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Canadian Stock Assessment Secretariat
Research Document 99/35

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Secrétariat canadien pour l'évaluation des stocks
Document de recherche 99/35

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Population structure of Atlantic cod (*Gadus morhua*) in the Newfoundland and Labrador area based on microsatellite variation

Terry D. Beacham, John Brattey*, Kristina M. Miller, Khai D. Le, and Ruth E. Withler

Department of Fisheries and Oceans,
Pacific Biological Station, Nanaimo, BC V9R 5K6

*Science Branch, Department of Fisheries and Oceans,
P.O. Box 5667, St. John's, NF A1C 5X1

ISSN 1480-4883
Ottawa, 1999

Canada

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 and the synaptophysin (SypI) 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 2,700 cod from 13 putative populations. Two populations were sampled over two or more years, and variation among populations was on average about 33 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, Trinity Bay, and Bonavista Bay, providing no evidence of distinct "bay" stocks of cod along the northeast coast of Newfoundland. However, cod from the inshore sites were genetically distinct from most offshore samples of northern cod. The offshore samples were more heterogeneous, and there may be at least two 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 generally distinct from offshore samples of northern cod. The Burgeo Bank sample may have been a composite of fish from at least two spawning populations. Simulated mixed-stock fishery samples of northern cod suggested that variation at the seven microsatellite loci and synaptophysin locus should provide reasonably accurate estimates of stock composition (inshore vs. offshore) when the inshore component comprises at least 50% of the mixture. Characters affording greater differentiation among the sampling sites in Subdiv. 3Ps are required to increase the accuracy and precision of the estimated contributions of inshore and offshore cod for practical applications.

Résumé

La présente étude avait pour objet de décrire la structure des populations et de déterminer s'il était possible d'identifier génétiquement les stocks de morue de l'Atlantique (Gadus morhua) de Terre-Neuve et du Labrador à l'aide de locus de microsatellites et du locus pour la synaptophysine (SypI). Les variations à sept locus de microsatellites (Gmo3, Gmo8, Gmo19, Gmo34, Gmo35, Gmo36, et Gmo37) et au locus SypI ont été déterminées chez 2 700 morues environ provenant de 13 populations supposées. Deux populations ont fait l'objet de prélèvements pendant au moins deux ans et l'écart noté entre ces populations était en moyenne 33 fois environ plus élevé que la variation annuelle au sein des populations. Une structuration régionale des populations était visible, due au fait que les populations de géniteurs côtiers et hauturiers forment des groupes distincts. La population du Bonnet Flamand était la plus distincte du groupe hauturier et celle de la baie Gilbert, au Labrador, la plus distincte du groupe côtier. Dans les divisions 2J3KL, aucune différenciation génétique significative n'a été décelée entre les groupes de morues côtiers échantillonnés aux sites des baies Notre Dame, Trinity et Bonavista, ce qui ne fournit aucune indication de la présence de stocks de morue de « baie » distincts le long de la côte nord-est de Terre-Neuve. Par ailleurs, les échantillons de morue des sites côtiers étaient génétiquement distincts de la plupart des échantillons hauturiers de morue du nord. Les échantillons hauturiers étaient plus hétérogènes et il pourrait exister au moins deux populations de géniteurs hauturiers distinctes de morue du nord. Dans la sous-division 3Ps, une différence génétique a été notée entre les échantillons côtiers des baies Placentia et Fortune, et l'échantillon de la baie Placentia était généralement distinct des échantillons hauturiers de morue du nord. L'échantillon du banc Burgeo pourrait réunir des poissons provenant d'au moins deux populations de géniteurs. Des échantillons simulés de pêche de stocks mixtes de morue du nord portent à croire que la variation notée aux sept locus de microsatellites et au locus pour la synaptophysine devrait fournir une estimation raisonnablement exacte de la composition des stocks (côtiers et hauturiers) lorsque la composante côtière représente au moins la moitié du mélange. Il faudra disposer de caractères permettant de mieux différencier les sites d'échantillonnage dans la sous-division 3Ps afin d'accroître l'exactitude et la précision des apports estimés des morues côtières et hauturières et ainsi s'en servir à des fins pratiques.

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 the best 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. For Atlantic cod, analysis of genetic variation 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). 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). 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. We 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? We also examined the degree of genetic differentiation between inshore-spawning populations and offshore-spawning populations to determine whether they constitute separate stocks. Finally, we evaluated the utility of using microsatellite 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 2,700 fish in several locations around Newfoundland (Table 1). All inshore samples were collected during the spring spawning season, but all offshore northern cod samples were collected in the fall. (An additional sample labelled "Grand Bank" (N=30) was also included in the original analyses presented at the Regional assessment meetings in St. John's in March 1999; this sample has been excluded from the present analyses because it was subsequently found to be mis-labelled and contained a mixture of inshore and offshore samples. Exclusion of this sample did not affect the conclusions of the study). 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).

New loci developed at the Pacific Biological Station were amplified via PCR namely: *Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, and *Gmo*37 (Table 2), as well as the synaptophysin locus (SypI) (Fevolden and Pogson 1997). Primers were developed for trinucleotide repeat and tetranucleotide repeat loci for which there were a moderate number (<25) alleles observed (Miller et al. unpub.). For all primer sets except *Gmo*35, PCR was conducted in 25-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 $MgSO_4$, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, and 0.1 mg/ml nuclease-free Bovine Serum Albumin. For *Gmo* 35, PCR was conducted in 12.5-µl reactions. All PCR in this study was preceded by an initial denaturation step of three min at 94°C. All cycle extension (30 cycles for all loci) steps were for 60 sec at 72°C and all cycle denaturation steps were for 20 sec at 94°C. PCR 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 was conveniently multiplexed in the same 25-µl reactions. SypI PCR products were digested with DraI (New England Biolabs, Ontario, Canada) for 2 hours at 37°C.

Gel electrophoresis and band analysis

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 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 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.

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 stock 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 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). Principal components of composite arrays of allele frequencies for eight loci were calculated with the PRINCOMP procedure in SAS (SAS 1989).

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). 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. 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 3). 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.

Variation within populations

Observed heterozygosities of the loci examined over all populations were as follows: *Gmo*3 0.31 (population range 0.23-0.43), *Gmo*8 0.86 (0.77-0.90), *Gmo*19 0.91 (0.86-0.94), *Gmo*34 0.45 (0.16-0.74), *Gmo*35 0.73 (0.65-0.80), *Gmo*36 0.59 (0.38-0.73), *Gmo*37 0.81 (0.64-0.96), and SypI 0.55 (0.18-0.70)(Table 2). The observed heterozygosity of the Gilbert's Bay population was the least of all 14 populations examined at three (*Gmo*3, *Gmo*8, and *Gmo*37) of the seven microsatellite loci surveyed.

Genotypic frequencies observed in 13 populations at the seven microsatellite loci surveyed in our study were those expected for populations in Hardy-Weinberg equilibrium with a few exceptions. 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. In the Burgeo Bank sample, genotypic frequencies at three microsatellite loci (*Gmo*3, *Gmo*19, and *Gmo*37) were not in Hardy-Weinberg equilibrium due to an excess of homozygous fish at all three loci. This indicates that this sample may have contained fish from two separate spawning populations (homozygous excess due to the Wahlund effect).

At SypI, significant departures (correction for 13 tests per locus, α=0.0038) from the expected Hardy-Weinberg distribution of genotypic frequencies were observed in eight populations. In each case, there was an excess of heterozygotes observed indicating that balancing selection favouring heterozygotes may operate at SypI in some populations.

The Notre Dame Bay and Bonavista Bay populations had been sampled in multiple years (the 1996 Trinity Bay sample was not considered due to small (n=9 fish) sample size). No significant annual variation in allele frequencies was observed in either the Notre Dame Bay or Bonavista Bay populations (all P>0.10).

Variation among populations

Genetic differentiation at all loci was observed among the putative cod populations surveyed in our study. For example, the frequency of $Gmo3^{186}$ in offshore populations (Flemish Cap 0.59, 2J3KL <0.80) was generally less than that observed in inshore populations (>0.80), with the Gilbert's Bay population having the highest observed frequency (0.88)(Fig. 2a). The Gilbert's Bay sample was similarly distinctive at *Gmo*8, with the frequency of $Gmo8^{144}$ in this population (0.50) substantially higher than in other populations (<0.16)(Fig. 2b). The cod populations in Division 2G had higher frequencies of $Gmo19^{144}$ than did other populations (all other populations < 0.10)(Fig. 2c). Offshore populations in Divs. 2J3KL and Flemish Cap had higher frequencies of $Gmo34^{98}$ (e.g. Hawke Channel 0.79, Funk Island Bank 0.81, 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 (0.35) (Fig. 2d). Differentiation between inshore and offshore populations in 2J3KL was also observed at *Gmo*35 and *Gmo*36 (Figs. 2e, 2f). The Hawke Channel population tended to have higher frequencies of $Gmo37^{256}$ (0.22) than did other populations

(usually < 0.15) (Fig. 2g). At SypI, offshore populations had higher frequencies of the allele possessing the DraI restriction site than did inshore populations (Fig. 2h).

Population differentiation was examined by comparing allele frequencies of cod in different areas. Cod from offshore 2J3KL were compared to those from Flemish Cap, with the expectation that genetic differentiation should be observed, as Flemish Cap is considered to be a distinct stock based upon tagging studies and other biological characters. The pooled offshore 2J3KL population (Hawke Channel, Funk Island Bank, northern Grand Bank) was significantly different from the Flemish Cap population at five of the eight loci surveyed, strongly indicative of restricted gene flow between these two groups and their identification as separate breeding stocks (Table 4). Having demonstrated that the microsatellite loci and SypI locus surveyed were capable of detecting significant population differentiation strongly supported by other types of data, we then compared allele frequencies of offshore 2J3KL and inshore 3KL (Notre Dame Bay, Bonavista Bay, and Trinity Bay) cod. Significant differentiation was observed at five of eight loci surveyed (Table 4), illustrating that inshore and offshore cod from these two areas likely constitute separate breeding stocks. Pairwise probability tests revealed significant differences at 1-5 loci between inshore sites and two of the three offshore sites sampled within 2J3KL; the exception was samples from northern Grand Bank which were not significantly different from inshore sites at any of the loci tested. Do the offshore 2J3KL populations comprise a single breeding population? Significant differentiation at two of the microsatellite loci suggest that there is more than one distinct breeding population (Table 4). Pairwise population comparisons of the allele frequencies (eight tests per comparison, α=0.0063) suggest that there may be at least two breeding populations or stocks in offshore 2J3KL (Table 4). No genetic differentiation was observed among the inshore 3KL populations, perhaps indicative of a single breeding population. However, these populations were quite distinct from the inshore population at Gilbert's Bay, with significant differentiation observed at six of eight loci surveyed (Table 4).

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 observed at two of the eight loci examined (Table 4). Genetic differentiation at some loci was observed between the two inshore samples (Fortune Bay and Placentia Bay), the two offshore samples (Burgeo Bank, Halibut Channel), and between the inshore and offshore samples (eight tests, α=0.0063).

We examined population structure of the differentiated inshore populations surveyed in this study (eight tests, α=0.0063). The Inshore 3KL population (samples from Notre Dame, Bonavista, and Trinity bays combined) was clearly differentiated from Gilbert's Bay in inshore 2J, and from the Fortune Bay population in 3Ps (significant differences at two of eight loci, Table 4). No genetic differentiation was observed between the Placentia Bay population and the inshore 3KL population, even though there is some geographic separation between the two groups. The two inshore populations in 3Ps, Fortune Bay and Placentia Bay, displayed genetic differentiation at one of the eight loci surveyed (Table 4). Based upon an evaluation of the distance measure among populations, the Gilbert's Bay population was the most distinctive of all populations surveyed (Table 5).

Allele frequencies were compared between the Placentia Bay population and offshore spawning populations in 2J3KL and Flemish Cap (eight tests, α=0.0063). There was little similarity between the Placentia Bay and offshore cod, with significant genetic differentiation observed between the Placentia Bay population and three of the four offshore samples examined (Table 4).

Population Structure

The relative levels of temporal variation in allele frequencies within inshore 3KL sampling locations was compared with differentiation among all sampling sites. Differentiation among sampling sites exceeded annual variation within two sampling sites at all loci by at least three-fold, with an average over all eight loci surveyed of 33-fold (Table 6). Although the estimate of temporal variation was based on only two putative populations, the results indicate a relative stability of the genetic characteristics surveyed. This supports the general designation of sampling sites as populations in this study. Temporal

stability of allele frequencies at offshore spawning sites should also be addressed in future studies. However, individual bay samples from northeastern Newfoundland may constitute a single population, and Burgeo Bank sample may contain cod from two or more separate spawning populations. F_{ST} estimates by locus were: *Gmo*3 0.0148, *Gmo*8 0.0066, *Gmo*19 0.0031, *Gmo*34 0.0250, *Gmo*35 0.0013, *Gmo*36 0.0054, *Gmo*37 0.0028, and SypI 0.0702, with the mean over all loci of 0.0117. The historical average number of migrants between the inshore-spawning populations of Div. 3KL and the offshore-spawning populations of Divs. 2J3KL was estimated at 45 fish per generation.

The basic structure of the sampled populations in Divisions 2J3KL was that of an offshore and an inshore component (Fig. 3). 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 was the most unique surveyed in our study (Fig. 4). Of all inshore samples, Fortune Bay was the one with the highest similarity to offshore samples. Of all offshore samples, northern Grand Bank was the one with the highest similarity to inshore samples.

Estimation of stock composition

We tested the utility of the microsatellite loci and SypI to estimate 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 span 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 10% when it comprised only 20% of the mixture (Table 7). 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 lack of differentiation in the inshore Divs. 3KL populations, precision of estimated stock compositions of individual inshore populations was relatively low. Precision of the estimates increased when the inshore contributions to the mixture increased.

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). This reflects the decreased genetic differentiation among 3Ps populations as compared with the differentiation observed between inshore and offshore populations, and among offshore populations, in Divs. 2J3KL (Fig. 3). Characters affording greater differentiation among the putative populations in Subdiv. 3Ps or, possibly, better characterization of stock structure in this region, are required to increase the accuracy and precision of the estimated stock compositions for practical applications.

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 population. In that sample, genotypic frequencies were not in Hardy-Weinberg equilibrium at three of seven microsatellite loci surveyed due to an excess of homozygous individuals at each locus. As this sample was collected between April 6-7, 1998, this may have reflected sampling 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).

To assess divergence among putative populations correctly, sampling must be adequate to establish population divergence. Smouse and Chevillon (1998) suggested that a modest number of independent loci is best, each locus with a modest number of alleles, and with each allele in modest frequency. They indicated that "it would be silly to have many more characters sampled than individuals per population". If the number of characters is the total number of alleles at all loci minus the number of loci, then the number of individuals sampled in a population should be at least one more than the number of characters in order to estimate a non-singular within-population covariance matrix (Smouse and Chevillon 1998). The maximum number of alleles observed at the seven microsatellite loci surveyed in our study was 104 alleles (106 alleles including SypI), and sample sizes for the populations surveyed were usually close to (except for Division 2G) or in excess of the minimum recommended by Smouse and Chevillon (1998).

Although only two locations were sampled in at least two years, 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 and the SypI locus did not reveal any significant differentiation among the cod populations in Notre Dame Bay, Bonavista Bay, or Trinity Bay. A previous survey at five different microsatellite loci (187 total alleles (Bentzen et al. 1996)) indicated that there was no differentiation among cod from Trinity Bay (302 fish), St. Anthony Basin (48 fish), and the Notre Dame Channel (48 fish)(Ruzzante et al. 1998). Moreover, the Placentia Bay sample of cod was very similar to the northeastern Newfoundland inshore populations sampled in this study. The genetic surveys conducted to date provide no evidence to indicate that there are separate bay stocks of cod along the northeast coast of Newfoundland. Levels of migration (resulting in gene flow) among cod spawning in the different bays 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 and the SypI locus 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 seven different microsatellite loci, significant differences were observed at four of the seven loci, as well as at the SypI locus, convincingly demonstrating significant genetic differentiation between inshore- and offshore-spawning populations. The microsatellite loci 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 spawning populations, in spite of extensive mixing in the inshore during summer. 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 suggest that there could be at least two spawning stocks of cod in offshore Divs. 2J3KL, the offshore 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? We estimated an historical average of 45 genetically-effective migrants per generation move between the inshore and offshore-spawning components of northern cod, similar to an estimate of up to 250 individuals reported by Ruzzante et al. (1998). This number is the product of the proportion of individuals that migrate and the effective population size. Historical population sizes of the inshore and offshore stocks were large (in the millions of fish), meaning that the proportion of migrants per generation was typically very small (<0.1%). Unless the patterns of migration between the inshore and offshore locations have changed greatly in recent years, it is unlikely that the inshore-spawning stock will contribute significantly to recovery in abundance of the offshore-spawning stocks. Similarly, the Flemish Cap population would unlikely contribute significantly to recovery of the offshore populations, given its degree of genetic differentiation (significant allele frequency differences at four of seven microsatellite loci and SypI). For the microsatellite loci such differentiation indicates a significant restriction of gene flow between cod of the two areas. At the SypI locus, 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 (significant differences at four of seven microsatellite loci and SypI). 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 one microsatellite locus and the SypI locus, indicative of some degree of restricted gene flow between these two populations. Offshore cod have a higher frequency of the SypI allele possessing the DraI restriction site (Fevolden and Pogson 1997; current study) which may confer some survival advantage in offshore habitats, making the relatively high frequency of this allele in the Fortune Bay population unexpected. The Burgeo Bank sample was differentiated from other Subdiv. 3Ps samples. However, this sample was likely drawn from a composite mixture of at least two spawning populations (at least one of which was not sampled in this study), making interpretation of this differentiation problematic. The Halibut Channel population was distinct from the Placentia Bay population, but not from the Fortune Bay population. The Placentia Bay population was differentiated from the other populations sampled in Subdiv. 3Ps. However, confirmation of differentiation among the putative populations in this region needs to be confirmed by sampling in subsequent years.

Is the Placentia Bay population distinct from offshore-spawning populations in Divs. 2J3KL? Significant genetic differentiation between the Placentia Bay population and the offshore populations was observed at from two to four loci in comparisons with three of the four 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 10% 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 was 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.

Acknowledgements

We would like to acknowledge technical staff from the Department of Fisheries and Oceans and commercial fishers who assisted in the collection of adult Atlantic cod samples. Dr. J. S. Wroblewski of Memorial University collected the Gilbert's Bay sample. Dr George Rose, Fisheries Conservation Chair, Memorial University, and R. Rideout kindly collected the sample from Hawke Channel. J. Candy assisted in the figure preparation.

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Table 1. Samples collected and analyzed from cod populations in the Newfoundland region. Number in parentheses is population reference number (see Fig 1.) N is sample size in each year.

Area	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) Hawke Channel	-	June 13-16	1998	52.75	53.00	236	236
inshore	(3) Gilberts Bay	-	May 29	1997	52.58	55.83	86	86
3K offshore	(4) Funk Island Bank	-	Nov 11-21	1996	-[1]	-	97	97
inshore	(5) Notre Dame Bay	Miles Cove	June 18-July 3	1997	49.54	55.77	126	339
		Fogo	June 18	1998	49.65	54.05	213	
3L offshore	(6) Northern Grand Bank	-	June 25-27	1998	-[2]	-	113	113
inshore	(7) Bonavista Bay	Open Hall	May 29-June 11	1997	48.51	53.51	147	309
		Plate Cove	June 4	1998	48.52	53.52	162	
	(8) Trinity Bay	Hopeall	June 25-28	1996	47.63	53.56	9	164
		West Trinity Bay	April 23-26	1997	48.18	53.61	155	
3M	(9) Flemish Cap	-	Sept 25 – Oct 12	1996	-[3]	-	103	103
3Ps offshore	(10) Halibut Channel	-	April 2-4	1998	45.10	55.20	214	214
	(11) Burgeo Bank	-	April 6-7	1998	46.75	57.65	250	250
inshore	(12) Placentia Bay	Bar Haven,	April 22-26	1998	47.73	54.19	223	523
		Warehams Rock	May 1-3	"	47.70	54.15	205	
		Paradise Sound	April 27-29	"	47.51	54.49	95	
	(13) Fortune Bay	Pools Cove	May 21-28	1998	47.70	55.38	264	264

[1] collected at various locations on or adjacent to Funk Island Bank during Teleost trip 40 and Wilfred Templeman trip 198.

[2] collected at various locations in offshore northern 3L north of 47.6°N during Wilfred Templeman trip 198.

[3] 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.

Table 2. Repeat type, number of alleles per locus, allele size range (bp), and observed heterozygosity for the microsatellite loci surveyed.

Locus	Repeat Type	No. of alleles	Allele size range	Heterozygosity
Gmo 3	GACA, imperfect	9	150-200	0.305
Gmo 8	GACA, perfect	23	110-205	0.862
Gmo 19	GACA, perfect	24	120-220	0.909
Gmo 34	GACA, perfect	8	80-120	0.446
Gmo 35	ACC, perfect	12	110-145	0.725
Gmo 36	GGT, imperfect	13	170-210	0.594
Gmo 37	GACA, imperfect	15	220-290	0.809

Table 3. 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 4. 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
Divisions 2J3KL									
Flemish	offshore 2J3KL	**0.0000**	**0.0005**	0.0262	**0.0000**	0.0904	**0.0000**	0.0657	**0.0000**
inshore 3KL	offshore 2J3KL	**0.0043**	0.1114	**0.0019**	**0.0000**	0.2520	**0.0019**	0.0816	**0.0000**
Funk Island Bank	Notre Dame	**.0081**	.0132	.8397	.0120	.3983	**.**0510	**.0073**	**.0000**
	Trinity	**.0000**	.3726	.5558	.1095	.7152	.2086	.2908	**.0002**
	Bonavista	**.0000**	**.0069**	.6056	.0128	.3485	.1977	.0433	**.0000**
Hawke Channel	Notre Dame	.8868	.0505	.1856	**.0004**	.1950	**.0000**	**.0000**	**.0000**
	Trinity	.3028	.1607	.0131	.0432	.3669	**.0023**	**.0069**	**.0000**
	Bonavista	.0998	.2766	**.0007**	**.0002**	.1756	**.0000**	**.0010**	**.0000**
N. Grand Bank	Notre Dame	.4161	.6028	.7931	.5068	.0112	.4378	.6963	.0573
	Trinity	.5986	.3291	.3784	.4788	.0886	.8680	.9074	.4443
	Bonavista	.3640	.3175	.1694	.0939	.0917	.6958	.8639	.0895
Single offshore pop. 2J3KL?		0.0348	0.3555	0.9681	0.8003	0.0398	**0.0003**	**0.0000**	0.0148
Hawke Ch.	Funk Island	0.0508	0.0333	0.9835	0.9047	0.0456	0.0117	**0.0000**	0.5423
	Northern GB	0.5405	0.8797	0.7955	0.4377	0.0460	0.0611	0.0644	0.0111
Funk Is.	Northern GB	**0.0018**	0.0513	0.9673	0.3560	0.0125	0.2871	0.0408	0.0103
Single inshore pop 3KL?		0.4211	0.2377	0.0160	0.7400	0.3466	0.9977	0.6807	0.4901
inshore 3KL	Gilbert's Bay	0.3505	**0.0000**	**0.0000**	**0.0000**	**0.0010**	**0.0049**	**0.0000**	-
Subdivision 3Ps									
Single population?		0.5279	0.5465	0.0931	0.2818	0.1342	**0.0031**	0.5614	**0.0000**
Burgeo Bank	Halibut Chan.	0.0327	0.1248	0.0070	0.6483	0.4804	0.0809	0.7746	0.2624
	Fortune Bay	0.1157	0.6949	0.5893	0.7175	0.2482	**0.0029**	0.3961	0.6825
	Placentia Bay	0.4878	0.6440	0.9440	0.1837	0.1756	0.3577	0.4472	**0.0000**
Halibut Chan	Fortune Bay	0.6934	0.2478	0.0275	0.1805	0.5166	0.1048	0.8570	0.4313
	Placentia Bay	0.8812	0.0973	**0.0013**	0.3056	0.5840	0.7208	0.4514	**0.0069**
Fortune Bay	Placentia Bay	0.2940	0.9266	0.3968	0.2415	0.0662	0.0075	0.6958	**0.0000**
Inshore population structure									
3KL	Gilbert's Bay	0.3505	**0.0000**	**0.0000**	**0.0000**	**0.0010**	**0.0049**	**0.0000**	-
	Fortune Bay	0.1708	0.4982	0.2663	0.1018	0.6894	**0.0000**	0.3994	**0.0000**
	Placentia Bay	0.0415	0.0385	0.4497	0.7556	0.0142	0.2021	0.3361	0.2473
Gilbert's Bay	Fortune Bay	0.2913	**0.0000**	**0.0000**	**0.0000**	**0.0002**	0.0128	**0.0000**	-
	Placentia Bay	0.2786	**0.0000**	**0.0000**	**0.0000**	0.1881	0.0250	**0.0000**	-
Fortune Bay	Placentia Bay	0.2940	0.9266	0.3968	0.2415	0.0662	0.0075	0.6958	**0.0000**
Placentia Bay and offshore populations									
Placentia	Flemish Cap	**0.0000**	**0.0044**	0.0899	**0.0000**	0.1804	**0.0000**	0.0436	**0.0000**
	Hawke Ch.	0.4825	0.2462	0.0759	**0.0006**	0.2162	**0.0010**	**0.0004**	**0.0000**
	Funk Island	**0.0001**	**0.0015**	0.9063	0.0524	0.0625	0.4563	**0.0004**	**0.0000**
	Northern GB	0.8595	0.8595	0.6616	0.3222	0.1058	0.7743	0.9720	0.2366

Table 5. Pairwise Cavalli-Sforza and Edwards (1967) chord distance among 14 populations of Atlantic cod derived from seven microsatellite loci and the synaptophysin locus.

Population	Notre	Flemish	Trinity	Bona	Gilbert	Burgeo	Halibut	Fortune	Placentia	Hawke	2G	Northern
Funk Island	0.0058	0.0082	0.0057	0.0060	0.0391	0.0054	0.0058	0.0058	0.0059	0.0065	0.0186	0.0071
Notre Dame		0.0083	0.0028	0.0019	0.0348	0.0020	0.0027	0.0028	0.0015	0.0035	0.0175	0.0033
Flemish Cap			0.0100	0.0086	0.0374	0.0079	0.0081	0.0096	0.0079	0.0096	0.0224	0.0079
Trinity Bay				0.0021	0.0373	0.0030	0.0036	0.0030	0.0023	0.0045	0.0187	0.0041
Bonavista Bay					0.0353	0.0022	0.0030	0.0025	0.0018	0.0039	0.0162	0.0033
Gilbert's Bay						0.0347	0.0354	0.0358	0.0354	0.0388	0.0588	0.0354
Burgeo Bank							0.0033	0.0031	0.0016	0.0039	0.0174	0.0034
Halibut Channel								0.0030	0.0025	0.0049	0.0171	0.0044
Fortune Bay									0.0021	0.0040	0.0161	0.0034
Placentia Bay										0.0032	0.0172	0.0022
Hawke Channel											0.0188	0.0034
2G												0.0162

Table 6. Hierarchical gene-diversity analysis of 13 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	0.3386	0.3308	0.9772	0.0005	0.0223	47.4
*Gmo*8	0.9166	0.9047	0.9871	0.0018	0.0112	6.2
*Gmo*19	0.9335	0.9241	0.9899	0.0003	0.0098	33.9
*Gmo*34	0.4577	0.4365	0.9537	0.0000	0.0462	-
*Gmo*35	0.7677	0.7640	0.9952	0.0012	0.0036	3.0
Gmo36	0.6028	0.5952	0.9874	0.0000	0.0126	-
Gmo37	0.8638	0.8539	0.9886	0.0013	0.0150	11.6
SypI	0.4787	0.4144	0.8657	0.0000	0.1343	-
All			0.9739	0.0008	0.0253	33.3

Table 7. Estimated percentage composition of three simulated mixtures of inshore and offshore NAFO Divisions 2J3KL Atlantic cod incorporating variation at seven microsatellite loci and the SypI locus. 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
Notre Dame Bay	30	31.1 (12.2)	20	21.5 (10.7)	10	13.3 (7.8)
Trinity Bay	20	16.5 (10.0)	20	16.0 (9.3)	5	8.8 (6.3)
Bonavista Bay	30	29.2 (12.0)	10	14.0 (9.6)	5	7.7 (8.4)
S Inshore	**80**	**76.8 (7.8)**	**50**	**51.7 (7.7)**	**20**	**29.8 (8.6)**
Hawke Channel	10	11.6 (6.6)	25	24.6 (6.8)	40	36.5 (8.2)
Funk Island Bank	5	4.0 (3.0)	15	10.6 (4.8)	20	16.6 (6.4)
Northern Grand Bk	5	7.6 (6.2)	10	13.1 (7.3)	20	17.1 (7.1)
S Offshore	**20**	**23.2 (7.8)**	**50**	**48.3 (7.7)**	**80**	**70.2 (8.6)**

Table 8. Estimated percentage composition of three simulated mixtures of inshore and offshore NAFO Subdivision 3Ps Atlantic cod incorporating variation at seven microsatellite loci and the SypI locus. 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	15	17.6 (10.5)	20	22.4 (11.3)	30	24.3 (9.3)
Placentia Bay	25	33.7 (11.7)	40	42.7 (13.4)	50	49.4 (12.1)
S Inshore	**40**	**51.2 (13.1)**	**60**	**65.1 (13.2)**	**80**	**73.7 (12.2)**
Burgeo Bank	35	29.2 (10.3)	10	13.0 (9.1)	10	12.3 (8.6)
Halibut Channel	25	19.6 (11.4)	30	21.9 (12.2)	10	14.0 (10.1)
S Offshore	**60**	**48.8 (13.1)**	**40**	**34.9 (13.2)**	**20**	**26.3 (12.2)**

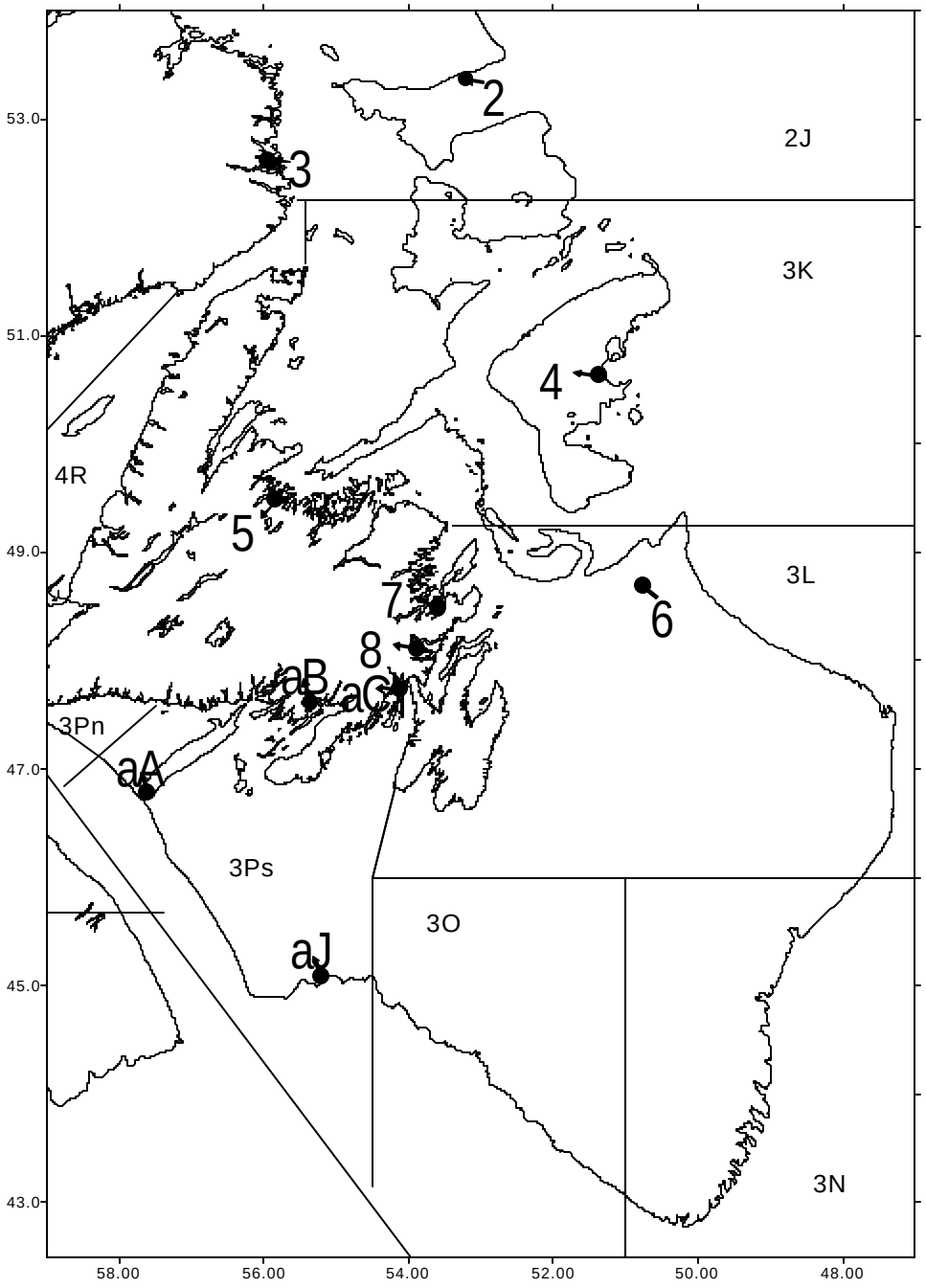

Figure 1. Approximate locations of cod populations sampled. Other sampling details are summarized in Table 1. Population 1 (Division 2G) and population 9 (Flemish Cap, Division 3M) are not shown and are north and west of the area shown, respectively.

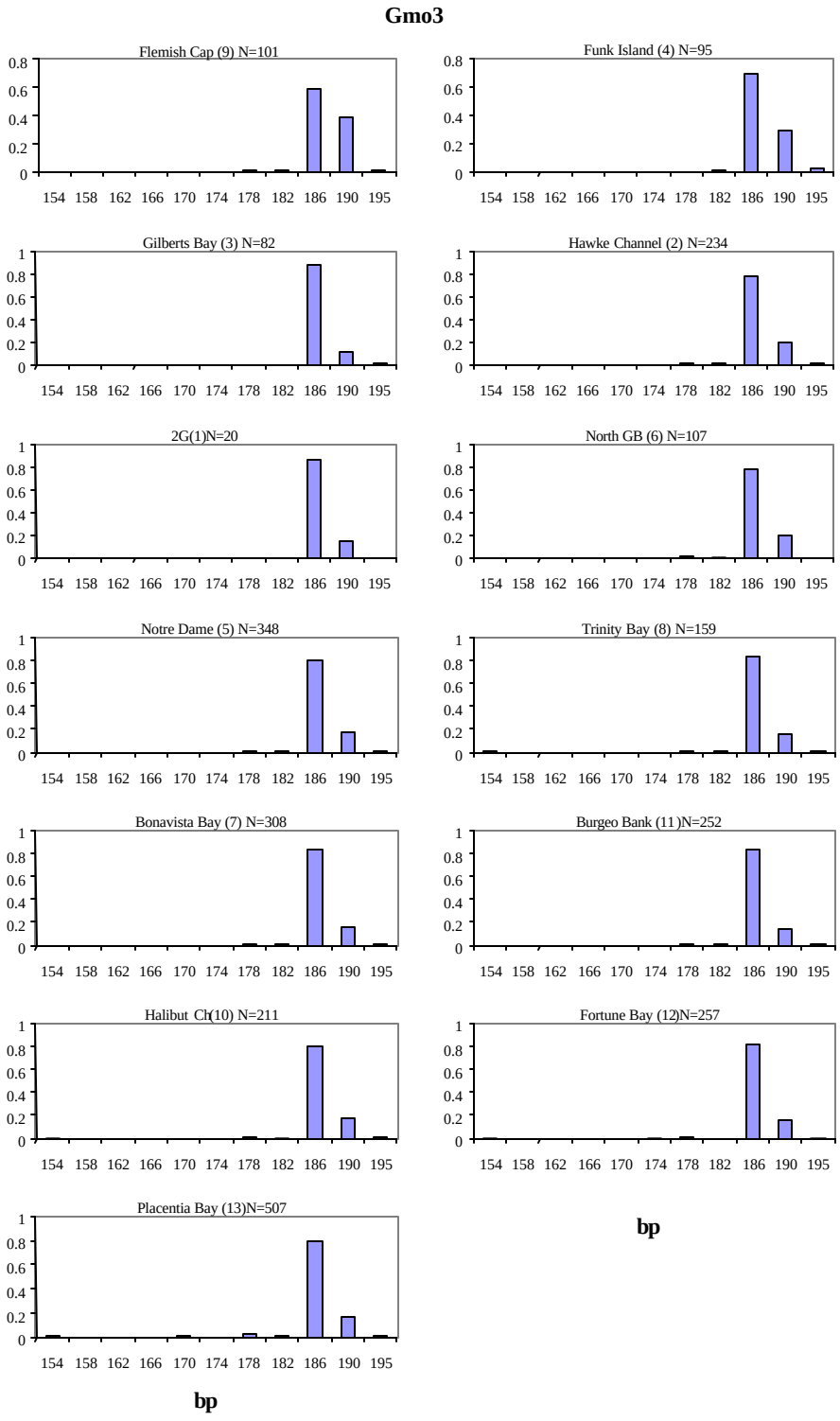

Fig 2a. – Allele frequencies of Gmo3 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

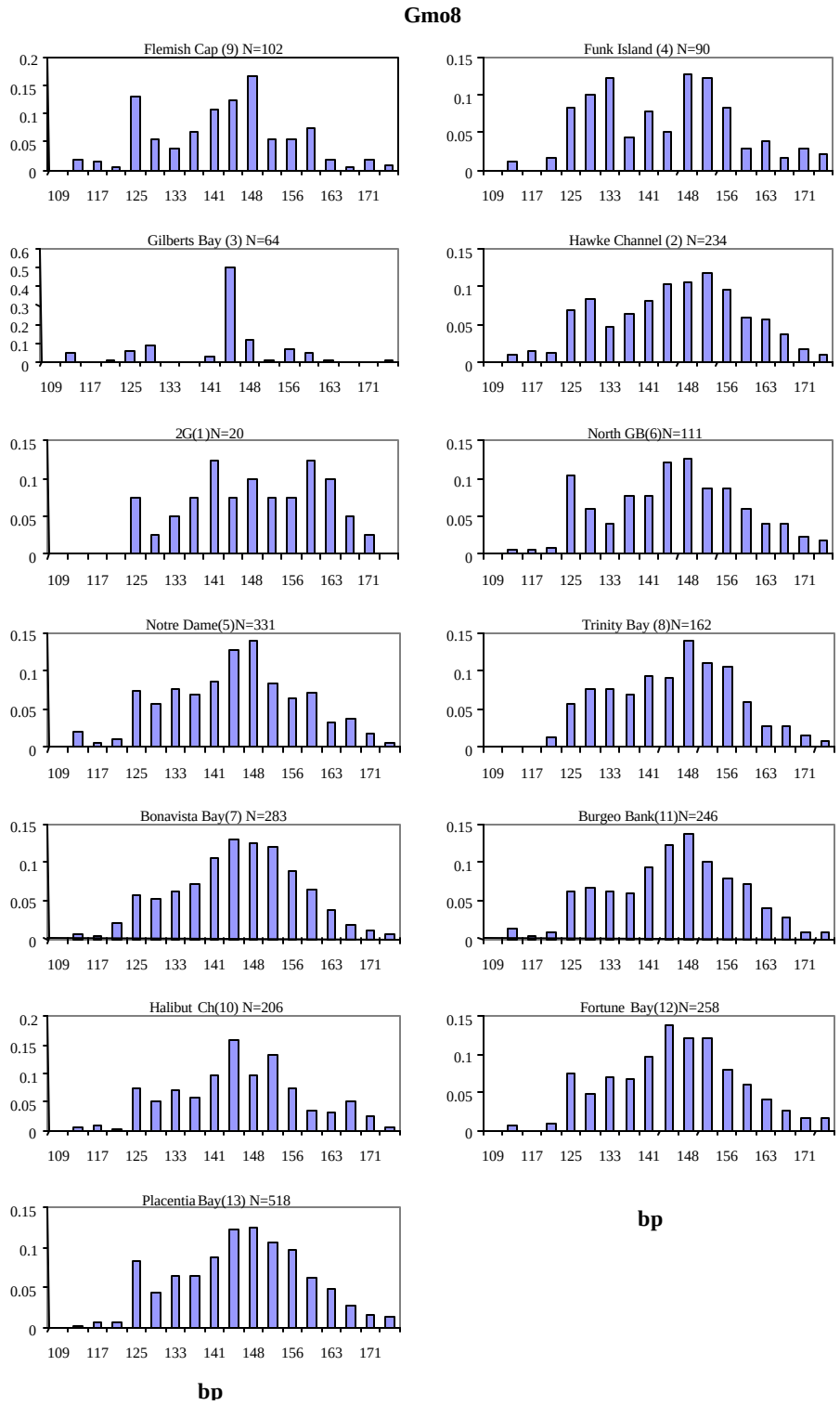

Fig 2b.- Allele frequencies of Gmo8 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

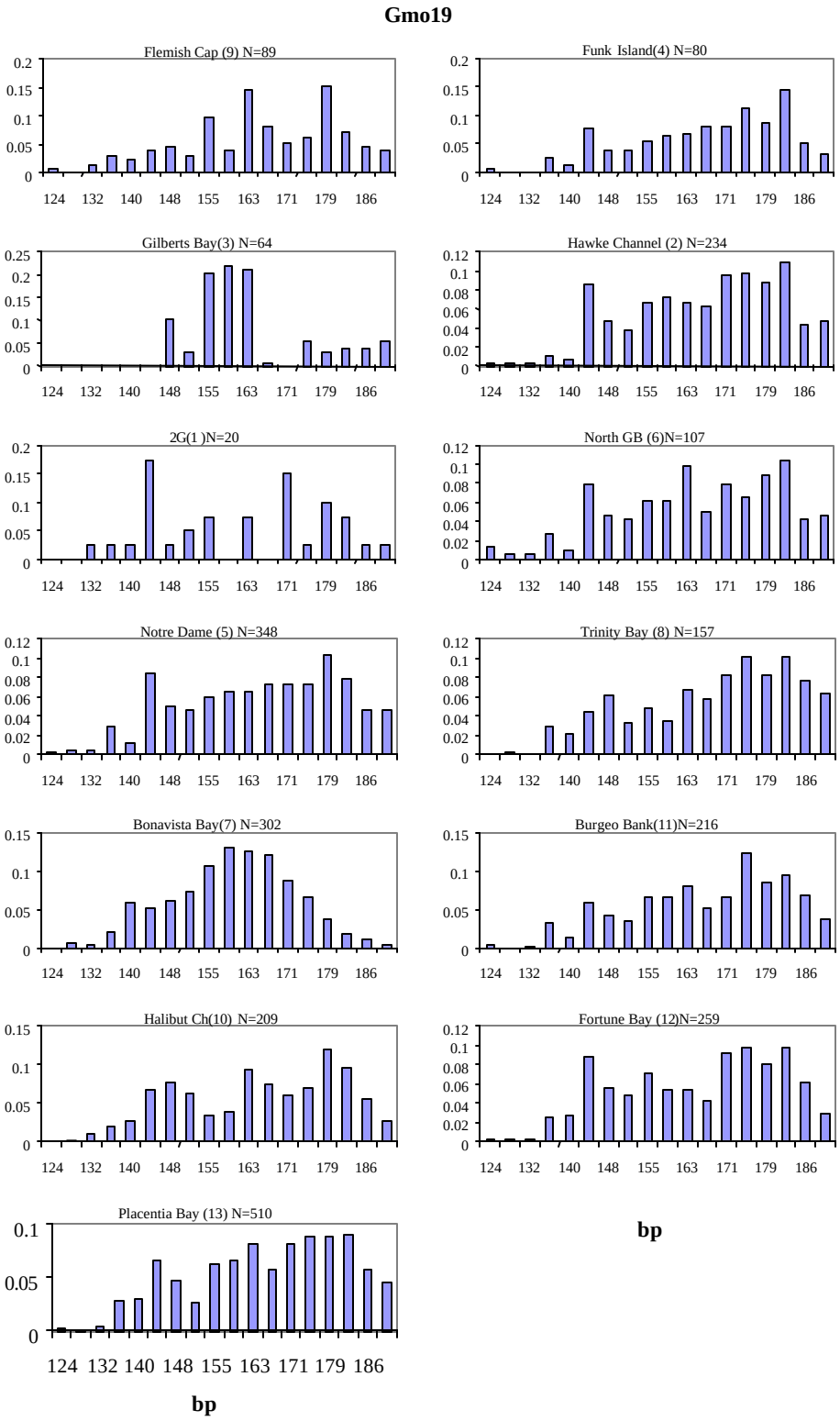

Fig 2c. – Allele frequencies of Gmo19 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

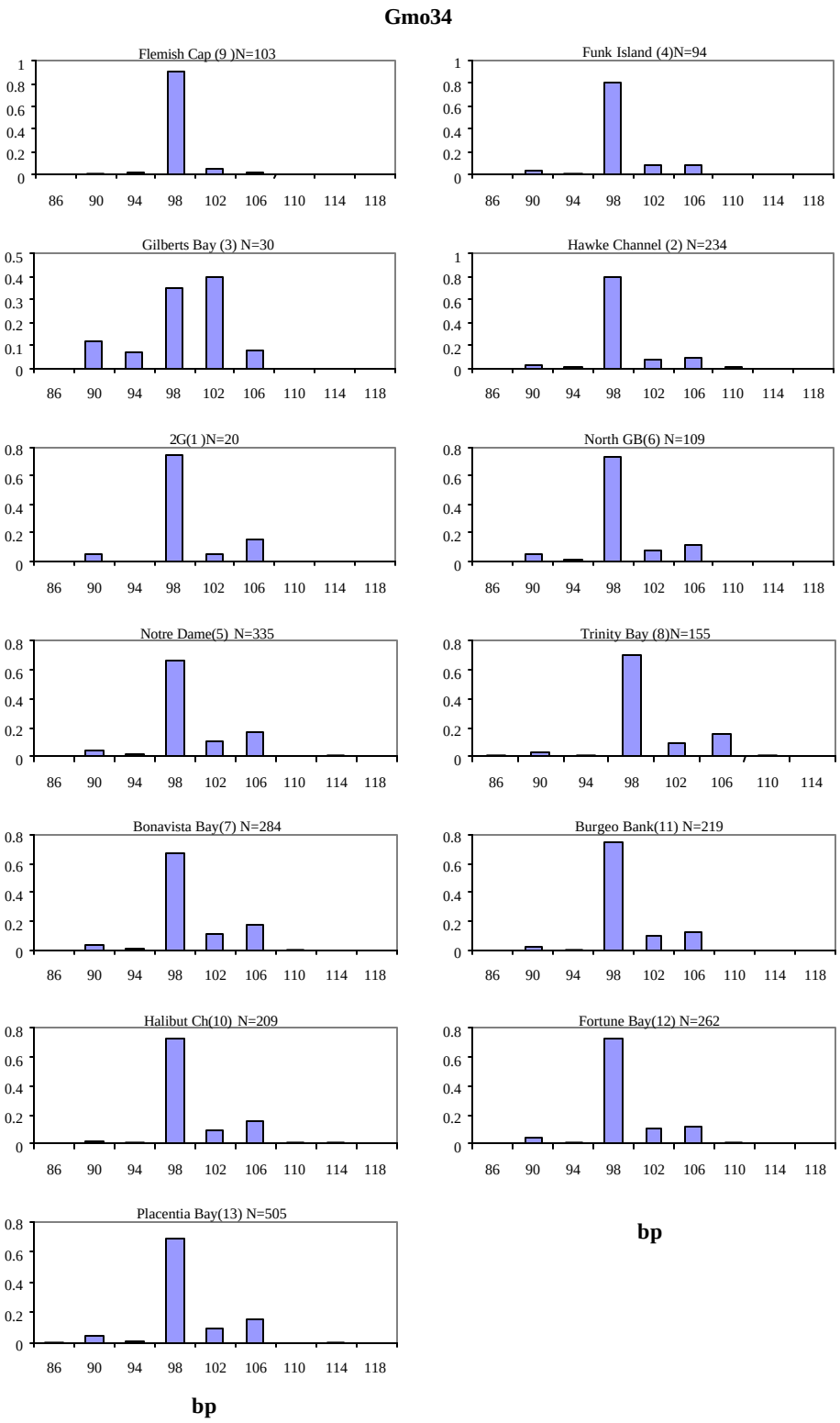

Fig 2d. – Allele frequencies of Gmo34 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

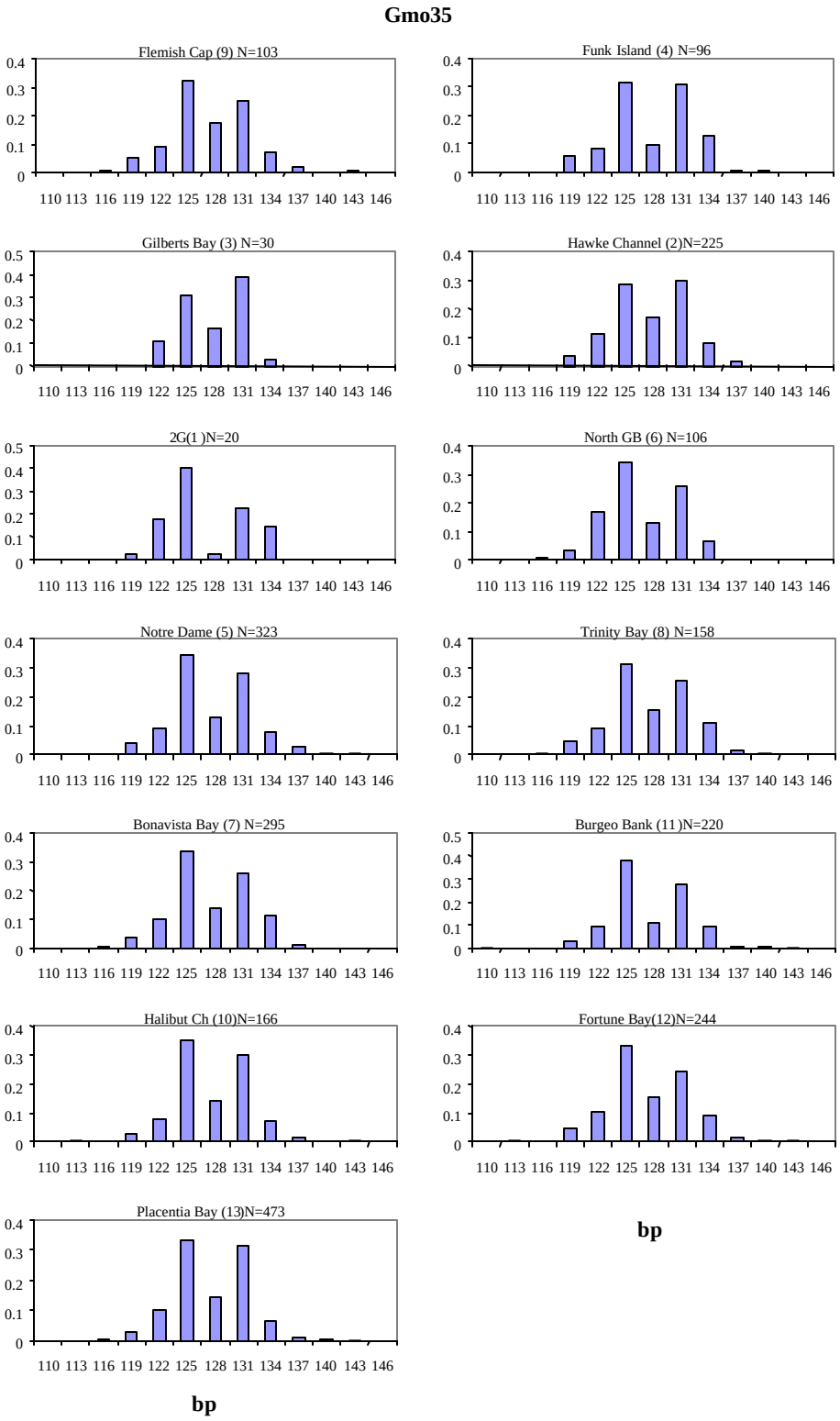

Fig.2e – Allele frequencies of Gmo35 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

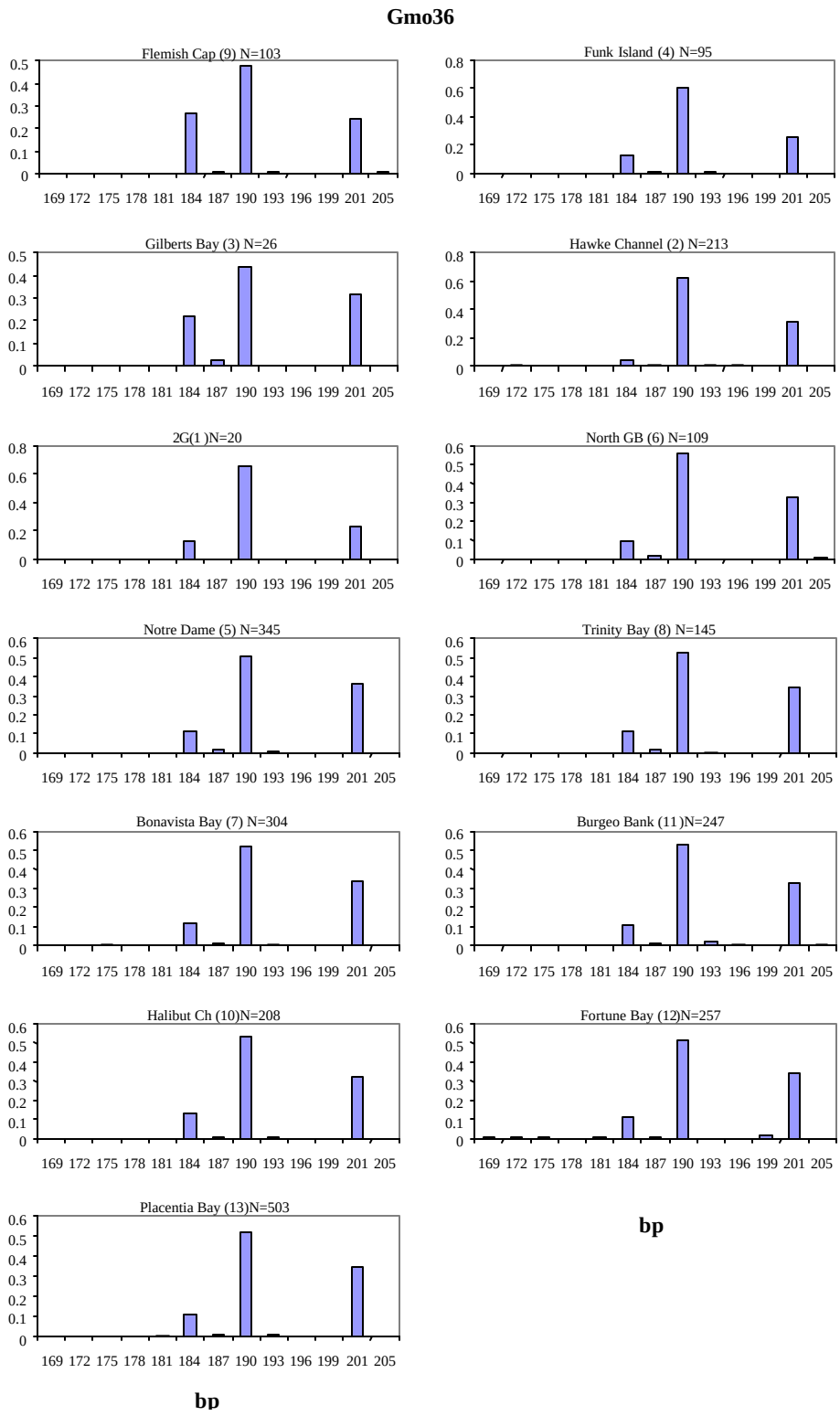

Fig 2f. – Allele frequencies of Gmo36 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

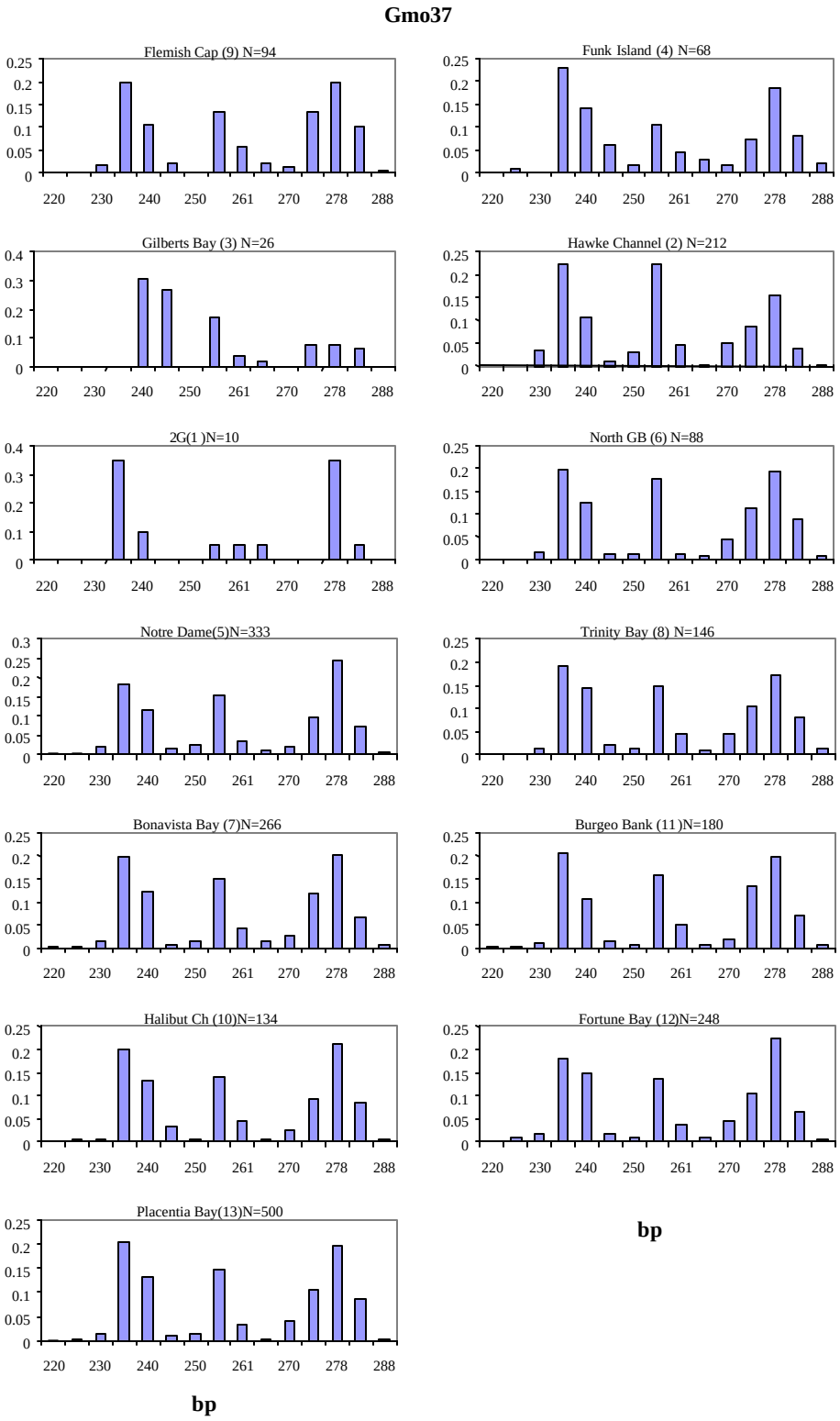

Fig 2g. – Allele frequencies of Gmo37 in 13 populations of Atlantic cod. Number in parenthesis is the population reference number from Table 1 and Fig. 1. N is the number of cod sampled in each population. Alleles were designated by the lower limit (bp) of the allele bin used to define the alleles.

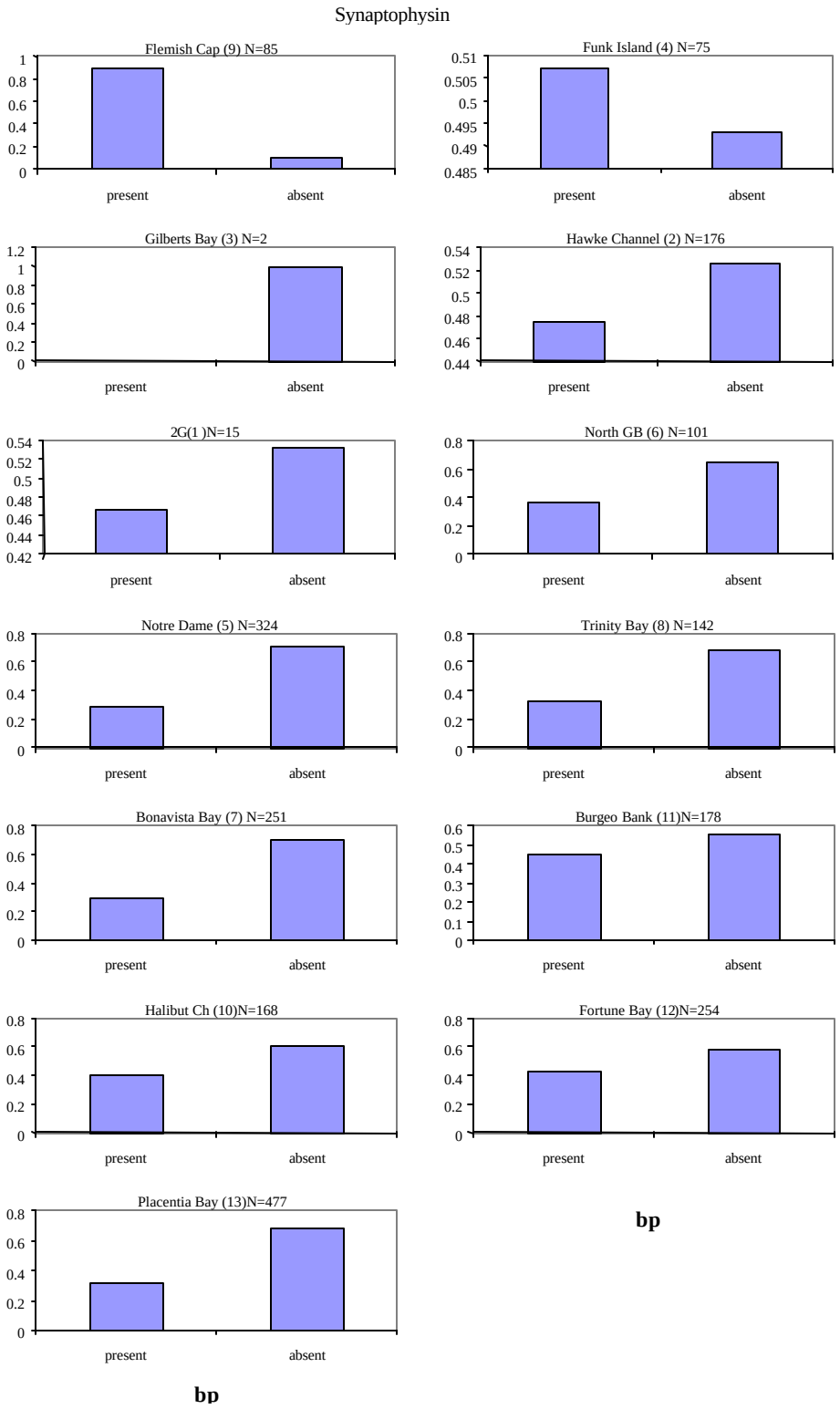

Fig 2h. – Allele frequencies of synaptophysin in 13 populations of Atlantic cod. N is the number of cod sampled in each population. Number in parenthesis is the population reference number from Table 1 and Fig. 1.

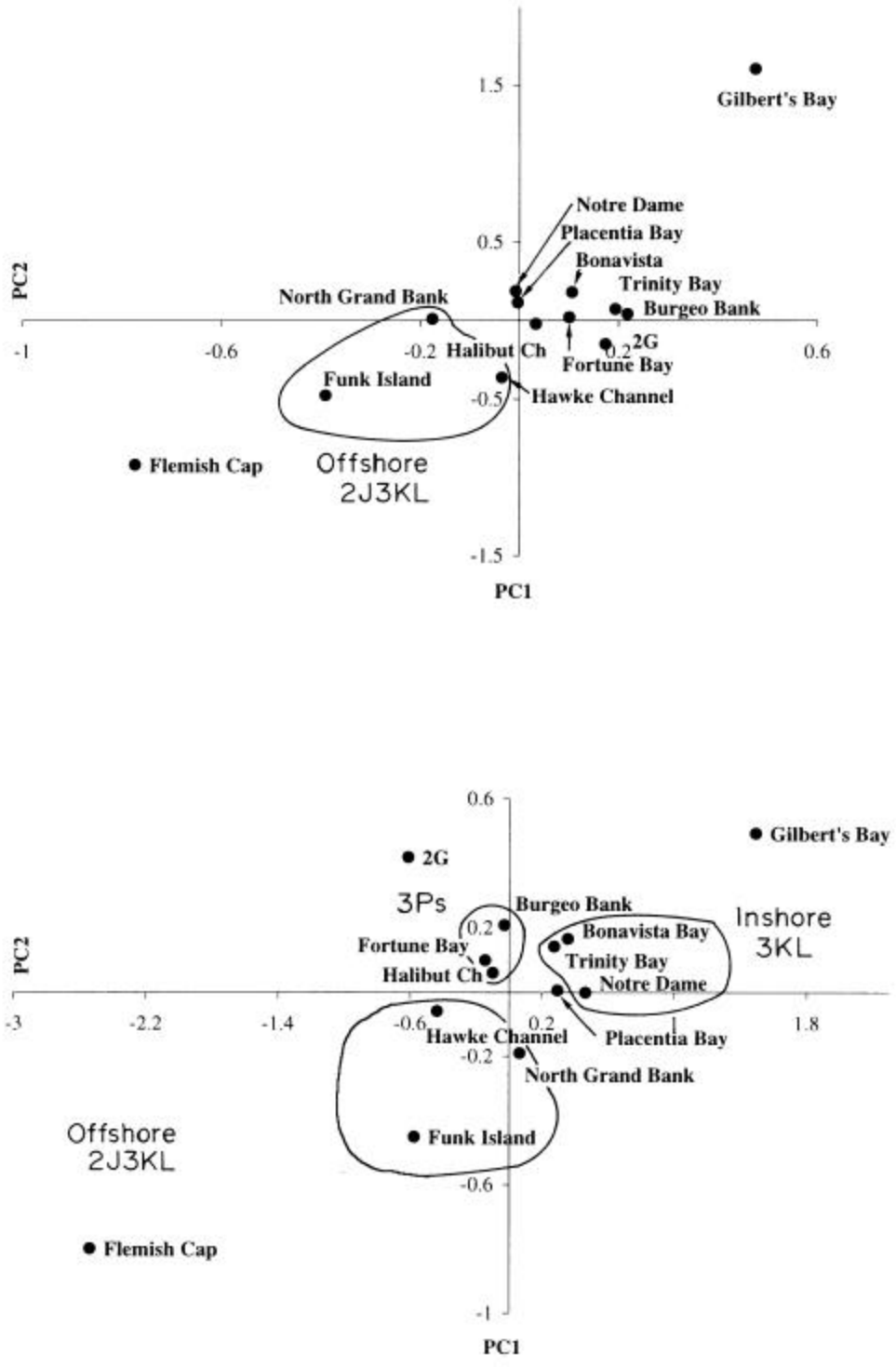

Figure 3. – Plot of the first two principal components incorporating variation at: A seven microsatellite loci and B seven microsatellite loci and sypI for 13 populations of Atlantic cod.

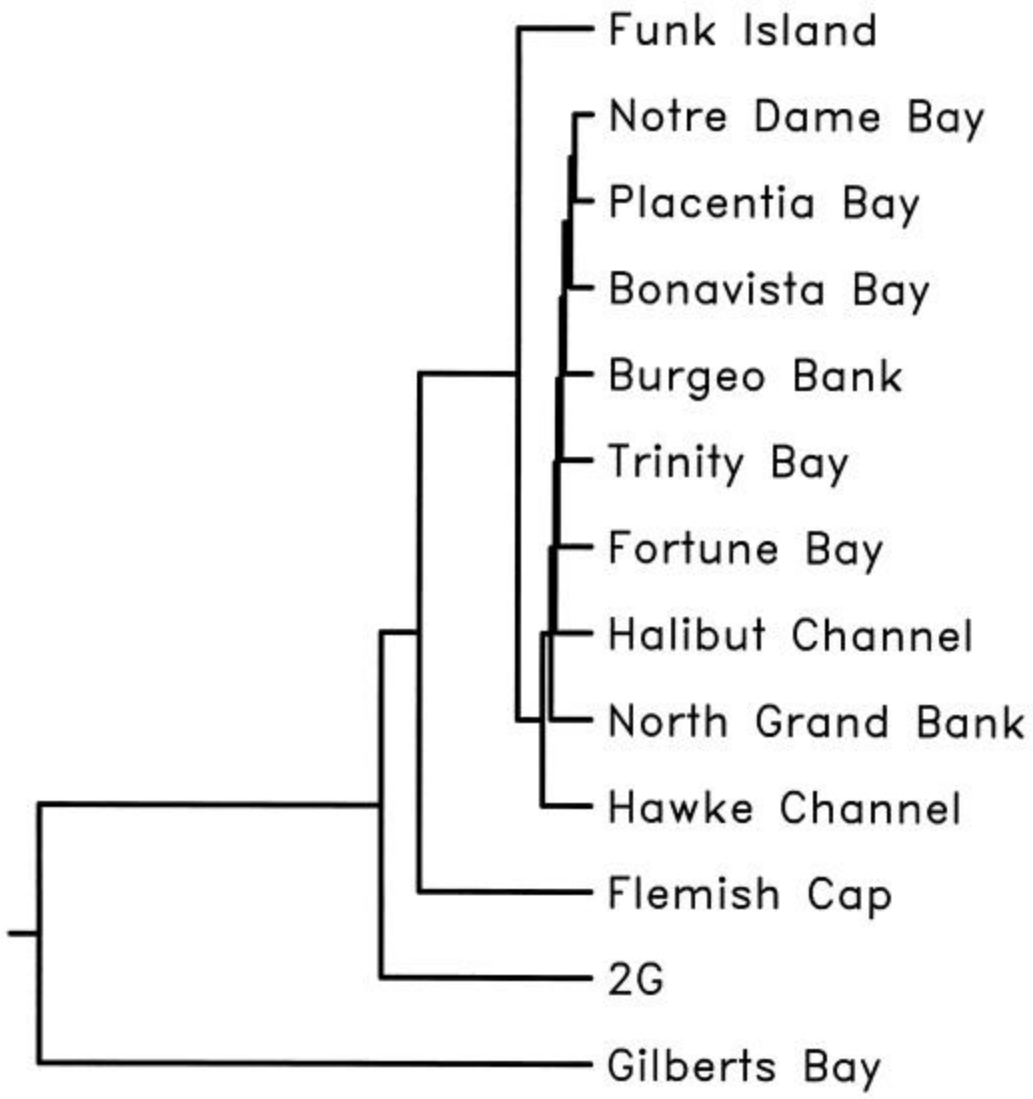

Fig 4. Unrooted UPGMA dendrogram outlining relationship of 13 Atlantic cod populations.