Northwest Atlantic Fisheries Organization

Serial No. N2694 NAFO SCR DOC. 96/21

SCIENTIFIC COUNCIL MEETING - JUNE 1996

Microsatellite Polymorphism and the Population Structure of Atlantic Cod (*Gadus morhua*) in the Northwest Atlantic

by

Paul Bentzen,(*) Christopher T. Taggart,(†,‡) Daniel E. Ruzzante,(ŧ) and Doug Cook(§)

(*) Marine Molecular Biotechnology Laboratory and School of Fisheries, University of Washington, 3707 Brooklyn Ave. NE, Seattle, WA 98105-6715, USA. Internet: pbentzen@fish.washington.edu

(†)Science Branch, Canada Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, PO Box 5667, St. John's, NF, A1C 5X1
(‡)current address: Department of Oceanography, Dalhousie University, Halifax, N.S., B3H 4J1. Internet: chris.taggart@dal.ca

(ŧ) Department of Biology, Dalhousie University, Halifax, NS, B3H 4J1. Internet: ruzzante@cs.dal.ca

(§) Marine Gene Probe Laboratory, Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1. Internet: cookd@ac.dal.ca

ABSTRACT

Allelic variation in six highly polymorphic microsatellite loci (mean heterozygosity, 86%) provided evidence that cod (*Gadus morhua*) in the northwest Atlantic belong to multiple genetically distinguishable populations, and further, that genetic differences may also exist between northwestern and southeastern cod aggregations within the northern cod stock complex off Newfoundland. Cod were sampled from winter aggregations ranging from Hamilton Bank to the northern Grand Bank in the northern cod complex, and from the Flemish Cap, the Scotian Shelf and the Barents Sea. Tests of allele frequency homogeneity (χ^2), F_{ST} , and $(\delta\mu)^2$, allele sharing and Rogers' distance measures revealed significant differences among northern cod, Flemish Cap, Scotian Shelf and Barents Sea samples. Within the northern cod complex, two pooled samples, NORTH (Hamilton, Funk and Belle Isle Banks) and SOUTH (northern Grand Bank area) were distinguishable using χ^2, $(\delta\mu)^2$ and allele sharing measures. Both $(\delta\mu)^2$ and Rogers' distances clustered western Atlantic samples in two groups distinct from the divergent Barents Sea sample; one comprised NORTH, SOUTH and Scotian Shelf, and the other, Flemish Cap.

INTRODUCTION

Despite considerable study in the past, basic features of the population structure of Atlantic cod (*Gadus morhua*) remain controversial. Although spawning areas for cod in many parts of its North Atlantic range are known to be relatively discrete, stable over time, and often relatively distant from other spawning areas (Brander 1994; Serchuck et al. 1994; Frank et al., 1994; Taggart et al. 1994; Buch et al. 1994; Schopka 1994), whether or not such spawning aggregations constitute reproductively and genetically distinct populations remains uncertain. Proper resolution of these uncertainties is important for a detailed understanding of the biology of the species, and ultimately, for successful management (Angel et al. 1994). These issues are exemplified by the seriously depleted northern cod stock complex (Northwest Atlantic Fisheries Organization (NAFO) Divisions 2J, 3K, and 3L).

The distribution and migration patterns of northern cod are complex. Most mature northern cod overwinter in warm (> 2 °C) slope water along the margin of the continental shelf, but some remain inshore all winter, relying on serum antifreeze proteins to survive in cold (< 0 °C) coastal water (Goddard et al. 1994). Spawning occurs over a 3-4 month period from winter through early summer, and generally begins earlier

in the north than in the south (Myers et al. 1993). Spawning takes place in offshore bank areas and along the continental shelf break, but some also occurs in the interior of the Grand Bank and in coastal regions (reviewed in Taggart et al. 1994). Cod that have overwintered and spawned offshore migrate inshore in the spring to coastal feeding grounds, then return offshore in late autumn/early winter (Templeman 1966). Juvenile cod may remain inshore for several years before adopting the migratory behaviour of mature cod around age 4 (Lear and Green 1984; Rose 1993).

Existing data on population structure within the northern cod are equivocal. On the one hand, geographic surveys (Hutchings et al. 1993), vertebral data (Templeman 1981, Lear and Wells 1984) and tag recovery data (Templeman 1974, 1979; Lear 1984; reviewed in Lear and Green 1984 and Taggart et al. 1995) all suggest that northern cod are divided into several distinct offshore spawning units; on the other hand, none of these observations constitute evidence of reproductive isolation among spawning units. Cod-tagging studies have provided evidence of population fidelity to particular overwintering/spawning areas on offshore banks and in some inshore bays, as well as evidence of vagrant movements among these areas (Lear 1984; Taggart et al. 1995; Wroblewski et al. 1996). However, tag recovery data yield no direct evidence of the extent of gene flow among spawning units.

In theory, allele frequency data from neutral genetic markers may provide the most stringent test of population differentiation. Thus far, however, evidence of population structure gained from genetic studies of northern and other western Atlantic cod has been mixed, and subjected to various interpretations. Data from a variety of allozyme (Mork et al. 1985) and mitochondrial DNA (mtDNA) studies (Smith et al. 1989; Carr and Marshall 1991a,b; Pepin and Carr 1993; Carr 1995; Carr et al. 1995) have not permitted rejection of the null hypothesis that no genetic differentiation exists among various samples drawn from northwest Atlantic populations. In contrast, highly significant differences among populations have been detected with a small number of protein loci (Jamieson 1975; Cross and Payne 1978), more recently with a suite of nuclear DNA restriction fragment length polymorphism (RFLP) loci (Pogson et al. 1995), and most recently with nuclear microsatellite loci (Ruzzante et al. 1996a).

Several authors have invoked selection (non-neutrality of genetic loci) as an explanation for the contrast in the results obtained with different genetic markers, but differ in their view of the direction taken by selective forces. Some (e.g., Mork and Sundnes 1985; Mork et al. 1985) have suggested that selection is acting to increase heterogeneity at some loci (thereby exaggerating the distinctiveness of populations), whereas Pogson et al. (1995) have argued that balancing selection may be acting to homogenize variation expressed by some protein loci across different locales (thereby under-representing the isolation of populations).

Notwithstanding the possible role of selection, loci that exhibit significant geographic variation in cod have tended to exhibit moderate to high levels of polymorphism, whereas those that do not have tended to be less polymorphic, with low heterozygosities and/or low frequency of variant alleles or haplotypes (Jamieson 1975; Cross and Payne 1978; Mork et al. 1985; Smith et al. 1989; Carr and Marshall 1991a; Pepin and Carr 1993; Carr 1995; Carr et al. 1995; Pogson et al. 1995). This would suggest that studies of cod population structure at less than ocean basin-scales might be more informative if highly polymorphic, and preferably neutral, markers are employed.

We have initiated such studies utilizing highly polymorphic microsatellite markers. The attributes of microsatellites as genetic markers and their application to fisheries have been reviewed elsewhere (Park and Moran 1994; Wright and Bentzen 1994; O'Reilly and Wright 1995). Briefly, microsatellites are repetitive arrays of di-, tri- or tetranucleotide sequences in the nuclear genome. They exhibit high levels of allelic polymorphism in repeat number, are presumed selectively neutral (but see Warren 1996) and are readily assayed from small amounts of fresh or preserved tissue using the polymerase chain reaction (PCR). Recent studies employing microsatellites have detected high levels of genetic variation in cod (Brooker et al. 1994; Ruzzante et al. 1996a), as well as genetic differences among some moderately distant locations in the western Atlantic (S.K. McConnell, Dept. Biology, Dalhousie University, pers. com.) and even between northern cod aggregations overwintering inshore and offshore spawning aggregations (Ruzzante et al. 1996a).

Here we report on polymorphism at six microsatellite loci in northern cod sampled from offshore overwintering/spawning locations spanning their range from the northern Grand Bank northwest to Hamilton Bank off Labrador, as well as from two other northwest Atlantic locations, Flemish Cap and the Scotian Shelf, and from one northeastern Atlantic location in the Barents Sea. We report results that lead us to reject the null hypothesis of no genetic differentiation among cod from various northwest Atlantic regions, and hence to conclude that northwest Atlantic cod belong to multiple, genetically distinguishable populations. Further, we describe evidence that northern cod may comprise more than one population. We also use the results of our study to address questions concerning the relative utility of microsatellites as genetic markers for cod, and the various approaches that can be taken in analyzing microsatellite data.

MATERIALS AND METHODS

Sample collections

Northern cod were collected during January and February of 1992 and 1993 from nine different sample populations stretching from Hamilton Bank in the north to the "Nose" of the Grand Bank in the south (Fig. 1, Table 1). These samples were collected from pre-spawning aggregations using an otter trawl fishing at depths between 300 and 500 m. Tissue samples of cod from the Flemish Cap (Fig. 1, Table 1) were secured in the summer of 1993 from the commercial trawl fishery bycatch (nominal depths of 300 m) and were collected by on-board fisheries observers retained by the Canada Department of Fisheries and Oceans.

Samples from the Scotian Shelf (Fig. 1, Table 1) comprised recently hatched cod larvae collected within a gyre-like water mass on Western Bank in November of 1992 (Taggart et al. 1996; Ruzzante et al. 1996b). Tissue samples of Barents Sea cod were taken from frozen cod-heads that were off-loaded from a Russian freezer trawler and were provided by National Sea Products Ltd, Arnolds Cove, NF in July 1993 (Lot #686). The fish had been captured some time during the first half of 1993 at an unknown location in the Barents Sea (International Council for the Exploration of the Seas; ICES Area I).

The average fork lengths of cod were highly variable among samples (Table 1) and (excluding the larval cod sample) ranged from 30 cm (Nose of the Grand Bank in 1993) to 60 cm (Flemish Cap in 1993). The estimated ages of these cod (derived from length at age relationships) were equally variable (Table 1) and ranged from two to six years old. The sex ratio of the analyzed samples was approximately 1:1 for samples where the sex of the individual was known, although the majority of samples were from immature or first time spawning fish (Table 1).

Sample tissues and genetic analyses

Blood and muscle from juvenile and mature cod, and eye tissues from larval cod were used as the DNA source for genetic analysis. Blood samples were collected from live, or recently dead, cod using a sterile 2 mL hypodermic needle (21 gauge) inserted ventrally into the blood vessels that run in the haemal arches of the vertebrae between the anal fin and the caudal peduncle, and ~1 mL of blood was withdrawn and preserved immediately in 5 mL of 95% ethanol and stored. Muscle tissue samples for genetic analysis were taken from the anterior of the tongue of dead cod and preserved in 95% ethanol. Larvae were preserved in liquid nitrogen immediately following collection at sea (Taggart et al. 1996). DNA extraction from alcohol preserved tissue was as described in Bentzen et al. (1990) and Ruzzante et al. (1996a). DNA was released from eyeballs of larval cod as described in Ruzzante et al. (1996b).

PCR was carried out on 1-2 µL of purified or crude cod DNA as previously described by Brooker et al. (1994) using six sets of cod microsatellite primers: Gmo2, Gmo132, and Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993) and Gmo120 (Ruzzante et al. 1996a), and Gmo141 (Ruzzante et al. 1996b). Gmo2, Gmo4, Gmo120, Gmo132 and Gmo141 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). The PCR products were resolved on 6.5% denaturing polyacrylamide gels and the alleles sized relative to a sequence ladder generated from M13mp18 .

Data analyses

Tests for departures from Hardy-Weinberg equilibrium (HWE) and deficiencies or excess of heterozygotes, and tests for heterogeneity in allele frequencies among samples were conducted using the chi-square pseudo-probability programs, CHIHW and CHIRXC (Zaykin and Pudovkin 1993). Both programs use randomization procedures to estimate the significance of the χ^2 statistic (Roff and Bentzen 1989). In all cases, 1,000 randomizations were used to estimate probability (P) values.

Genetic distances among population samples were estimated using Rogers' (Rogers 1972), allele sharing (Bowcock et al. 1994), average squared (Goldstein et al. 1995a) and $(\delta\mu)^2$ (Goldstein et al. 1995b) measures. Estimates of subpopulation structure were obtained using F_{ST} following Weir and Cockerham (1984) and the related R_{ST} measure recently proposed for microsatellite data by Slatkin (1995). Significance values for estimates of genetic distance and sub-population structure were determined as described in Ruzzante et al. (1996b) using Monte Carlo simulations and the bootstrap method (minimum 1,000 resampling trials per individual comparison; Manly 1991), and critical significance levels for simultaneous statistical tests were assessed using sequential Bonferroni adjustments as outlined in Rice (1989).

The genetic relationships among cod populations were evaluated using the various genetic distance measures listed above as inputs for Neighbour-joining and Fitch-Margoliash trees constructed using programs in the PHYLIP software package (Version 3.5C; Felsenstein 1994). As these two methods of tree construction yielded the same topologies only the Fitch-Margoliash trees are presented here.

RESULTS

Variability of microsatellite loci within and among populations

All six microsatellite loci surveyed were highly polymorphic in all cod populations. The number of alleles observed per locus ranged from 24 for Gmo132 to 58 for Gmo141 (mean ± SD = 40.8 ± 14.9; Table 2). Observed heterozygosities (H_O) were correspondingly high, ranging from 0.369 for Gmo132 in the Barents Sea sample to 1.0 for Gmo4, -120, -141 and -145 in several populations (mean = 0.86; Table 3). In all cases variation among the microsatellite alleles occurred in size increments consisting of multiples of two base pairs (bp).

We pooled some samples of northern cod to facilitate further statistical analyses, including broader geographic comparisons. Pair-wise comparisons using χ^2 pseudo-probability tests were made first across sampling years at the same location, and then among geographically related sampling areas. No significant differences in allele frequencies were found among any of the six microsatellites among the 1992 and 1993 samples from Hamilton, Belle Isle and Funk Island Banks; hence, these samples were pooled to form a single sample (hereafter, NORTH). Similar analyses revealed no significant differences among the 1992

and 1993 samples from the North Cape, Grand Bank and Nose of the Bank, so these samples were combined to form a single pooled sample (SOUTH). A sample from the NE Spur area located between the northern Grand Bank and the Flemish Cap did differ from SOUTH in allele frequencies for Gmo141 (P = 0.005; Table 4) and consequently was excluded from the pooled SOUTH sample.

Pseudo-probability tests revealed no significant departures from Hardy-Weinberg equilibrium for the pooled NORTH and SOUTH or Scotian Shelf samples (Table 3). Pseudo-probability tests also indicated that measures of heterozygote excess or deficiency (D) did not deviate significantly from zero for five of the microsatellites in all populations. The exception was Gmo141, for which D was negative (indicating a deficiency of heterozygotes) in five of six population samples; four of these departures were significant (Table 3).

Geographic variation in alleles: size and frequency

The size-frequency distributions of microsatellite alleles varied among NORTH, SOUTH and the four unpooled samples (Table 3, Fig. 2). Variation in mean allele size was significant (Kruskal Wallis test, $P \leq 0.006$) for all loci except Gmo2 among the six populations (Tables 2, 3).

Pair-wise pseudo-probability tests revealed significant differences in allele frequencies at one or more loci for each pair of populations, with the exception of the NORTH vs NE Spur comparison (Table 4). Pair-wise comparisons involving the Barents Sea sample were most likely to be significant: 16 of 30 comparisons involving this population revealed significant differences in allele frequencies. With one exception, Gmo120, all six microsatellites exhibited significant heterogeneity in allele frequencies in at least some pair-wise comparisons.

Broad-scale geographic variation was strongest for Gmo132 (Fig. 2). Although the allele distributions for NORTH, SOUTH and NE Spur were very similar ($P \geq 0.133$, Table 4), they differed sharply from those seen in other populations. In the three northern cod samples (N, S, NE Spur) the allele distribution for Gmo132 consisted of a single mode dominated by two alleles of 111 and 117 bp at 24-28% and 38-44% frequency, respectively. In contrast, the frequencies of the 111 and 117 bp alleles were 76% and 5% in the Barents Sea sample, and 55% and 19% in the Flemish Cap sample. The Scotian shelf sample exhibited multiple modes of alleles at this locus, separated from each other by intervals of approximately 20 bp. One of these modes, dominated by 111 and 117 bp alleles, corresponded to the only mode seen in the other populations. The shape of this mode was similar to that seen in the northern cod samples. The two modal allele groups unique to the Scotian shelf sample comprised alleles close in size to 137 bp and 157 bp, respectively, with a collective frequency of 22.1%.

The heterogeneity of Gmo132 allele distributions across the range of populations examined was apparent in the results of the pair-wise pseudo-probability tests (Table 4). Among a total of 15 pair-wise comparisons between populations, 10 tests were significant for Gmo132; whereas, the number of significant results obtained with the other five loci ranged from 0 to 5 (mean = 3).

Temporal variability

The majority (67%) of the cod in the NE Spur sample were estimated to be from the 1990 year class (age 2+ in 1993), and most of the remaining fish were from the '89 and '91 year classes (17% and 10%, respectively; Fig. 3). In contrast, cod in the pooled SOUTH sample were broadly distributed among year classes '85 to '90 (mean age = 5+ in 1993), with small contributions from '91 and pre-'85 year classes as well. Finally, we estimated that the pooled NORTH sample was composed primarily of the three year classes '88, '89 and '90 (17%, 32% and 35%, respectively), along with a small number of '86, '87 and '91 fish.

We used χ^2 pseudo-probability tests to evaluate whether variation in allele frequencies among year classes might confound the interpretation of genetic variation among geographic samples. Although the estimated year class compositions of the NE Spur, SOUTH and NORTH samples differed from each other (Fig. 3), heterogeneity of allele frequencies among age classes was not significant for any of the six microsatellite loci in a pooled sample of SOUTH and NE Spur fish (adjusted $\alpha = 0.008$, $P \geq 0.027$). Likewise, allele frequencies did not vary significantly for any of the loci among the '89 and '90 year classes of cod in the NORTH sample ($P \geq 0.143$).

To further evaluate whether temporal variability of allele frequencies was a factor in our results, we split the NORTH and SOUTH pools into their 1992 and 1993 components, then conducted pairwise χ^2 pseudo-probability tests of allelic heterogeneity for Gmo4, the locus that differed between NORTH and SOUTH. Pairwise comparisons revealed significant differences in allele frequencies for the following comparisons, NORTH92 vs SOUTH92, NORTH92 vs SOUTH93, NORTH93 vs SOUTH93, but not for NORTH92 vs NORTH93, NORTH93 vs SOUTH92 and SOUTH92 vs SOUTH93 (Table 5).

Estimates of genetic distance

When applied to our cod data, four measures of genetic distance (allele sharing, $(\delta\mu)^2$, Rogers', and average squared) yielded results that varied markedly in at least two ways (Table 6). First, the number of pair-wise distances that were significantly greater than zero differed among the measures. Among 10 pair-wise comparisons, all 10 genetic distances were significantly greater than zero for the $(\delta\mu)^2$ and allele sharing

measures. For the Rogers' and average squared distances, the corresponding numbers of significant tests were 9 and 0, respectively (Table 6). The second difference was apparent when we summarized the pair-wise distances using Fitch-Margoliash trees. Both the $(\delta\mu)^2$ and the Rogers' tree aligned northwest Atlantic populations into two groups distinct from the relatively divergent Barents Sea sample (Figs. 4A and 4C, respectively). One group comprised the NORTH, SOUTH and Scotian Shelf samples, and the other consisted of the Flemish Cap sample. In contrast, the allele sharing tree presented little evidence of hierarchical population structure, with only a weak indication of a NORTH, SOUTH, Scotian Shelf cluster and no indication of divergence between the Barents Sea sample and the four western Atlantic samples (Fig. 4B). Finally, the average squared distance tree portrayed the four western and one eastern Atlantic population as an unresolved polytomy, with a single central node and terminal branches of similar length (Fig. 4D).

Estimates of population substructure

F_{ST} estimates calculated for six different populations ranged from 0.0 for Gmo120 to 0.092 for Gmo132 (Table 7). Except for Gmo2, all non-zero F_{ST} values were significant, as was the combined F_{ST} (= 0.015, $P < 0.001$). We found that excluding the small NE Spur sample from the analysis had little overall effect (combined $F_{ST} = 0.016$, $P < 0.001$). F_{ST} values were larger between a pooled western Atlantic sample (NORTH, SOUTH and Scotian Shelf, but excluding the Flemish Cap sample) and the Barents Sea (F_{ST} = 0.037, $P < 0.001$). On a finer geographic scale, F_{ST} values for a comparison between all northern cod samples and Flemish Cap were only marginally smaller than in the larger scale six population analysis, but only significant due to a large contribution from Gmo132 ($F_{ST} = 0.090$, $P < 0.001$). The overall F_{ST} between all northern cod and the Scotian Shelf sample was significant ($F_{ST} = 0.003$, $P < 0.001$) but also entirely dependent on Gmo132 ($F_{ST} = 0.021$, $P < 0.001$). Finally, the overall F_{ST} was significant for a comparison among samples in the NORTH group ($F_{ST} = 0.007$, $P = 0.002$), but not for a comparison among samples in the SOUTH group ($F_{ST} = 0.002$, $P = 0.080$) or between NORTH and SOUTH ($F_{ST} = 0.000$).

The R_{ST} analyses (Table 7) presented a more complex picture than that obtained with F_{ST}. In various analyses, the single-locus R_{ST} estimates ranged from large and positive to large and negative values for all loci except Gmo145. The most extreme example of this effect involved Gmo132, for which the estimated R_{ST} was 0.208 ($P < 0.001$) in a three-way comparison between NORTH, SOUTH and Scotian Shelf but assumed a value of -0.131 when all northern cod were pooled into a single sample and compared to Scotian Shelf .

In comparison to the results obtained with F_{ST}, fewer single- and multi-locus R_{ST} values were significantly greater than zero (Table 7). The overall R_{ST} was not significant in comparisons of six (mean $R_{ST} = 0.034$, $P = 0.060$) or five populations (excluding NE Spur, mean $R_{ST} = 0.021$, $P = 0.126$) despite large positive (and highly significant) contributions from Gmo132 and Gmo145. The overall R_{ST} was also not significant in a comparison between pooled NORTH, SOUTH and Scotian Shelf samples and the Barents Sea (mean R_{ST} = 0.037, $P = 0.119$). Overall R_{ST} values were negative and hence not significantly different from zero in comparisons between pooled northern cod and Flemish Cap, and between pooled northern cod and Scotian Shelf. By contrast, the overall R_{ST} was positive (= 0.018) and significant ($P = 0.003$) in a three-way comparison between NORTH, SOUTH and Scotian Shelf, due to contributions from Gmo132 ($R_{ST} = 0.208$, $P < 0.001$) and Gmo145 ($R_{ST} = 0.027$, $P = 0.015$). Single- and multi-locus R_{ST} values were not significant in other comparisons within NORTH or SOUTH, or between NORTH and SOUTH (Table 7).

DISCUSSION

Our results lead us to reject the null hypothesis of no genetic differentiation among northern cod in particular, and northwest Atlantic cod in general - a result contrary to that obtained in previous studies employing less variable mtDNA or allozyme markers (Mork et al. 1985; Smith et al. 1989; Carr and Marshall 1991a,b; Árnason et al. 1992; Carr 1995; Carr et al. 1995; but see Cross et al. 1978; Pogson et al. 1995). We employed a variety of measures to assess genetic differentiation and population structure in cod using the microsatellite data, and most, but not all of the measures, revealed significant genetic differences among population samples at large (ocean-basin) and meso (regional bank) scales. Our results, therefore, have various implications for the population biology of cod, the management of cod stocks, the utility of microsatellites as genetic markers in population studies, and the statistical methods used to interpret the data. We consider these issues below.

Population structure: ocean basin-scales

We observed the strongest differentiation between the Barents Sea sample and the northwest Atlantic samples, a feature seen in χ^2 tests of allele frequency homogeneity, $(\delta\mu)^2$, and Rogers' genetic distances. This result is consistent with genetic differentiation of cod at Atlantic basin-scales that has been previously documented using allozymes (Mork et al. 1985), mtDNA (Carr and Marshall 1991a,b; Carr 1995), and nuclear RFLP markers (Pogson et al. 1995). Cod from the northwest Atlantic during the 1950's and 1960's, but not since 1966, have been known to stray to Greenland, Iceland, the North sea and the Barents Sea, but with extremely low and decreasing (from west to east) frequency (Taggart et al. 1995).

MtDNA studies have revealed differing levels of genetic diversity across the eastern and western portions of the range of cod. Nucleon diversities (analogous to heterozygosity for nuclear loci) off Newfoundland and Nova Scotia are lower (18 - 38%) than they are in the northeast Atlantic (82 - 85%), a pattern that suggests that cod in the northwest Atlantic have been subject to one or more population bottlenecks (Carr

and Marshall 1991a,b; Carr 1995; Carr et al. 1995). Such a loss of genetic variation in northwest Atlantic cod is not evident in the microsatellite loci: mean expected heterozygosity among all six loci in our northwest Atlantic samples was 88%, compared to 84% for the Barents Sea sample. Allozyme and cDNA loci similarly show no evidence of a genetic bottleneck in the northwest Atlantic (Mork et al. 1985; Pogson et al. 1995), implying that any bottleneck(s) that may have occurred only influenced genetic diversity in mtDNA.

Population structure: northwest Atlantic

Comparisons of single locus allele frequencies, single and multilocus F_{ST} values, $(\delta\mu)^2$, allele sharing, and Rogers' genetic distances revealed significant genetic differences among northwest Atlantic population samples. Flemish Cap, Scotian Shelf and northern cod samples all differed significantly from each other. Each of these regions are separated from each other by wide and deep submarine trenches. Within the northern cod complex, χ^2, $(\delta\mu)^2$, and allele sharing analyses suggested the presence of at least two genetically distinguishable groups: one comprising our pooled NORTH samples (Hamilton, Belle Isle and Funk Island Banks; NAFO Divs. 2J, 3K) and the other consisting of our SOUTH samples from the northern Grand Bank area (North Cape, Grand Bank, and Nose of the Bank; NAFO Div. 3L). One other northern cod sample, NE Spur (Div. 3L), also differed at one locus (Gmo141) from the adjacent SOUTH pool of northern cod samples but not from the NORTH pool; this result could be an artifact of the small sample size for this group (Table 3) and/or allele scoring problems affecting this sample (see below).

The genetic variation we observed in northwest Atlantic cod could conceivably result from three different causes: 1) selective effects on non-neutral genetic markers; 2) temporal or "chaotic" (*cf.* Hedgecock 1994) variation in allele frequencies among samples not associated with stable spatial differentiation; or 3) divergence through the effects of genetic drift and mutation acting on reproductively isolated populations. We consider each in turn below.

We have no direct evidence of the neutrality or non-neutrality of the microsatellite markers employed in this survey, but since they all consist of dinucleotide repeats, they can not be part of the protein-coding regions of genes. It is possible that they might be indirectly involved in selection through close linkage to a selectively important gene, but this is unlikely, at least statistically. Moreover, the extreme degree of *polymorphism exhibited* by these microsatellites argues against fitness effects associated with particular alleles.

We evaluated the relative significance of temporal or chaotic variation vs stable geographic variation to our results in two ways. First, we conducted χ^2 tests of allele frequency heterogeneity among the inferred year classes of cod within the NORTH and SOUTH (in this case including NE Spur) pools of samples and we observed no significant variation among the year classes for any of the six loci. Second, we split the SOUTH and NORTH pools into their 1992 and 1993 components, then conducted pairwise χ^2 tests of allele frequency heterogeneity for Gmo4, the single locus that differed between the NORTH and SOUTH pools. Differences in allele frequency were significant for NORTH92 vs SOUTH92, NORTH92 vs SOUTH93 and NORTH93 vs SOUTH93 comparisons, but not significant for NORTH93 vs SOUTH92, NORTH92 vs NORTH93 and SOUTH92 vs SOUTH93 (Table 5). In general, these results are more consistent with the hypothesis that the difference seen with Gmo4 between the NORTH and SOUTH pools reflects stable spatial rather than temporal or chaotic variation among samples; however, the lack of significance in the NORTH93 vs SOUTH92 comparison leaves some uncertainty over this issue. The χ^2 results and direct inspection of the allele frequency data both suggest the following two points: 1) The SOUTH92 and SOUTH93 pooled samples were closely similar and 2) The NORTH93 sample was somewhat more similar to the two SOUTH samples than was the NORTH92 sample. Clearly, the tentative conclusion that the difference between NORTH and SOUTH is a stable phenomenon needs independent testing using surveys of additional samples drawn from the areas and populations we have examined here.

Our data are not sufficient to permit a direct evaluation of the relative importance of spatial vs temporal variation for comparisons outside of northern cod. However, a number of independent surveys have confirmed the presence of the large (> 131 bp) Gmo132 alleles in Scotian Shelf cod and their absence in northern cod (Brooker et al. 1994; Taggart 1995; Ruzzante et al. 1996a,b), indicating that this difference between northern and Scotian Shelf cod is a stable phenomenon.

The genetic differentiation we observed among northwest Atlantic cod populations using microsatellite markers stands in contrast with the results of surveys of mtDNA sequence variation in northwest Atlantic cod (Carr and Marshall 1991a,b; Pepin and Carr 1993; Carr 1995; Carr et al. 1995). Conversely, our microsatellite analyses show some similarities to studies of Cross and Payne (1978) and Pogson et al. (1995) which surveyed protein and nuclear RFLP loci, respectively. Both of these studies detected highly significant genetic differences among northwestern Atlantic populations of cod.

Cross and Payne (1978) studied variation at two loci, transferrin (*Tf*) and phosphoglucose isomerase (*PGI-1*) across a range of locations that included several that corresponded closely to the locations we sampled in the northwest Atlantic, but some 20 years earlier. In Cross and Payne's study, as in ours, significant genetic differences were detected between Flemish Cap, Scotian Shelf and northern cod populations. Cross and Payne's results suggested a split between populations south of the Laurentian channel (including the Scotian Shelf) and those to the north. In our study, such a distinction is only weakly suggested by the $(\delta\mu)^2$ distances, and not by the other distance measures. However, as noted above, the Laurentian channel does mark a discontinuity in geographic variation at the Gmo132 locus, with alleles larger than 131 bp frequently observed south of the Laurentian channel, but never to the north (Fig. 3).

With respect to the three conceivable explanations outlined earlier for the genetic variation that we detected in our analyses of northwest Atlantic cod, and based on the ensuing arguments, we conclude that (3) "divergence through the effects of genetic drift and mutation acting on reproductively isolated populations" is the simplest and most consistent explanation.

Our results also show some similarity to those of Cross and Payne (1978) regarding relationships among populations within the northern cod complex. Their results, like ours, suggest a subdivision between cod from the NE Newfoundland Shelf (corresponding to our NORTH pool) and cod from the more southerly Grand Bank areas. Their study, however, included samples from the southern Grand Bank. Moreover, cod in Cross and Payne's study that came from our SOUTH area (North Cape and northern Grand Bank) showed mixed affinities between the more northerly and southerly groups.

The genetic differentiation between northern cod, Flemish Cap and Scotian Shelf populations suggests that the deep ocean trenches that separate these populations act as barriers to gene flow. A similar conclusion was reached for haddock (*Melanogrammus æglefinus*) in the western Atlantic by Zwanenburg et al. (1992) on the basis of mtDNA data.

The isolating effect of ocean channels or other hydrographic features appears most extreme for the Flemish Cap. In both our study and that of Cross and Payne, Flemish Cap cod were more divergent from geographically proximate Grand Bank samples than the latter were from more spatially distant Scotian Shelf and Labrador samples. Cross and Payne (1978) speculated that the distinctiveness of Flemish Cap cod might result from their separate descent from an isolated glacial refuge around Flemish Cap. Regardless of the historical and hydrographic factors involved, the genetic distinctiveness of the Flemish Cap cod demonstrate that genetic differentiation of cod populations can occur on geographic scales of 200 km or less. The relative isolation of cod on the Flemish Cap is also supported by tagging studies that show all but 4% of cod tags reported recaptured (n=132) from a release (n=704) on the Flemish Cap were reported from the Flemish Cap. In addition, of the tens of thousands of cod tagged and released over the last 40 years in the Newfoundland and Grand Banks region (excluding Flemish Cap), none (0%) of 4,836 recaptures in the 1950's, four (0.03%) of 13,600 recaptures in the 1960's, and four (0.02%) of 17,620 recaptures in the 1970's and 80's were reported as captured from the Flemish Cap (Taggart et al. 1995), despite a history of intensive fishing activity on the Cap.

Although our results are consistent with the existence of reproductively isolated populations of cod, they also indicate that the extent of genetic differentiation between populations is of low magnitude. Both direct inspection of allele frequency distributions (see Fig. 2) and F_{ST} and R_{ST} measures indicate that the proportion of total genetic variation occurring among populations is small: approximately 1.5% and 3% for range-wide estimates of F_{ST} and R_{ST} , respectively (Table 7, col. 1). The low level of genetic differentiation in cod undoubtedly stems, at least in part, from gene flow (straying), but may also reflect the relatively recent origin of cod populations (Pogson et al. 1995) and/or the effects of convergent mutations acting on the microsatellite loci (see below).

Utility of microsatellites as genetic markers

We observed an average of 40.8 alleles per locus in the six microsatellites that we surveyed in cod. Heterozygosities averaged 86% across all loci and population samples, and reached 100% in some cases. This level of variation is doubly remarkable. First, as noted elsewhere, cod microsatellites appear unusually variable relative to comparable dinucleotide microsatellites in many other species (Brooker et al. 1994; see also O'Reilly and Wright 1995). Second, the variation exhibited by the cod microsatellites substantially exceeds that seen with most other genetic markers in cod, although it is comparable to that observed with a minisatellite locus assayed in cod using the PCR (Galvin et al. 1995). By contrast, average heterozygosity and the average number of alleles per locus for 10 polymorphic allozyme loci were 13% and 1.9, respectively, for a basin-scale survey of cod (Mork et al. 1985). In a similar study involving nuclear RFLP loci detected with cDNA probes, average heterozygosities and numbers of alleles per locus (36% and 4.3) were intermediate between allozyme and microsatellite loci (Pogson et al. 1995).

Our microsatellite survey, as well as that of Ruzzante et al. (1996a) of cod microsatellite loci detected genetic differentiation in northwest Atlantic cod populations not evident with less variable genetic markers. These results suggest that hypervariable microsatellite markers offer some advantages of sensitivity in contexts where genetic variation exhibited by other genetic markers is low, or where genetic differentiation, at least in terms of neutral markers, is marginal. In other circumstances, however, hypervariability may be suboptimal for at least two reasons: 1) large numbers of alleles create statistical problems in the form of large sampling errors. We countered this problem by pooling some samples, at the cost of forgoing both serial replication and analysis on some spatial scales for the northern cod complex (e.g., among the multiple marine banks encompassed within the NORTH pool); 2) hypervariability implies high mutation rates at these loci. The step-mutational process believed to prevail in microsatellites may result in many convergent or parallel mutations; hence, identity of allelic state does not necessarily denote identity by descent (Di Rienzo et al. 1994). In this regard, it is worth noting cases where the microsatellites did not reveal genetic differentiation. Although northwest Atlantic populations were individually distinguishable from the Barents Sea sample at 2 to 5 loci, only one microsatellite locus, Gmo132, distinguished all northwest Atlantic populations from the Barents sample. Since eastern and western Atlantic cod are known to be well differentiated at protein, nuclear RFLP and mtDNA loci, the lack of consistent east-west differences in microsatellite loci could reflect the action of convergent mutations erasing the effects of genetic drift, and in a practical sense, it suggests that hypervariable microsatellites may not be the markers of choice for broad-scale surveys.

The least variable microsatellite, Gmo132, exhibited the greatest genetic differentiation in comparisons outside the northern cod complex. In the basin-scale comparisons of five populations (excluding NE Spur), F_{ST} and R_{ST} values for Gmo132 were 45- and 37-fold higher than the averages for the other five loci, respectively (Table 7). For the most part, basin-scale differences in the allele distributions of Gmo132 were due to marked differences in frequencies of alleles common to multiple populations (Fig. 3), a pattern consistent with divergence primarily by genetic drift. One population however, Scotian Shelf, exhibited a unique group of large alleles, indicating a role of (divergent) mutations in establishing genetic differentiation in this case. The unusual Gmo132 alleles present in the Scotian Shelf cod may have arisen through a mutational mechanism involving changes in the flanking sequence (D. Cook unpublished observation) rather than within the repeat sequence, making this aspect of Gmo132 differentiation atypical.

Two complications that may attend the use of some microsatellite loci in population surveys are the occurrence of null alleles (alleles that fail to amplify) and the presence of artifactual "stutter" bands on gels that can make some loci difficult to score (both reviewed in O'Reilly and Wright 1995). Both phenomena can lead to scoring errors marked by an apparent deficiency of heterozygotes. We tested the inheritance of the microsatellite markers used in this study with captive families involving six parents, and the reproducibility of gel scoring with blind comparisons between two gel readers. The former test revealed no evidence of null alleles at any locus; the latter supported the reproducibility of gel scores (D. Cook, unpublished data). Nonetheless, one of the microsatellite loci we assayed, Gmo141, exhibited a significant deficiency of heterozygotes in four populations (see Table 3), suggesting the occurrence of null alleles and/or scoring problems. Either problem could bias the results of allele frequency comparisons. In this regard, it may be noteworthy that the genetic difference which led to the exclusion of the NE Spur sample from the SOUTH pool involved Gmo141; NE Spur also showed the strongest heterozygote deficiency at this locus (Table 3).

For the reasons outlined above, the more moderately variable nuclear RFLP loci studied by Pogson et al. (1995) may present statistical and analytical advantages for population discrimination over the microsatellites employed in this study. On the other hand, the practical and economic advantages of PCR-based microsatellite assays over the Southern blot-based RFLP technology are compelling (Wright and Bentzen 1994; O'Reilly and Wright 1995). Indeed, only PCR-based methods could allow the assay of the disparate tissue sources (including the larval cod eyeballs) used in this study.

The speed and modest cost of microsatellite analyses allowed us to achieve relatively large sample sizes in this study. This factor, coupled with differing levels of genetic variability may account for the difference between our results and those obtained with mtDNA. The sample sizes in this study, which averaged 169 for NORTH, SOUTH and Scotian Shelf, are at least five-fold greater than those reported to date for similar locations in the mtDNA studies (Carr 1995; Carr et al. 1995). Further, this study surveyed six (presumably) unlinked loci, whereas mtDNA is equivalent to a single locus. Finally, although the mtDNA studies have detected numerous haplotypes, the frequency distribution of these haplotypes around Newfoundland and Nova Scotia is characterized by a single haplotype which prevails everywhere at frequencies close to 85%, with individual variant haplotypes frequently observed only once, and invariably at ≤ 3% frequency (Carr and Marshall 1991a,b; Carr 1995; Carr et al. 1995). Skewed haplotype distributions of this type may not offer the statistical power needed to detect the low (but significant) levels of population differentiation revealed with microsatellite and RFLP markers.

Statistical methods for analyzing microsatellite data

We employed a variety of analyses to reach the conclusions above. In general, familiar measures such as χ^2 contingency tests, F_{ST} and Rogers' genetic distance not only proved to be sensitive indicators of genetic differentiation when evaluated using Monte Carlo methods, they also yielded similar results. The genetic distance measure, $(\delta\mu)^2$, recently proposed by Goldstein et al. (1995b) for microsatellites, also revealed significant genetic differentiation in a pattern largely congruent with the previous methods. Three other measures proposed specifically for microsatellite data gave variable results; these included two other genetic distance measures, allele sharing (Bowcock et al. 1994) and average squared (Goldstein et al. 1995a), as well as R_{ST}, an analog of F_{ST} (Slatkin 1995).

R_{ST}, like F_{ST}, estimates the proportion of total genetic variation that occurs between populations. Unlike F_{ST}, however, R_{ST} incorporates information about the size of microsatellite alleles. The inclusion of allele size is expected to make R_{ST} more informative (Slatkin 1995). However, variances of estimates of R_{ST} in pairwise comparisons can be relatively large when sample sizes differ, even when both samples are large. By contrast variances on F_{ST} estimates appear much less affected by unequal sample sizes (Ruzzante unpublished). In this study, R_{ST} estimates were relatively variable in comparison to F_{ST} estimates (Table 7). The variability inherent in the R_{ST} estimate was most striking in the different results obtained for two analyses, one in which NORTH, SOUTH and Scotian Shelf were compared, and another in which the northern cod complex was pooled as a single group and compared to Scotian Shelf. R_{ST} estimates for Gmo132 in these two analyses were 0.208 and -0.131, respectively (Table 7). In contrast, F_{ST} estimates varied little from each other (0.015 and 0.021, respectively) and were both statistically significant ($P <$ 0.001, Table 7). A comparison of the R_{ST} and F_{ST} estimates from the present study with those from Ruzzante et al. (1996a) suggests the two estimates may perform differently under different conditions such as number of populations considered, nature of the differences among populations, and degree of population subdivision, etc. It appears however, that for analyses of R_{ST} it may be important to equalize sample sizes (Ruzzante unpublished).

Two criteria, the number of pair-wise distances significantly greater than zero, and concordance with previous genetic data and zoogeographic factors, suggest that the $(\delta\mu)^2$ and Rogers' genetic distance were approximately equally effective in resolving relationships among cod populations, and that the average squared distance was not useful. The number of pair-wise distances significantly greater than zero among 10 comparisons were 10, 10, 9 and 0 for the $(\delta\mu)^2$, allele sharing, Rogers', and average squared distance, respectively (Table 6). Further, the $(\delta\mu)^2$ and Rogers'trees were both in accord with previous protein, mtDNA and nuclear RFLP analyses as well as geographic considerations in depicting the Barents Sea sample as most divergent, and the three populations from the northwest Atlantic continental shelf (NORTH, SOUTH and Scotian Shelf), as closely related (Fig. 4A,C). This pattern was not evident in the allele sharing tree (Fig. 4B). It should be added that the allele sharing distance has the limitation that its expectation is greater than zero even for within populaton comparisons. Finally, the average squared distance tree revealed no structure at all among cod populations (Fig. 4D).

The Rogers' tree was very similar to a maximum likelihood tree based on allele frequencies (not shown). The latter approach to tree construction corresponds to a model in which populations diverge by genetic drift alone, and is insensitive to the genealogical relationships of the alleles involved (Felsenstein 1994). In contrast, the $(\delta\mu)^2$ measure is based on differences in mean allele size between populations, and hence is explicitly based on the step mutational model (Goldstein et al. 1995b). Hence, for these populations and loci, drift and mutation-based models appear to work equally well in portraying reasonable relationships among populations.

Implications for northern cod

This study was motivated in large part by interest in the underlying population structure of the northern cod stock complex which has undergone intense fishing pressure over the last four decades and has reached an all time low in abundance (Taggart et al. 1995). It was recently suggested by deYoung and Rose (1993) that northern cod population structure is formed by extensive egg and larval drift, followed by opportunistic (and nonphilopatric) recruitment of juveniles to whatever adult assemblages they find themselves near. Consequently, deYoung and Rose predicted that population structure in northern cod is neither genetically based nor temporally stable, particularly following what they showed is an apparent southward shift in abundance occurring after 1988. Our data, which include cod from a variety of years classes, but a majority from post-1988, are inconsistent with both predictions. We have shown evidence for at least two genetically discernable groups within the northern cod complex, our NORTH and SOUTH pools that correspond to the 2J3K and the 3L management divisions. A related microsatellite study demonstrated genetic differences between two overwintering cod aggregations within this complex in NAFO Div. 3L, one inshore and the other offshore (Ruzzante et al. 1996a). The differences occurred despite the fact that cod of both migratory types intermingle inshore during the summer as a result of the feeding migration by offshore cod. As noted above, these joint findings deserve independent testing with additional samples, and ideally, with additional markers. Such work, now in progress at Dalhousie University, will serve to refine our knowledge of population structure within northern the northern cod complex. At present, however, the results reported here, those of Ruzzante et al. (1996a), as well as the previous work of Cross and Payne (1978) collectively support, with some weight, the conclusion that northern cod are not comprised of a single, panmictic assemblage, but rather are composed of genetically distinguishable subunits, each of which appear to be geographically affiliated with spawning area. This suggests it may be wise to seriously reconsider the current (and historical) management scheme for northern cod which presumes one stock distributed throughout the inshore and offshore regions stretching from Hamilton Bank to the Grand Bank through NAFO Divs. 2J, 3K and 3L (Taggart et al. 1994).

ACKNOWLEDGMENTS

We are grateful to C. George and the Captains, crews and technical staff of the vessels WILFRED TEMPLEMAN and PETREL V for their assistance in securing the cod samples. We are equally grateful to B. Warham of National Sea Products Ltd., Arnold's Cove, NF for the Barents Sea cod samples and the DFO Fisheries Observers for their assistance in securing cod samples from the Flemish Cap. Funding for this research was provided the Canada Department of Fisheries and Oceans, northern cod Science Programme and by the Ocean Production Enhancement Network (OPEN) and through the OPEN-Interim Funding Research Programme (IFRP) which is supported by the Government of Canada and by the Canada/Newfoundland and Canada/Nova Scotia COOPERATION agreements. Programs (Splus) for a number of genetic distances used here are available from D. Ruzzante (ruzzante@cs.dal.ca).

REFERENCES

Angel, J.R., Burke, D.L., O'Boyle, R.N., Peacock, F.G., and Sinclair, M. 1994. Report of the workshop on Scotia-Fundy Groundfish Management from 1977 to 1993. Can. Tech. Rep. Fish. Aquat. Sci. 1979: 181 p.

Árnason, E., Pálsson, S., and Arason, A. 1992. Gene flow and lack of population differentiation in Atlantic cod, *Gadus morhua* L. from Iceland, and comparison of cod from Norway and Newfoundland. J. Fish Biol. 40:751-770.

Bentzen, P., Cook, D., Denti, D., Harris, A., Hofman, J., and Wright, J.M. 1990. One tube DNA extraction procedure for molecular fingerprinting. Fingerprint News 2:17-21.

Bowcock, A.M., Ruiz-linares, A., Tomfohrde, J., Minch, E., Kidd, J.R., and Cavalli-sforza, L.L. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455-457.

Brander, K.M. 1994. Patterns of distribution, spawning, and growth in North Atlantic cod: the utility of inter-regional comparisons. ICES Mar. Sci. Symp. 198:406-413.

Brooker, A.L., Cook, D., Bentzen, P., Wright, J.M., and Doyle, R.W. 1994. Organization of microsatellites differs between mammals and cold-water teleost fish. Can. J. Fish. Aquat. Sci. 51:1-9.

Buch, E., Horsted, S.A, and Hovgård, H. 1994. Fluctuations in the occurence of cod in Greenland waters and their possible causes. ICES Mar. Sci. Symp. 198:158-174.

Carr, S. 1995. Cod genetics and Physiology: mtDNA. p.40-42, IN: OPEN, The Interim Funding Research Programme, Final Report. Office of Research Services, Dalhousie University, Halifax, NS. 92 p.

Carr, S.M., and Marshall, H.D. 1991a. Detection of intraspecific DNA sequence variation in the mitochondrial cytochrome *b* gene of Atlantic cod (*Gadus morhua*) by the polymerase chain reaction. Can. J. fish. Aquat. Sci. 48:48-52.

Carr, S.M., and Marshall, H.D. 1991b. A direct approach to the measurement of genetic variation in fish populations: applications of the polymerase chain reaction to studies of Atlantic cod, *Gadus morhua*. J. Fish Biol. (Suppl. A) 39:101-107.

Carr, S.M., Snellen, A.J., Howse, K.A., and Wroblewski, J.S. 1995. Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (*Gadus morhua*) from bay and offshore locations on the Newfoundland continental shelf. Mol. Ecol. 4:79-88.

Cross, T.F., and Payne, R.H. 1978. Geographic variation in Atlantic cod, *Gadus morhua*, off eastern North America: a biochemical systematics approach. J. Fish. Res. Board Can. 35:117-123.

Dahle, G., and Jørstad, K.E. 1993. Haemoglobin variation in cod - a reliable marker for Arctic cod (*Gadus morhua* L.). Fish. Res. 16:301-311.

deYoung, B., and Rose, G.A.. 1993. On recruitment and distribution of Atlantic cod (*Gadus morhua*) off Newfoundland. Can. J. Fish. Aquat. Sci. 50:2729-2741.

Di Rienzo, A., Peterson, A.C., Garza, J.C., Valdes, A.M., Slatkin, M., and Freimer, N.B. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proc. Natl. Acad. Sci. USA 91:3166-3170.

Felsenstein, J. 1994. PHYLIP Manual Version 3.5. Department of Genetics, University of Washington, Box 357360, Seattle, WA, 98195.

Frank, K.T., Drinkwater, K.F., and Page, F.H. 1994. Possible causes of recent trends in fluctuations in Scotian/Gulf of Maine cod stocks. ICES Mar. Sci. Symp. 198:110-120.

Galvin, P., Mcgregor D., and Cross,T. 1995. A single locus minisatellite as a hypervariable genetic marker in gadoid species. Aquaculture 137:31-40

Goddard, S.V., Wroblewski, J.S., Taggart, C.T., Howse, K.A., Bailey, W.L., Kao, M.H., and Fletcher, G.L. 1994. Overwintering of adult Northern Atlantic cod (*Gadus morhua*) in cold inshore waters as evidenced by plasma antifreeze glycoprotein levels. Can. J. Fish. Aquat. Sci. 51:2834-2842.

Goldstein, D.B., Linares, A.R., Cavalli-sforza, L.L., and Feldman, M.W. 1995a . An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463-471.

Goldstein, D.B., Linares, A.R., Cavalli-sforza, L.L., and Feldman, M.W. 1995b. Genetic absolute dating based on microsatellites and the origin of modern humans. Proc. Natl. Acad. Sci. USA 92:6723-6727.

Hedgecock, D. 1994. Temporal and spatial genetic structure of marine animal populations in the California current. CalCOFI Rep. 35:73-81.

Hutchings, J.A., Myers, R.A., and Lilly, G.R. 1993. Geographic variation in the spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 50:2457-2467.

Jamieson, A. 1975. Enzyme type of Atlantic cod stocks on the North American banks, p. 491-515. *In* C.L. Markert [ed.] Isozymes. IV. Genetics and Evolution. Academic Press, New York.

Lear, W.H. 1984. Discrimination of the stock complex of Atlantic cod (*Gadus morhua*) off southern Labrador and eastern Newfoundland, as inferred from tagging studies. J. Northw. Atl. Fish. Sci. 5:143-159.

Lear, W.H., and Green, J.M. 1984. Migration of the northern Atlantic cod and the mechanisms involved. p. 309-315 *In* J.D. McCleave, G.P. Arnold, J.J. Dodson and W.W. Neil. [ed.] Mechanisms of Migration in Fishes. Plenum, New York.

Lear, W.H., and Wells, R. 1984. Vertebral averages of juvenile cod, *Gadus morhua*, from coastal waters of eastern Newfoundland as indicators of stock origin. J. Northw Atl. Fish. Sci. 5:23-31.

Manly, B.F.J. 1991. Randomization and Monte Carlo methods in Biology. Chapman and Hall, New York.

Mork, J., and Sundnes, G. 1985. Haemoglobin polymorphism in Atlantic cod (Gadus morhua): allele frequency variation between yearclasses in a Norwegian fjord stock. Helgoländer Meeresunters. 39:55-62.

Mork, J., Ryman, N., Ståhl, G., Utter, F., and Sundnes, G. 1985. Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. Can. J. Fish. Aquat. Sci. 42:1580-1587.

Myers, R.A., Mertz, G., and Bishop, C.A. 1993. Cod spawning in relation to physical and biological cycles of the northern North-West Atlantic. Fish. Oceanogr. 2:154-165.

O'Reilly, P., and Wright, J.M. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol. 47 (suppl. A):29-55.

Park, L.K., and Moran, P. 1994. Developments in molecular genetic techniques in fisheries. Rev. Fish Biol. Fish. 4:272-299.

Pepin, P., and Carr, S.M. 1993. Morphological, meristic, and genetic analysis of stock structure in juvenile Atlantic cod (*Gadus morhua*) from the Newfoundland shelf. Can. J. Fish. Aquat. Sci. 50:1924-1933.

Pogson, G.H., Mesa, K.A., and Boutilier, R.G. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics 139:375-385.

Roff, D.A., and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: Chi-square and the problem of small samples. Mol. Biol. Evol. 6:539-545.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genet. VII. Univ. Texas Publ. 7213:145-153.

Rose, G., 1993. Cod spawning on a migration highway in the north-west Atlantic. Nature 366:458-461.

Ruzzante, D.E., Taggart, C.T., Cook, D., and Goddard, S. 1996a. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. 53 (in press)

Ruzzante, D.E., Taggart, C.T., and Cook, D. (1996b). Spatial and temporal variation in the genetic composition of an aggregation of larval cod (*Gadus morhua*) on the Scotian Shelf: cohort contribution and evidence of larval retention based on microsatellite DNA polymorphism. Can. J. Fish. Aquat. Sci. 53 (in press)

Schopka, S.A. 1994. Fluctuations in the cod stocks off Iceland during the twentieth century in relations to changes in the fisheries and environment. ICES mar. Sci. Symp. 198:175-193.

Serchuck, F.M., Grosslein, M.D., Lough, R.G., Mountain, D.G., and O'Brien, L. 1994. Fishery and environmental factors affecting trends and fluctuations in the Georges Bank and Gulf of Maine Atlantic cod stocks: an overview. ICES mar. Sci. Symp. 198:77-109.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

Slatkin, M., and Barton, N.H. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349-1368.

Smith, P.J., Birley, A.J., Jamieson, A., and Bishop, C.A. 1989. Mitochondrial DNA in the Atlantic cod, *Gadus morhua*: lack of genetic divergence between eastern and western populations. J. Fish Biol. 34:369-373.

Taggart, C.T. 1995. Cod genetics and Physiology; microsatellite and cDNA. p.23-39, IN: OPEN, The Interim Funding Research Programme Final Report. Office of Research Services, Dalhousie University, Halifax, NS. 92 p.

Taggart, C.T., Anderson, J., Bishop, C., Colbourne, E., Hutchings, J., Lilly, G, Morgan, J., Murphy, E., Myers, R., Rose, G., and Shelton, P. 1994 Overview of cod stocks, biology, and environment in the Northwest Atlantic region of Newfoundland, with emphasis on northern cod. ICES Mar. Sci. Symp. 198:140-157.

Taggart, C.T., Penney, P., Barrowman, N., and George, C. 1995. The 1954-1993 Newfoundland cod-tagging data base: statistical summaries and spatial-temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. 2042. 441 p.

Taggart, C.T., S.E. Lochmann, D.A. Griffin, K.R. Thompson and G.L. Maillet. 1996. Abundance distribution of larval cod (*Gadus morhua*) and zooplankton in a gyre-like water mass on the Scotian Shelf. p 127-145. In: Watanabe, Y., Y. Yamashita and Y. Oozeki (eds.). Survival strategies in early life stages. of marine resources. Proc. Internat. Workshop, Yokohama. Balkema Press, Rotterdam.

Tautz, D. 1989. Hypervariability of simple sequences as a source for polymorphic DNA markers. Nucleic Acids Res. 17:6463-6471.

Templeman, W. 1966. Marine resources of Newfoundland. Bull. Fish. Res. Board Can. 154:170 p.

Templeman, W. 1974. Migrations and intermingling of Atlantic cod (*Gadus morhua*) stocks of the Newfoundland area. J. Fish. Res. Board. Can. 31:1073-1092.

Templeman, W. 1979. Migrations and intermingling of stocks of atlantic cod, *Gadus morhua*, of the Newfoundland and adjacent areas from tagging 1962-1966. ICNAF Res. Bull. 14:5-50.

Templeman, W. 1981. Vertebral numbers in Atlantic cod, *Gadus morhua*, of the Newfoundland and adjacent areas, 1947-1971, and their use for delineating cod stocks. J. Northw. Atl. Fish. Sci. 2:21-45.

Warren, S.T. 1996. The expanding world of trinucleotide repeats. Science 271:1374-1375.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wright, J.M. 1993. DNA fingerprinting of fishes. p 57-91 *In* P.W. Hochachka and T. Mommsen [ed.] Biochemistry and molecular biology of fishes, vol. 2. Elsevier Science Publishers B. V. Amsterdam, The Netherlands.

Wright, J.M., and Bentzen, P. 1994. Microsatellites: genetic markers for the future. Rev. Fish Biol. and Fish. 4:384-388.

Wroblewki, J.S., Smedbol, R.K., Taggart, C.T., and Goddard, S.V. 1996. Movements of farmed and wild Atlantic cod (*Gadus morhua* L.) released in Trinity Bay, Newfoundland. Mar. Biol. 124:619-627.

Zaykin, D.V., and Pudovkin, A.I. 1993. Two programs to estimate significance of χ^2 values using pseudo-probability tests. J. Hered. 84:152.

Zwanenburg, K.C.T., Bentzen, P., and Wright., J.M. 1992. Mitochondrial DNA differentiation in western Atlantic populations of haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 49:2527-2537.

Table 1. Cod sample collection statistics.

Collection Location & Date	N	Mean Location[1]		Length (cm)		Estimated Age (y)		Number
		latitude	longitude	range	mean	range	mean	mature
Hamilton & Belle Isle Bank, 1992	8	53.31	-52.38	25-48	33.4	1.7-4.4	2.7	3
Funk Island Bank, 1992	30	51.25	-50.51	28-51	41.0	2.0-4.8	3.5	12
North Cape, 1992	84	48.49	-49.46	40-75	53.9	3.4-8.2	5.2	21
Northern Grand Bank, 1992	56	48.11	-48.50	25-63	40.0	1.7-6.4	3.4	20
Belle Isle Bank, 1993	30	53.11	-52.17	27-43	34.9	1.9-3.7	2.8	16
Funk Island Bank, 1993	60	51.22	-50.76	21-48	33.6	1.4-4.4	2.7	14
North Cape, 1993	30	48.4	-49.5	21-47	34.3	1.4-2.4	2.8	7
Northeast Spur, 1993	30	47.79	-47.26	23-58	32.0	1.5-5.7	2.5	4
Nose of Grand Bank, 1993	30	46.09	-47.59	23-69	30.1	1.5-7.3	2.3	1
Barents Sea, 1993	100	---	---	43-82	57.0	3.0-9.0	5.2	--
Flemish Cap, 1993	51	47.25	-45.75	38-94	60.1	3.1-11.4	6.1	3
Scotian Shelf, 1992	193	43.75	-61.35	larvae	---	< 30 days	---	0

[1] decimal degrees

Table 2. Single locus statistics for cod microsatellites.

Locus	N	n	H_e	P
Gmo2	664	25	0.803	0.123
Gmo4	646	57	0.957	< 0.001*
Gmo120	622	44	0.953	0.006*
Gmo132	672	24	0.769	< 0.001*
Gmo141	640	58	0.97	< 0.001*
Gmo145	647	37	0.938	< 0.001*
Mean	648.5	40.8	0.898	---

N, number of individuals; *n*, number of alleles; H_e, expected heterozygosity; *P*, probability that mean allele size is identical among NORTH, SOUTH, NE Spur, Flemish Cap, Scotian Shelf and Barents Sea samples, determined using Kruskal-Wallis tests (5 DF); Tests considered significant following adjustment for six simultaneous tests ($\alpha = 0.008$) are indicated with an asterisk.

Table 3. Summary statistics for genetic variation in Atlantic cod population samples

	Northern Cod					
	NE Spur	NORTH	SOUTH	Scotian Shelf	Flemish Cap	Barents Sea
Gmo2						
N	28	119	195	193	50	79
n	7	18	20	20	12	17
dlele	110.83	111.90	112.54	111.91	112.14	113.39
H_o	0.679	0.798	0.749	0.725	0.92	0.759
H_e	0.764	0.794	0.805	0.773	0.827	0.842
D	-0.112	0.005	-0.07	-0.062	0.113	-0.097
P (HW)	----	0.386	0.757	0.681	----	----
P (D)	0.345	0.797	0.031	0.074	0.11	0.037
Gmo4						
N	28	116	187	191	44	80
n	20	37	40	40	24	31
dlele	205.46	208.32	212.63	209.88	203.42	211.18
H_o	0.929	0.966	0.925	0.958	0.864	0.963
H_e	0.926	0.955	0.953	0.957	0.938	0.939
D	0.003	0.011	-0.03	0.001	-0.079	0.026
P (HW)	----	0.985	0.98	0.977	----	----
P (D)	0.763	0.686	0.021	1	0.022	0.495
Gmo120						
N	30	108	176	192	44	72
n	20	28	36	32	26	32
dlele	189.55	188.47	189.85	187.67	191.72	187.51
H_o	0.967	0.972	0.92	0.938	0.841	0.972
H_e	0.934	0.946	0.95	0.953	0.938	0.95
D	0.035	0.028	-0.031	-0.016	-0.104	0.023
P (HW)	----	0.985	0.98	0.977	----	----
P (D)	0.729	0.686	0.021	1	0.022	0.495
Gmo132						
N	30	118	197	193	50	84
n	7	10	12	18	8	10
dlele	115.325	114.887	114.912	120.434	113.660	111.858
H_o	0.767	0.729	0.766	0.824	0.62	0.369
H_e	0.746	0.723	0.751	0.797	0.646	0.414
D	0.028	0.008	0.021	0.033	-0.041	-0.109
P (HW)	----	0.356	0.116	0.341	----	----
P (D)	0.818	0.915	0.646	0.322	0.669	0.127
Gmo141						
N	28	114	194	193	32	79
n	26	46	47	53	28	39
dlele	168.05	174.26	172.44	172.91	173.15	181.16
H_o	0.75	0.93	0.897	0.933	0.844	0.975
H_e	0.947	0.966	0.967	0.967	0.952	0.96
D	-0.208	-0.037	-0.073	-0.036	-0.114	0.015
P (HW)	----	0.972	0.972	0.997	----	----
P (D)	<0.001*	0.021	<0.001*	0.009*	0.003*	0.617
Gmo145						
N	28	118	191	189	47	74
n	19	25	31	31	21	22
dlele	180.80	176.79	175.31	176.23	176.17	174.03
H_o	0.964	0.932	0.916	0.937	0.957	0.851
H_e	0.928	0.932	0.94	0.934	0.921	0.921
D	0.039	0.001	-0.025	0.002	0.04	-0.075
P (HW)	----	0.995	0.978	0.992	----	----
P (D)	0.591	1	0.152	0.88	0.464	0.011
Mean H_e	0.874	0.886	0.894	0.897	0.87	0.838
S.E.	0.038	0.042	0.038	0.036	0.049	0.086
Mean H_o	0.842	0.888	0.862	0.886	0.841	0.815
S.E.	0.051	0.041	0.033	0.038	0.048	0.096
Mean *D*	-0.036	0.003	-0.035	-0.013	-0.031	-0.036
S.E.	0.041	0.009	0.014	0.014	0.014	0.026

N, number of individuals; *n*, number of alleles; *dlele*, mean allele size; H_o, observed number of heterozygotes; H_e, expected number of heterozygotes; *D*, measure of heterozygote deficiency or excess $[(H_o-H_e)/H_e]$; *P*(HW) and *P*(D), estimated probability levels based on pseudo-probability tests. Tests considered significant following sequential Bonferroni adjustment for six tests per locus (initial $\alpha = 0.008$) are indicated with an asterisk (*).

Table 4. Probability of homogeneity of allele frequencies estimated from pair-wise pseudo-probability tests.

COMPARISON		Gmo2	Gmo4	Gmo120	Gmo132	Gmo141	Gmo145
NORTH	SOUTH	0.552	< 0.001*	0.443	0.643	0.195	0.214
NORTH	N.E. Spur	0.923	0.311	0.479	0.133	0.057	0.469
NORTH	Scotian	0.57	0.186	0.501	< 0.001*	0.923	0.238
NORTH	Flemish	0.455	0.21	0.71	< 0.001*	0.111	0.327
NORTH	Barents	0.002*	0.183	0.058	< 0.001*	0.073	< 0.001*
SOUTH	N.E. Spur	0.908	0.049	0.787	0.22	0.005*	0.321
SOUTH	Scotian	0.352	0.017	0.107	< 0.001*	0.121	0.311
SOUTH	Flemish	0.229	< 0.001*	0.269	< 0.001*	0.032	0.789
SOUTH	Barents	< 0.001*	< 0.001*	0.024	< 0.001*	< 0.001*	0.001*
N.E. Spur	Scotian	0.822	0.217	0.208	0.066	0.017	0.323
N.E. Spur	Flemish	0.533	0.184	0.775	0.023	< 0.001*	0.651
N.E. Spur	Barents	0.011	0.642	0.404	< 0.001*	0.001*	0.001*
Scotian	Flemish	0.123	0.058	0.035	< 0.001*	0.142	0.483
Scotian	Barents	< 0.001*	0.001*	0.262	< 0.001*	0.018	0.006
Flemish	Barents	0.707	0.012	0.405	< 0.001*	0.002*	0.005

Probability estimates considered significant following sequential Bonferroni adjustments for 15 simultaneous tests per locus (initial $\alpha = 0.003$) are indicated with an asterisk (*).

Table 5. Probability of homogeneity of allele frequencies for Gmo4 estimated from pair-wise pseudo-probability tests.

Comparison		*P*
NORTH92	NORTH93	0.116
NORTH92	SOUTH92	< 0.001*
NORTH92	SOUTH93	0.004*
NORTH93	SOUTH92	0.188
NORTH93	SOUTH93	0.036*
SOUTH92	SOUTH93	0.707

Probability (*P*) estimates considered significant are indicated with an asterisk (*).

Table 6. Genetic distances (above diagonal) and probability estimates (below diagonal).

A. $(\delta\mu)^2$

	NORTH	SOUTH	Flemish	Scotian	Barents
NORTH		5.37	7.77	6.24	13.35
SOUTH	0.008*		17.85	7.78	18.62
Flemish	0.042*	< 0.001*		16.85	27.54
Scotian	0.003*	< 0.001*	< 0.001*		29.03
Barents	< 0.001*	< 0.001*	< 0.001*	< 0.001*	

B. Allele Sharing

	NORTH	SOUTH	Flemish	Scotian	Barents
NORTH		0.303	0.386	0.286	0.326
SOUTH	0.010*		0.460	0.307	0.322
Flemish	0.015*	< 0.001*		0.448	0.383
Scotian	0.038*	< 0.001*	< 0.001*		0.344
Barents	0.004*	< 0.001*	0.013	< 0.001*	

C. Rogers'

	NORTH	SOUTH	Flemish	Scotian	Barents
NORTH		0.057	0.128	0.072	0.159
SOUTH	0.37		0.122	0.070	0.157
Flemish	< 0.001*	< 0.001*		0.138	0.126
Scotian	0.001*	< 0.001*	< 0.001*		0.159
Barents	< 0.001*	< 0.001*	< 0.001*	< 0.001*	

D. Average Squared

	NORTH	SOUTH	Flemish	Scotian	Barents
NORTH		312.045	336.892	341.100	321.870
SOUTH	0.948		346.102	341.778	326.275
Flemish	0.445	0.277		373.290	357.642
Scotian	0.332	0.298	0.037		364.872
Barents	0.787	0.715	0.139	0.034	

Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.005$) are indicated with an asterisk (*).

Table 7. F_{ST} and R_{ST} values for cod microsatellite loci.

A. F_{ST}

	1	2	3	4	5	6
Gmo2	0.003 (0.030)	0.011 (0.005*)	0.000 (NA)	0.000 (NA)	0.001 (0.130)	-0.002 (NA)
Gmo4	0.003 (< 0.001*)	0.005 (0.001*)	0.002 (0.132)	0.002 (0.003*)	0.000 (NA)	0.004 (0.004*)
Gmo120	0.000 (NA)	0.000 (NA)	0.001 (0.318)	0.000 (NA)	0.000 (NA)	0.000 (NA)
Gmo132	0.092 (< 0.001*)	0.200 (< 0.001*)	0.090 (< 0.001*)	0.015 (< 0.001*)	0.021 (< 0.001*)	-0.001 (NA)
Gmo141	0.003 (0.004*)	0.004 (0.001*)	0.002 (0.139)	0.000 (NA)	0.000 (NA)	0.000 (NA)
Gmo145	0.003 (0.004*)	0.007 (< 0.001*)	0.002 (0.177)	0.006 (0.179)	0.000 (NA)	0.000 (NA)
Overall	0.015 (< 0.001*)	0.037 (< 0.001*)	0.014 (< 0.001*)	0.003 (< 0.001*)	0.003 (< 0.001*)	0.000 (NA)

B. R_{ST}

Gmo2	0.102 (0.137)	-0.129 (NA)	-0.057 (NA)	0.014 (0.408)	-0.061 (NA)	-0.006 (NA)
Gmo4	0.026 (0.368)	0.085 (0.142)	-0.117 (NA)	0.008 (0.296)	-0.003 (NA)	0.006 (0.385)
Gmo120	0.0112 (0.391)	-0.090 (NA)	0.001 (0.496)	0.016 (0.137)	0.004 (0.407)	0.025 (0.093)
Gmo132	0.415 (< 0.001*)	0.387 (< .001*)	-0.034 (NA)	0.208 (< 0.001*)	-0.131 (NA)	0.013 (0.207)
Gmo141	-0.017 (NA)	0.039 (0.233)	-0.075 (NA)	-0.002 (NA)	-0.013 (NA)	-0.012 (NA)
Gmo145	0.098 (0.002*)	0.049 (0.216)	0.117 (0.024)	0.027 (0.015)	-0.012 (NA)	0.037 (0.009)
Overall	0.034 (0.060)	0.037 (0.119)	-0.051 (NA)	0.018 (0.003*)	-0.016 (NA)	0.005 (0.319)

Numbers in the table are R_{ST}/F_{ST} values and associated P values (in parentheses). 1, among six populations (NORTH, SOUTH, Flemish Cap, Scotian, Barents, NESpur); 2, between all Northern cod and Scotian Shelf (pooled) and Barents Sea; 3, between all Northern cod (pooled) and Flemish Cap 94, 4, between NORTH, SOUTH and Scotian Shelf; 5, between all Northern cod (pooled) and Scotian Shelf; 6, between NORTH and SOUTH. Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.005$) are indicated with an asterisk (*).

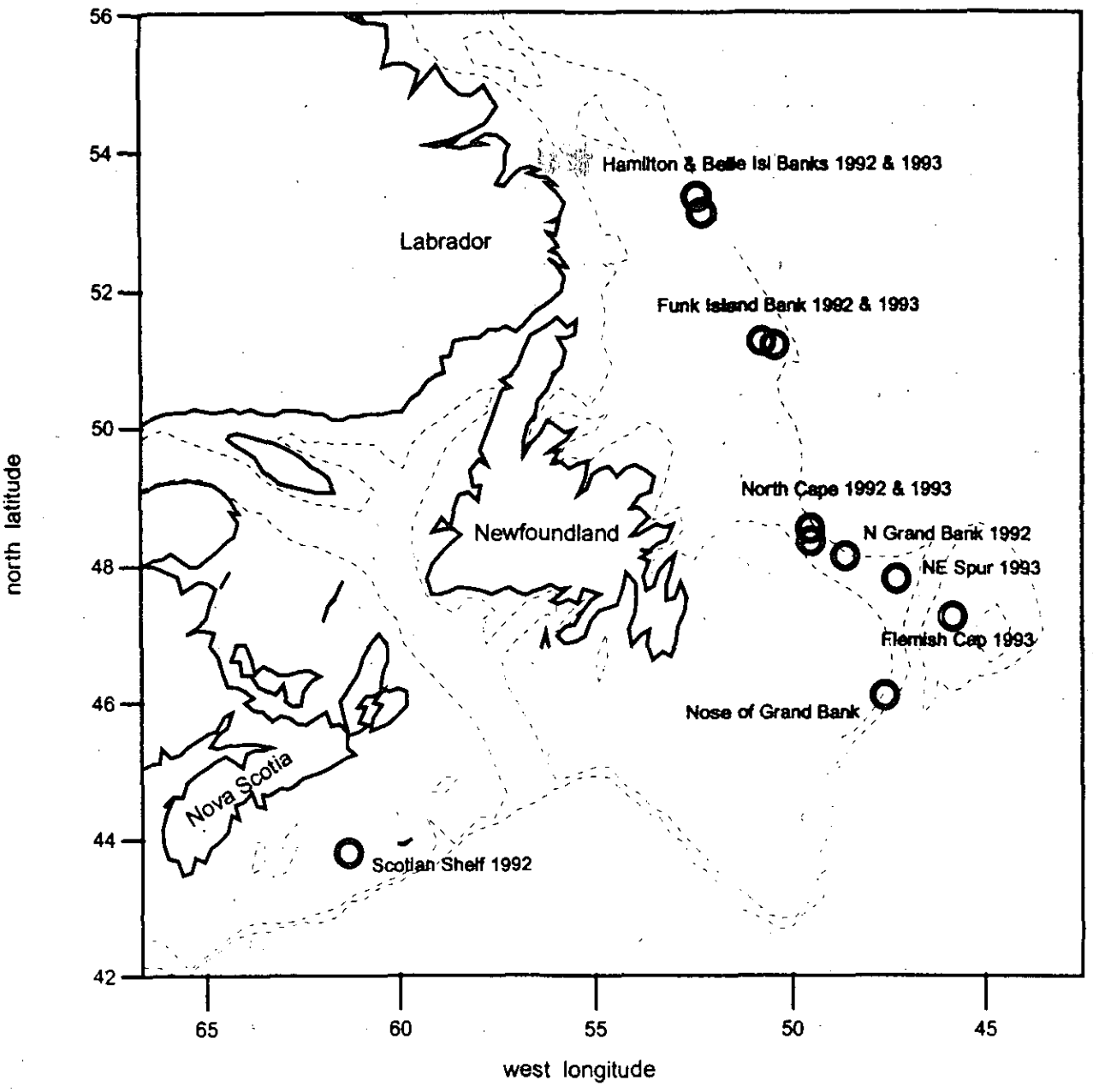

Figure 1.

Bathymetric chart (200 and 1000 m isobaths) of the northwest Atlantic shelf regions showing the dates and bank affiliations for the cod samples collected in 1992 and 1993 and used for microsatellite analyses.

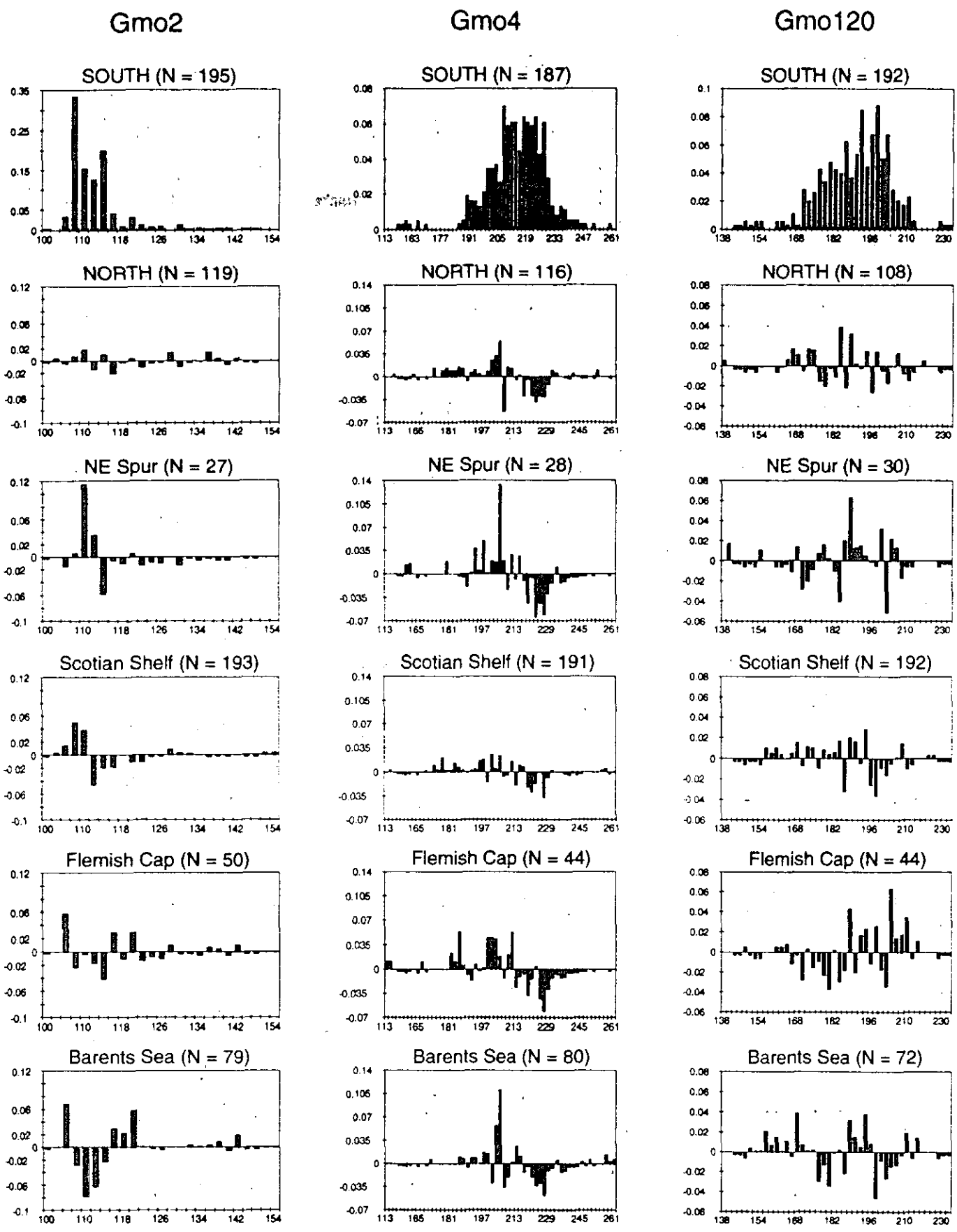

Figure 2.

Allele frequencies of microsatellite loci in cod populations. Shown are the absolute allele frequencies for SOUTH, and for other populations, the difference between the allele distribution in each population and the observed distribution in SOUTH (i.e., other-SOUTH). Absolute/relative frequencies and allele size in base pairs (bp) are given by the ordinate and abscissa, respectively for each locus.

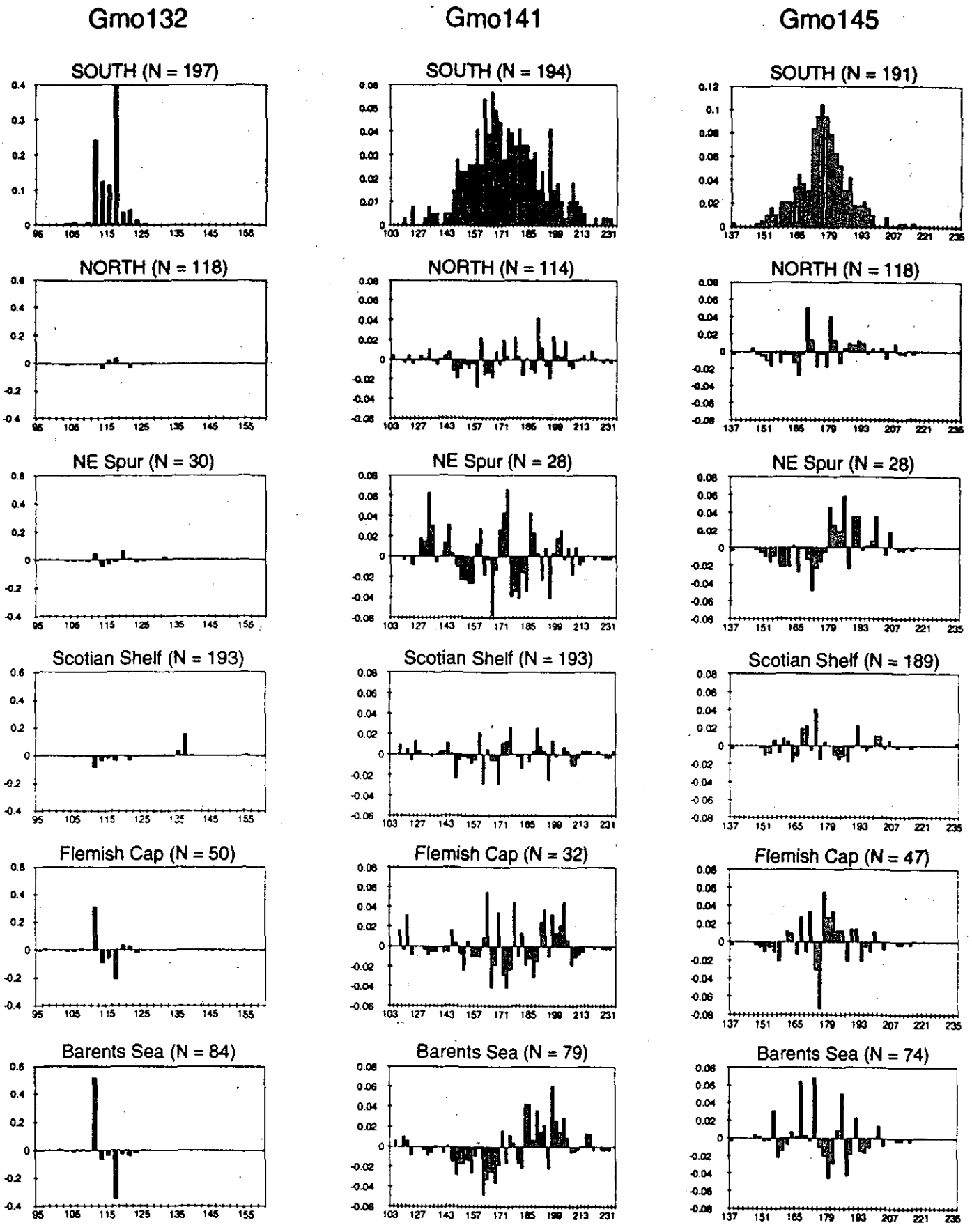

Fig. 2. (Continued).

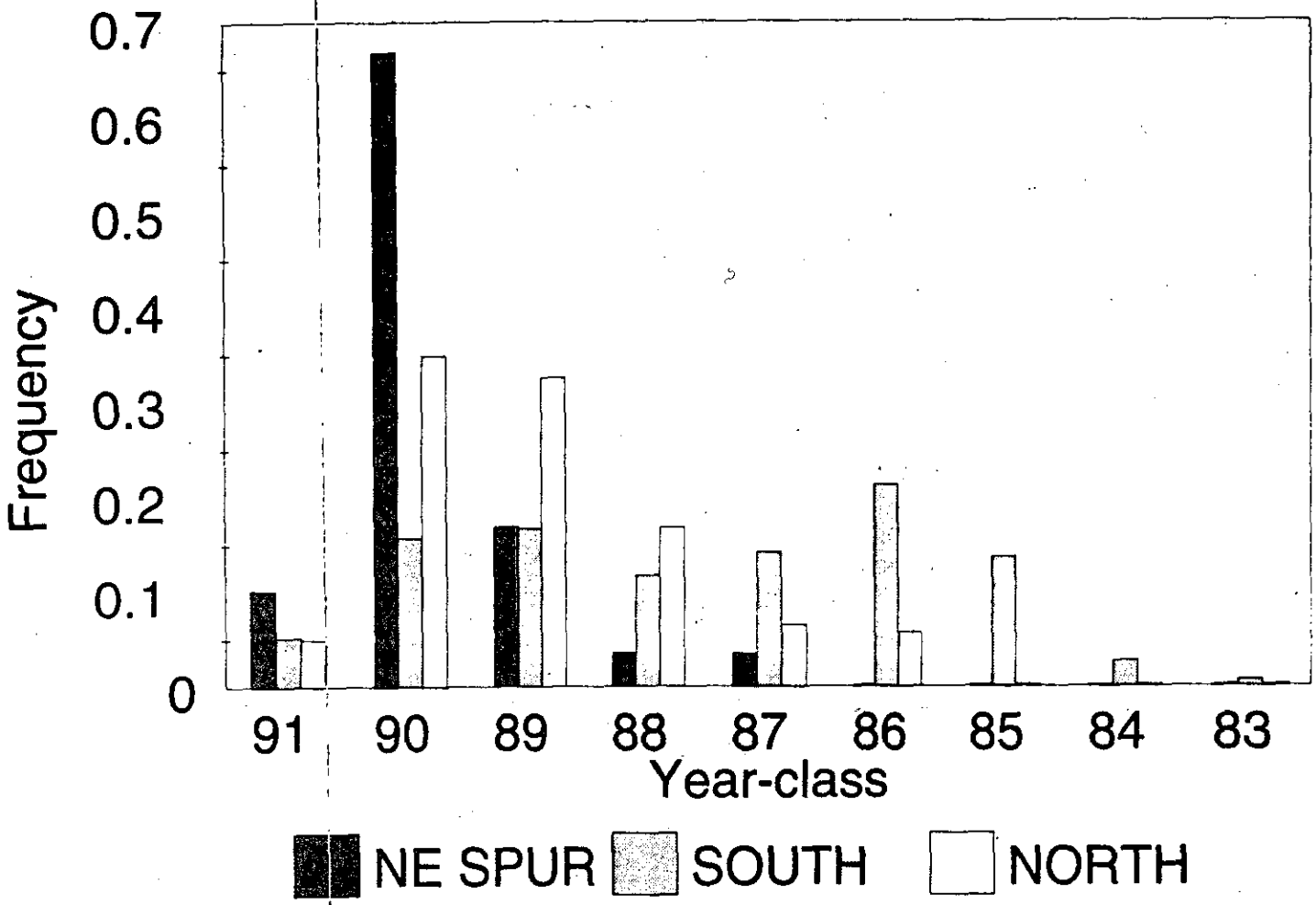

Figure 3.

Year-class contributions (distributions) for the northern cod samples as determined according to length at age estimates.

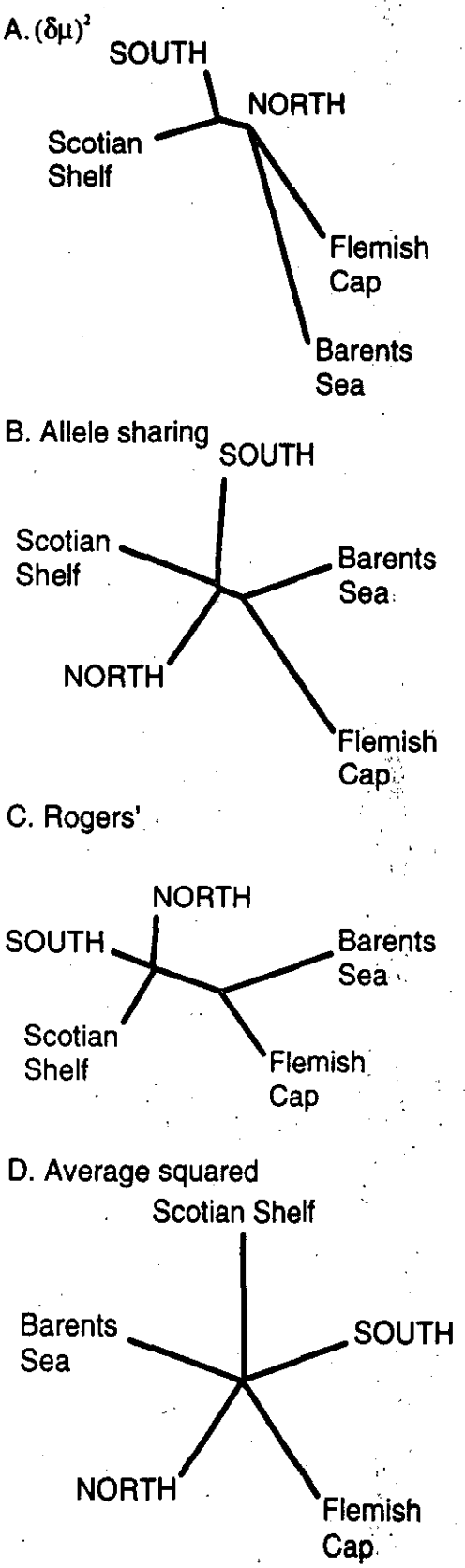

Figure 4.

Fitch-Margoliash phenograms depicting relationships among cod populations based on four measures of genetic distance.