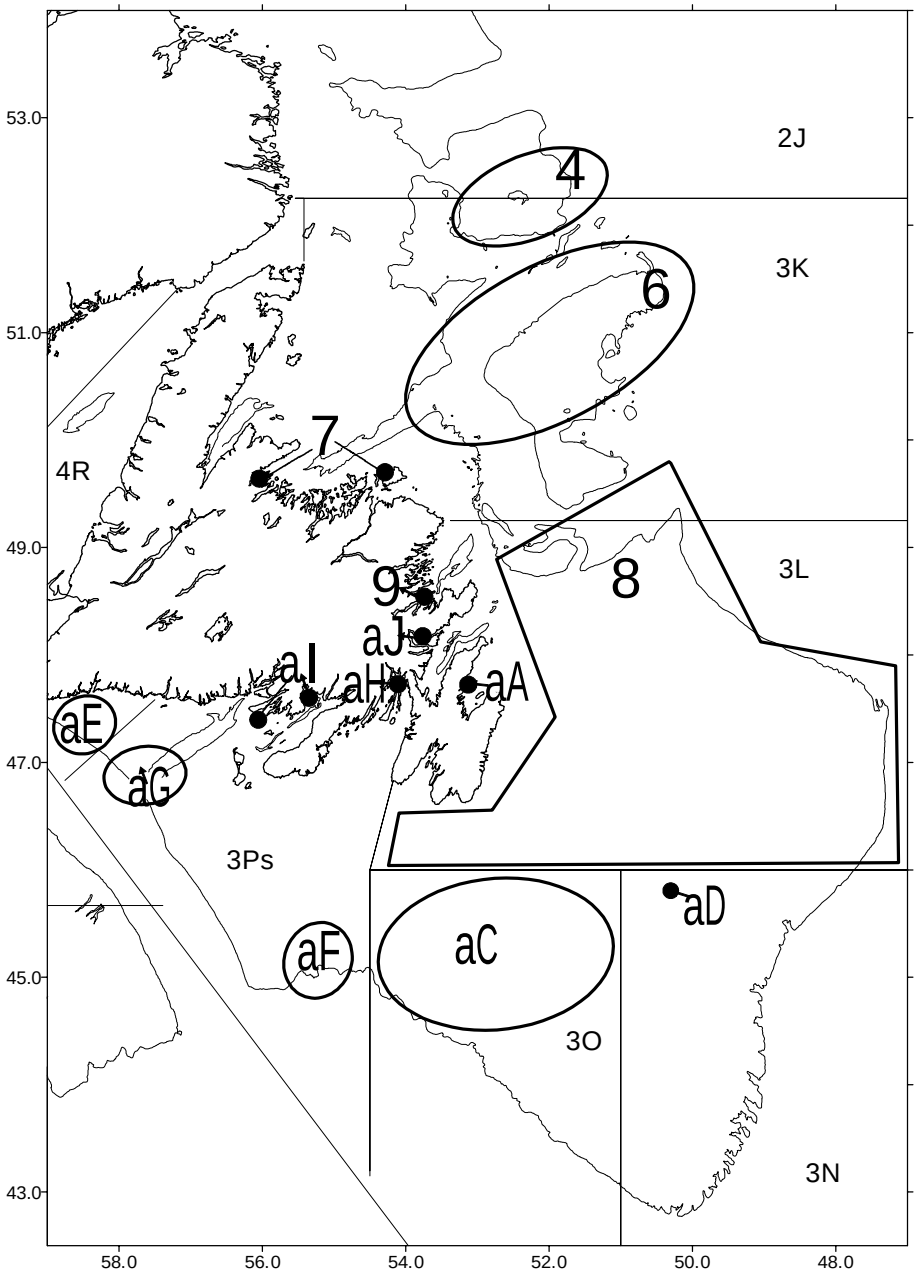

Fig. 1. Approximate locations of cod sampled in 1999. Numbers indicate reference locations listed in Table 1 which gives further details of sampling. Some sites sampled further northward (2J) are not shown.

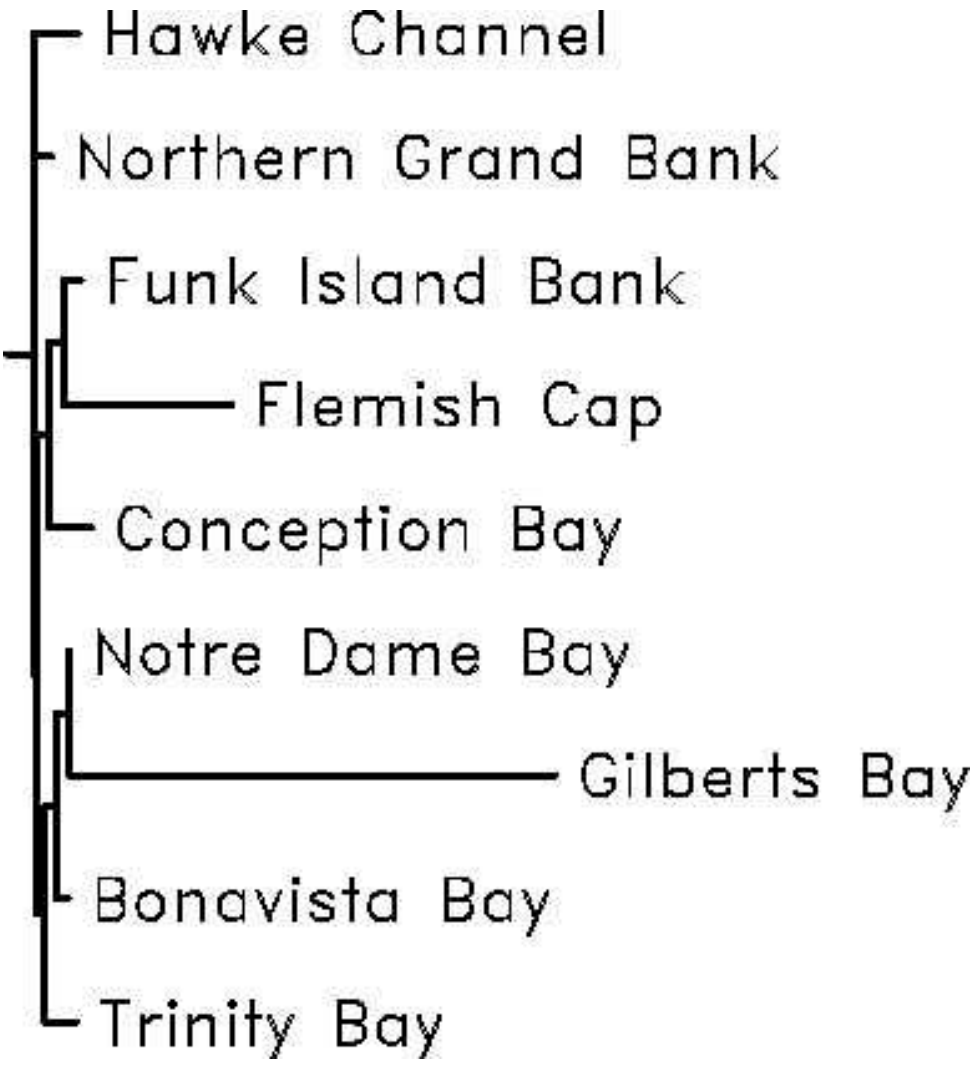

Fig. 2. Unrooted neighbour-joining dendrogram outlining relationships of various sub-components of the northern cod population and Flemish Cap cod.