Fisheries and Oceans Canada | Pêches et Océans Canada

Canadian Stock Assessment Secretariat
Research Document 99/55

Not to be cited without
permission of the authors[1]

Secrétariat canadien pour l'évaluation des stocks
Document de recherche 99/55

Ne pas citer sans
autorisation des auteurs[1]

Mixed-stock analysis of Atlantic cod (*Gadus morhua*) in the Cabot Strait approaches to the Gulf of St. Lawrence: a microsatellite DNA application

by

Daniel E. Ruzzante[1,3,4], Christopher T. Taggart[2,3], Shelley Lang[1,3], and Doug Cook[3]

[1]Department of Biology, Dalhousie University, Halifax, NS, B3H 4J1, Canada
[2]Department of Oceanography, Dalhousie University, Halifax, NS, B3H 4J1, Canada
[3]Marine Gene Probe Lab, Dalhousie University, NS, B3H 4J1, Canada

[4]Present Address: Danish Institute for Fisheries Research, Department of Inland Fisheries, Population Genetics Lab, Vejlsoevej 39, DK 8600 - Silkeborg - Denmark; Tel: +45 89213104; Fax: +45 89213150
e-mail: dr@dfu.min.dk

ISSN 1480-4883
Ottawa, 1999
Canada

ABSTRACT

The collapse of various stock complexes of cod (*Gadus morhua*) in the NW Atlantic have prompted a clarification of relationships among stock components. Here we examine the genetic composition of >2300 cod collected during 1994-1997 in the Gulf of St. Lawrence and its approaches to determine if: 1) stock components can be genetically identified; 2) population structure is temporally stable; 3) components are always separated and, if not, where and when are they mixed; and 4) if component contributions to mixtures can be estimated. We use polymorphism at 6 microsatellite DNA loci from cod collected on or near their spring and summer spawning grounds to examine structure and then employ maximum likelihood analyses to estimate contributions of each component to mixtures overwintering near the entrance to the Gulf. Estimates of genetic structure (F_{ST} and R_{ST}) reveal significant differences among cod populations during stock-separated periods and the structure appears to be temporally stable. Estimates of genetic distance (D_A) suggest that the structure results from differences among cod collected within the Gulf of St. Lawrence and those collected near the entrance to the Gulf on either side of the Laurentian Channel in the Cabot Strait, as well as among cod collected south of Newfoundland along the north side of the Channel. Weak genetic heterogeneity among seven regional mixed-stock collections during the overwintering period suggests that cod aggregations characteristically found in the overwintering region represent population mixtures that differ in the proportion of cod contributed to them by the various stock components. Maximum likelihood estimates indicate no significant temporal changes in component contributions to the mixed-stock samples between 1996 and 1997 when all of the winter mixed-stock samples were pooled. The combined contribution of cod from the southern and northern Gulf of St. Lawrence to the mixed-stock samples ranged between 46 % and 71 % (expected 64%). More precise estimates of contributions from these two regions is precluded by the weak genetic differentiation detected in our samples. The contribution by cod from the Cape Breton Island region was small and estimated at 3 %. Contributions by cod from the eastern Scotian Shelf, southwest Newfoundland and south-central Newfoundland were in the range of 13-14%, 4% and 8%, respectively. Contributions by inshore cod from Placentia and Fortune Bays in south Newfoundland were small to negligible (~3% each). The results indicate that future exploitation could be designed around the spatial and temporal scale of the stock structure identified during the stock-separated period and around the spatially varying contributions to the overwintering mixed-stock fishery.

RÉSUMÉ

L'effondrement de complexes de stocks de morue (*Gadus morhua*) du nord-ouest de l'Atlantique a incité à préciser les rapports existant entre les diverses composantes des stocks. Nous examinons ici la composition génétique de plus de 2 300 morues prélevées de 1994 à 1997 dans le golfe du Saint-Laurent et ses approches dans le but de déterminer si : 1) les composantes des stocks peuvent être identifiées de façon génétique ; 2) la structure des populations est stable dans le temps ; 3) les composantes sont toujours distinctes et, dans le cas contraire, le lieu et le moment du mélange et 4) l'apport des composantes peut être estimé. Nous avons utilisé le polymorphisme à six locus de microsatellites d'ADN de morues prélevées à proximité ou à l'intérieur de leurs zones de frai de printemps et d'été afin d'en examiner la structure et d'appliquer ensuite l'analyse par maximum de vraisemblance à l'estimation de l'apport de chaque composante aux concentrations mixtes se trouvant à proximité de l'entrée du Golfe. L'estimation de la structure génétique (F_{ST} et R_{ST}) montre des écarts significatifs entre les populations de morue pendant les périodes où les stocks sont distincts et la structure apparaît stable dans le temps. L'estimation de la distance génétique (D_A) porte à croire que cette structure résulte d'écarts entre les morues provenant du golfe du Saint-Laurent et celles provenant des environs de l'entrée du Golfe, de chaque côte du chenal Laurentien dans le détroit de Cabot, de même qu'entre les morues provenant du sud de Terre-Neuve le long du coté nord du chenal. L'existence d'une faible hétérogénéité génétique au sein des sept prélèvements régionaux de stocks mixtes pendant la période d'hivernage porte à croire que les concentrations de morue que l'on retrouve de façon caractéristique dans la région d'hivernage sont des mélanges de populations qui diffèrent par la proportion d'individus qu'elles ont obtenus des diverses composantes de stocks. Des estimations par maximum de vraisemblance indiquent l'absence de variation temporelle significative de l'apport des composantes aux échantillons de stocks mixtes de 1997 et 1997 lorsque tous les échantillons de stocks mixtes d'hiver sont regroupés. L'apport combiné de morues des parties sud et nord du golfe du Saint-Laurent aux échantillons de stocks mixtes variait entre 46 % et 71 % (valeur probable de 64 %). La faible différenciation notée au sein de nos échantillons nous interdit d'obtenir des estimations plus exactes des apports de ces deux régions. L'apport des morues de la région du Cap-Breton était faible et estimé à 3 %. Les apports des parties est du plateau Scotian, du sud-ouest de Terre-Neuve et du centre-sud de Terre-Neuve étaient, respectivement, de l'ordre de 13-14 %, 4 % et 8 %. L'apport des morues côtières des baies Placentia et Fortune, du sud de Terre-Neuve, variait de faible à négligeable (~3 % chacun). Les résultats obtenus montrent que l'exploitation pourrait être fondée sur les caractéristiques spatiales et temporelles de la structure des stocks déterminée pendant la période où ils sont distincts et en fonction des apports spatialement variables à la pêche des stocks mixtes en hivernage.

Introduction

Exploitation patterns that ignore or misidentify population structure within or among stock complexes can easily lead to the overexploitation of component populations and the erosion of genetic resources via the depletion of constituent spawning components. This problem is exacerbated in stock complexes with diverse, locally adapted, migratory components that intermingle seasonally yet are spatially and temporally managed under the critical assumption of panmixia. If the assumption is invalid then the smaller or less productive components, or those most readily exploited, are also those most readily eliminated (Larkin 1977, Iles and Sinclair 1982, Clark 1990, Policansky and Magnuson 1998). The elimination of stock components (populations) is detrimental to the stock because of the direct negative effects on recruitment potential, and to the species because of the resulting depletion of genetic diversity underlying resilience. Also, for stock complexes in a recovery phase, differential recovery among unidentified components can result in an inability to anticipate future patterns of recruitment that are necessary to define conservation strategies and management policy. In general, exploitation patterns that ignore genetic structure are functionally inconsistent with the principles of resource conservation and the maintenance of biodiversity (Hedrick and Miller 1992, Ryman et al. 1995).

The collapse of the Atlantic cod (*Gadus morhua*) fisheries throughout the northwest Atlantic prompted the imposition of commercial fishing moratoria of unspecified duration that began with northern cod in 1992 (Taggart et al. 1994). Subsequently, six of the seven major Canadian cod stock complexes have been subjected to a more-or-less permanent fishing closure (see Myers et al. 1996, 1997), and the limited recovery of these complexes has prompted a clarification of the relationships among them and their component populations (see Rice 1997).

The above concerns are directly relevant to the sustainability of cod stock complexes and their associated fisheries in the various NAFO (North Atlantic Fisheries Organization) management Divisions (Div.) in the Gulf of St. Lawrence and its approaches (**Figure** 1). There exist within this large region (~300,000 square km) a number of spawning populations of cod that are either known or suspected to migrate to common overwintering grounds in the Cabot Strait at the entrance to the Gulf and it is here that a substantial 'mixed-stock' fishery has traditionally taken place in Winter (Campana et al. 1998). Cod spawning areas are known to exist in the northern and southern regions of the Gulf (Div. 4RS and 4T respectively), in the Sydney Bight region off Cape Breton (Div. 4Vn) and on the eastern Scotian Shelf (Div. 4Vs). There are also suggestions of discrete spawning components off southern Newfoundland along the north side of the Laurentian Channel (Div. 3Pn and 3Ps). Winter (January) surveys in the Cabot Strait have been conducted since 1994 and have confirmed the historic observations of concentrated cod aggregations overwintering along the northern and southern flanks of the Laurentian Channel (Chouinard 1994, Campana et. al. 1998).

It has been known for some time that cod populations spawning in the northern and southern Gulf of St. Lawrence in early Summer migrate from the Gulf in the Autumn and overwinter in the Cabot Strait along with other putative stocks (those from Divs. 4Vn, 4Vs, 3Ps; see Jean 1964, Martin and Jean 1964). A recent compilation of results from tagging studies conducted in the region by Templeman (1974, 1979) show migration patterns that are consistent with those outlined above: cod tagged and released in the Cabot Strait off southern Newfoundland (Burgeo Bank) in the Spring are generally recaptured within the Gulf in the Spring and Summer of subsequent years and recaptured in the vicinity of original tagging in late Autumn and Winter (see Taggart et al. 1995,

Figure 11, p. 88, 136, 338). Similarly, cod tagged within the Gulf (Strait of Belle Isle) in late Summer and Autumn are recaptured during the Winter in the Cabot Strait along the southern coast of Newfoundland and in subsequent Summers are reported from the northern Gulf (Taggart et al. 1995, **Figure** 11, p. 160, 194, 196). However, tagging studies, unless conducted on spawners, provide little information on where a tagged fish actually spawns. This makes the interpretation of tag returns uncertain when spawning times differ among locations, as is the case for cod in the Gulf of St. Lawrence and approaches.

To determine the relative contribution to the overwintering area of cod from the various management Divisions cited above we first determine the genetic structure of the populations. We employ techniques similar to those used to demonstrate cod stock structure at a variety of scales across the distributional range of cod in the NW Atlantic (Ruzzante et al. 1998). The effort is focused on cod collected on or near their spawning grounds during the spring and summer months (April through July) of 1994 to 1997 (the stock-separated period) which allows the determination of the short term (2 to 3 yr) stability of the structure. We then employ the technique of mixed-stock analysis using maximum likelihood estimation (*sensu* Millar 1987) to examine the proportional contribution of each population component to cod aggregations collected during the winter "mixed-stock" periods (January 1996 and January 1997) in the Cabot Strait approaches to the Gulf of St. Lawrence.

Mixed-stock analysis: Overview, assumptions and limitations

Most mixed-stock analyses employ either likelihood estimation (Milner et al. 1981, Fournier et al. 1984, Beacham et al. 1985, Millar 1987, Pella and Milner 1987, Wood et al. 1987, Smouse et al. 1990) or quadratic programming techniques (Xu et al. 1994). For both methods the accuracy and precision of the composition estimates rely on the validity of assumptions concerning Hardy-Weinberg equilibrium within reference components (the putative structure) and concerning statistical distribution (mixture of multinomials; Milner et al. 1981, Fournier et al. 1984, Millar 1987, Wood et al. 1987). According to Utter and Ryman (1993) maximum likelihood methods are likely to give imprecise or biased results if: a) the sample sizes of the baseline ("learning" or "reference") or the mixed ("test") collections are small; b) the number of loci examined is small; c) there is little temporal stability in allele frequency differences among putative components; d) the differences among components is small; and/or d) not all groups present in the mixture are included in the putative population samples (Fournier et al. 1984, Millar 1987, 1990, Wood et al. 1987, Smouse et al. 1990). Smouse et al. (1990) examined the problem of incomplete sampling of baseline population data.

Mixed-stock analysis using genetic markers has been applied to a variety of fish (reviewed in Utter and Ryman 1993) and include Atlantic salmon (*Salmo salar*, Galvin et al. 1995); American shad (*Alosa sapidissima,* Epifanio et al. 1995, Brown et al. 1996); striped bass (*Morone saxatilis,* Wirgin et al. 1995, 1997); and most notably Pacific salmon (coho, *Oncorhynchus kisutch*, Milner et al. 1981, Millar 1987, Miller et al. 1996, chum, *O. keta*, Fournier et al. 1984, Beacham et al. 1985; sockeye, *O. nerka,* Grant et al. 1980, Wood et al. 1987; and chinook, *O. tsawytscha*, Smouse et al. 1990, Waples 1990, Beacham et al. 1996). The majority of these studies used allele frequency differences at allozyme loci and the more recent studies used either mtDNA (Epifanio et al. 1995, Brown et al. 1996, Wirgin et al. 1995, 1997) or nuclear DNA polymorphism (Beacham et al. 1996, Galvin et al. 1995, Miller et al. 1996 for minisatellite DNA; Wirgin et al. 1997 for single copy nuclear DNA (nDNA)). The potential for the application of mixed-stock

analyses to most marine species has been regarded as limited (Utter and Ryman 1993) because the level of population substructuring is typically low relative to other species (Ward et al. 1994). Although recommended several times (Herbinger et al. 1995, McConnell et al. 1995, Ferguson and Danzmann 1998), to our knowledge the study reported here is the first to employ highly polymorphic microsatellite DNA loci for a mixed-stock analysis.

Material And Methods

Sampling

We collected an excess of 2300 samples of individual cod tissues for genetic analyses over a four year period between 1994 to 1997. Approximately one half of the samples were collected from pre-, post-, or spawning aggregations during the spring and summer stock-separated period (**Table** 1, **Figure** 1). During the spawning period putative stocks or their components are presumed to be most separated and close to or on their spawning grounds in the southern (4T) and northern (4R) Gulf of St. Lawrence, the Sydney Bight region (4Vn), the eastern Scotian Shelf (4Vs) and southern Newfoundland (3Pn and 3Ps). The remaining half of the samples were collected during the mixed-stock period from winter aggregations representing seven of the regional management Divisions and sub-divisions near the entrance to the Gulf (**Table** 2, **Figure** 2). It is during this overwintering period that the putative stock components are presumed to be mixed. Care was taken to ensure that the geographic distribution and relative abundance of the mixed-stock samples reflected the geographic distribution of cod aggregations throughout the region as determined from surveys conducted in January 1996 and 1997. All mixed-stock and the majority of stock-separated samples were taken from cod collected with an otter trawl deployed to the bottom at depths as great as ~520 m (**Table** 2). The remainder were collected using "Sentinel Fishery" handlines and/or gillnets at depths as shallow as ~20 m (**Table** 1). The depressed state of all of the stock complexes resulted in unavoidable variation in the size, age, and reproductive state of cod within and among the collections (**Table** 1, 2). However, considerable effort was made to constrain the size (thus age) distribution within collections and to focus on spawning (ripe and running) individuals during the stock-separated period.

Tissue collection and DNA extraction

Cod blood (~1 mL) was the primary source of nuclear DNA and was collected from live or recently dead cod (details in Bentzen et al. 1996, Ruzzante et al. 1996a, 1996b, 1997). Blood samples were preserved immediately in ~5 mL of 95% ethanol. When blood was unavailable we employed soft muscle tissue generally taken from the posterior of the tongue and preserved in 95% ethanol.

DNA was extracted using a salting out procedure designed for nucleated cells. An aliquot of blood/alcohol equivalent to ~75 µl of blood was washed in high TE (100 mM Tris-HCl pH 8.0, 40 mM NaCl). Following the removal of the alcohol the extraction was as described by Miller et. al. (1988). The DNA precipitate was washed with cold 70% EtOH, air dried and resuspended in 100 uL TE.

PCR amplification of six microsatellite loci, Gmo2, Gmo132, Gmo145 (Brooker *et al.* 1994), Gmo4 (Wright 1993), and Gmo120 (Ruzzante et al. 1996a) and Gmo151 (this publication) were as detailed in Ruzzante et al. (1998). Primer sequences for Gmo151 are as follows: Gmo151a: *TTGTAGACAACATCCACTT* and Gmo151b: *GATACTGGTTCTGTAAGGT*, and annealing temperature was 48 °C.

Data Analysis

Population Genetics

We tested for the homogeneity of allele frequency distributions and for gametic linkage disequilibrium between any two loci using χ^2 pseudoprobability contingency tests following Weir (1996). Tests of homogeneity were done by randomization of alleles across individuals and populations (1000 bootstrap samples; Manly 1991). Tests of linkage disequilibrium were done by permutation of alleles across individuals for the entire data set. Estimates of subpopulation structure were obtained using F_{ST} (Wright 1951) and R_{ST} (Slatkin 1995). F_{ST} was estimated following Weir and Cockerham (1984). R_{ST} was calculated following Goodman (1997; see also Michalakis and Excoffier 1996) to minimize the variance due to sample size differences (see Ruzzante 1998). Allele sizes were standardized across the entire data set prior to estimation (Goodman 1997, eqn. 3, p. 882) to prevent differential influence among loci. Significance for both structure measures was estimated by bootstrapping genotypes across individuals and populations and for each locus separately. Multilocus estimates of F_{ST} and R_{ST} were calculated by first summing the variance components across loci (Weir and Cockerham 1984, Slatkin 1995, Goodman 1997), rather than averaging single locus R_{ST} or F_{ST} estimates over loci. Pairwise genetic distances among populations were estimated using D_A (Nei et al. 1983), a non-SMM (stepwise mutation model) estimate of genetic distance with low variance relative to other non-SMM measures (Takezaki and Nei 1996; see also Ruzzante 1998). Significance for the distance measure was estimated by bootstrapping genotypes (1000 resampling trials with replacement) across individuals and populations for each locus separately. We also used multidimensional scaling analysis of the D_A matrix to illustrate relationships among populations in more than two dimensions. In all cases significance levels were adjusted for multiple comparisons using the sequential Bonferroni approach (Rice 1989). All statistical tests and analyses of genetic distances and population structure were conducted using Splus© (MathSoft, Inc. 1996) standard code or functions written by D Ruzzante.

Mixed-stock Analysis

We used a maximum likelihood method (Millar 1987) to estimate the proportions of putative stock components (i.e. the reference or learning data; **Table** 1) contributing to samples collected during the winter mixed-stock periods of 1995 through 1997 (**Table** 2). Confidence intervals (95%) around the maximum likelihood estimates were obtained by bootstrapping both the mixed-stock samples and each of eight regional collections obtained on or near their respective spawning grounds during the stock-separated period.

Results

(1) Single locus statistics

The total number of cod analysed per locus for the entire data set (stock-separated and mixed-stock samples) ranged from N=2242 for Gmo145 to N=2333 for Gmo2, and the total number of alleles per locus for the entire data set ranged from n=21 for Gmo132 to n=94 for Gmo151 (**Table** 3). Observed and expected heterozygosities per locus ranged from 0.729 and 0.711 (both for Gmo132) to 0.982 and 0.956 for Gmo120 and Gmo4 respectively (**Table** 3). There was no evidence of linkage disequilibrium between pairs of loci in any of the 15 pairwise comparisons ($P \geq 0.068$ for 14 comparisons; $P=0.010$ for Gmo4 and Gmo145; $\alpha=0.05/15=0.0033$ with sequential Bonferroni correction for 15 simultaneous tests).

(2) Variation within and among samples

We next examine the variation within and among: ***(i)*** the stock-separated samples; and ***(ii)*** the mixed-stock samples.

(i) Stock-separated samples:

To facilitate statistical analysis, we first pooled samples (fishing sets) collected from neighbouring locations during a given collection trip. There were 10 collection trips with samples (fishing sets) from more than one location (**Table** 1; column 2: local groups 1, 2, 4, 5, 6, 7, 8, 9, 12, 18). Comparisons using χ^2 pseudoprobability tests indicated there was no evidence of heterogeneity in allele frequency distribution between groups of two or three sub-groups from neighbouring locations for 59 of 60 tests ($n=60$, $\alpha=0.05/60 \approx 0.0008$). The exception was locus Gmo145 in the comparison between sub-groups 3 and 4 from the western portion of the southern Gulf of St. Lawrence collected in June 1996 (**Table** 1, column 4: $N_3=52$ and $N_4=48$; $P<0.0001$). There was also no evidence of structure for any of the comparisons involving sets from neighbouring locations using F_{ST} or R_{ST} ($P \geq 0.051$ and $P \geq 0.048$, respectively, $\alpha=0.05/10=0.005$), or D_A ($P \geq 0.010$, $\alpha=0.05/14=0.0036$). As there was no consistent evidence of genetic heterogeneity or structure among these neighbouring sub-groups with any of the measures used, we pooled these 32 sub-groups (**Table** 1, column 4) into 20 local groups corresponding to sampling location and year of collection (**Table** 1, column 2).

We further pooled 17 of these 20 local groups into regional groups (**Table** 1, column 1). These regional groups correspond closely to management Divisions. Pooling into regional groups was done following analysis of the genetic composition using the same battery of tests as above (i.e. χ^2 pseudoprobability tests of allele frequency distributions, F_{ST} and R_{ST} estimates of genetic structure, and the D_A measure of genetic distance). The remaining three samples (local groups 18, 19, 20; **Table** 1) were from inshore and offshore locations in southern Newfoundland (Div. 3Ps) and were not pooled. Below we detail the results of these analyses for each region:

Southern Gulf of St. Lawrence (Div. 4T): χ^2 pseudoprobability tests indicated marginal evidence of heterogeneity in allele frequency distribution among the six samples (local groups 1-6; **Table** 1, column 2) collected in 1995, 1996 and 1997 ($P<0.05$ for 4 of 6 loci, although none was significant after sequential Bonferroni correction, $\alpha=0.05/6$ loci$=0.0083$). These 6 samples also

showed evidence of structure with F_{ST} (=0.0028, P<0.001) and R_{ST} (=0.0047, P=0.020). Pairwise comparisons using D_A showed that the sample collected on the western side of the southern Gulf of St. Lawrence in June 1997 (local group 4) was the most different of all, yet it was not significantly different from any of the other samples after sequential Bonferroni correction (P≥0.004; α=0.05/15=0.003). None of the remaining pairwise D_A distances, including those involving comparisons between the temporally spaced (1995-1997) samples from neighboring locations, or those between samples from the same year and different locations were significant (P≥0.050). However, when these same data were pooled within years there was evidence of temporal change across years with both F_{ST} (=0.0053, P<0.001) and R_{ST} (=0.0084, P<0.001). Analysis based on D_A indicated that this structure was largely caused by the pool of samples collected in 1997 [local groups 4 (N=50) and 6 (N=49), **Table** 1] but in particular by the sample from the western area of the southern Gulf (local group 4, **Table** 1) and not by that from the eastern area of the southern Gulf (local group 6). No structure or heterogeneity were detected with any of F_{ST} (= -0.0002), R_{ST} (=0.0023, P=0.120), or D_A (0.036, P=0.110) when the pool of samples from the western area of the southern Gulf (regional group 1a, **Table** 1) was compared to the pool of samples from the eastern area (regional group 1b). Thus, although there was some genetic heterogeneity among the 6 samples collected in the southern Gulf, this heterogeneity could not be systematically attributed to geographic location or temporal change in the genetic composition. We suspect that the heterogeneity may be caused by sampling effects as no individual sample is entirely representative of the whole southern Gulf cod spawning population. Thus, for the maximum likelihood analyses of mixed-stock composition we grouped all southern Gulf samples (regional groups 1a and 1b) into one southern Gulf (Div. 4T) regional pool (N=392).

Northern Gulf of St. Lawrence (Div. 4R): χ^2 pseudoprobability tests showed no evidence of heterogeneity in allele frequency distribution between the northern Gulf samples collected in April 1996 (local group 7) and April 1997 (local group 8; **Table** 1) for 5 of the 6 loci (P≥0.019, α=0.05/6=0.0083); the one exception being Gmo145 (P=0.006). No genetic structure was detected using either F_{ST} (=0.0018, P=0.084) or R_{ST} (=0.0049, P=0.138), although analysis based on D_A indicated the two samples to be marginally distinguishable (D_A=0.089, P=0.023). We grouped these two samples into one regional pool (regional group 2; **Table** 1).

Sydney Bight (Div. 4Vn): Three of the six loci examined showed either significant (Gmo151, P<0.001, α=0.008) or marginal (Gmo2 and Gmo145; P≤0.038) evidence of heterogeneity in allele frequency distribution among the three samples collected in 1996 and 1997 (local groups 9, 10, 11; **Table** 1a). There was evidence of genetic structure with both F_{ST} (=0.0032, P=0.009, N_1=100, N_2=48, N_3=48) and R_{ST} (=0.025, P<0.001). Analyses based on D_A indicated that the structure was due largely (though not exclusively) to the difference (P<0.001) between the early May 1996 sample (local group 9) and the late May 1997 sample (local group 11). The remaining two pairwise comparisons, including that between the early and late May 1997 samples, showed marginal heterogeneity (P≅0.036). The heterogeneity may, in part, be caused by the fact that the early May 1996 sample (local group 9) and the early May 1997 sample (local group 10) may include pre-spawning migrants (into the Gulf) of southern Gulf (Div. 4T) origin that are mixed with putative resident cod. The observation that both local groups had low proportions of spawners (~5% and ~40% respectively) and high proportions of pre-spawners (~68% and ~42%) relative to local group 11 (showing ~79% spawners and ~6% pre-spawners; **Table** 1) is consistent with this explanation. Thus, the analysis of genetic differentiation among the stock-separated

regional pools based on the D_A measure of genetic distance was conducted including and excluding the two samples presumed to contain migratory cod of 4T origin from the regional pool (see below and **Table** 5a).

Scotian Shelf (Div. 4Vs): Three loci (Gmo2, Gmo145, and Gmo151) showed evidence of marginal heterogeneity in allele frequency distribution ($P \leq 0.026$; otherwise $P \geq 0.134$) among the four samples (local groups 12-15; **Table** 1) collected in 1994, 1996 and 1997. There was no evidence of genetic structure when measured with F_{ST} (=0.001, P=0.145) whereas there was evidence when measured with R_{ST} (=0.011, P=0.002). Analyses based on D_A indicates the 1996 sample may be distinguishable (P=0.001; $\alpha=0.05/6=0.0083$) from one (but not both) of the 1997 samples and marginally (P=0.008) from the 1994 sample. No other pairwise comparison was significant. Analyses across years after pooling within years gave similar results: the 1996 sample was distinguishable from the 1997 and 1994 samples (P=0.002 and P=0.014; $\alpha=0.05/3=0.016$), but the latter two were not distinguishable from each other (P=0.083). Again, we suggest that the heterogeneity may be caused by sampling effects. We pooled the three samples into a single regional pool (regional group 4; N=243).

South Newfoundland (Div. 3Pn): There was marginal evidence of heterogeneity in allele frequency distribution between the 1996 and 1997 samples (local groups 16 and 17) for 3 of the 6 loci examined ($P \leq 0.040$), but no locus showed heterogeneity after sequential Bonferroni correction ($P \geq 0.021$ and $\alpha=0.05/6=0.0083$). No structure was detected when measured with F_{ST} (=0.0005, P=0.354) but some evidence of heterogeneity was suggested by R_{ST} (=0.042, $P<0.001$) and by D_A ($P<0.001$). We again suspect the potential heterogeneity (not consistent across measures) may be caused by sampling effects, particularly when considering the relatively small sample size of the 1997 sample (N=46 but only 31 and 34 with non-missing values for Gmo145 and Gmo151 respectively). For the purpose of the maximum likelihood analysis of mixed-stock composition we grouped these two samples into a single regional pool (regional group 5; N=107).

South Newfoundland (Div. 3Ps): Among the three samples collected from this region in 1996 and 1997 there was strong evidence of heterogeneity in allele frequency distribution for one locus (Gmo145, P=0.001, $\alpha=0.05/6=0.0083$) and marginal evidence for two others (Gmo120, P=0.037; Gmo151, P=0.048; both insignificant after sequential Bonferroni correction). Structure was evident, though weak, when measured with F_{ST} (=0.0019, P=0.049) and evident when measured with R_{ST} (=0.045, $P<0.001$). The analysis based on D_A indicates the structure results from differences among all three samples ($P \leq 0.009$; ($\alpha=0.05/3=0.017$). The evidence of differentiation using all measures compelled us to consider the three samples as representing three different populations. Furthermore, two of the samples were collected in the inshore areas of Fortune and Placentia Bays on the southern coast of Newfoundland and may represent inshore cod populations that have been described elsewhere in coastal Newfoundland (see Ruzzante et al. 1996b, 1997, 1998, Taggart et al. 1998). The third sample was collected well offshore. For the mixed-stock analyses we estimated the separate contribution by each of the local groups (18-20) identified within this management division during the stock-separated period.

To summarize, we found evidence of genetic heterogeneity (frequently marginal) in a limited number of comparisons involving temporally spaced samples from related geographic areas. However, in most cases the evidence was not consistent across all measures used suggesting the degree of heterogeneity was small and may have resulted from sampling effects. Using the results

detailed above we pooled 17 of the 20 local groups into 5 regional pools comprising regional groups 1a and 1b (Southern Gulf, N=392), 2 (Northern Gulf, N=148), 3 (Sydney Bight, N=196), 4 (Eastern Scotian Shelf, N=243), and 5 (South Nfld, Div. 3Pn, N=107). We kept local groups 18, 19, and 20 (Div. 3Ps) separated (**Table** 1). In pooling some of the samples, we explicitly acknowledge that there may be some genetic heterogeneity within some of the resulting regional pools as would be expected for temporally spaced samples (see Waples and Teel 1990). The pooling of samples for the purpose of describing genetic structure despite (weak) heterogeneity is a conservative approach given that we are interested in detecting whether there is genetic heterogeneity at larger spatial scales, i.e. among management Divisions. The detection of large-scale structure in the presence of weak heterogeneity at smaller scales (within management Divisions) implies that the large-scale structure is unlikely to result from sampling effects.

Estimates of genetic structure among the 8 regional pools:

An analysis of F_{ST} revealed evidence of population genetic structure among the eight regional pools of cod collected during the stock-separated period. The magnitude of the F_{ST} estimate overall loci was low (F_{ST}=0.0017), but significant (P<0.001) and due to the collective influence of all loci (i.e. all single locus estimates were >0; **Table** 4). However, Gmo132 (F_{ST}=0.0044) and Gmo151 (F_{ST}=0.0024; **Table** 3) and to a lesser extent Gmo120 and Gmo145 were the most influential in determining both the magnitude of the estimate and its significance. Genetic structure among the eight regional pools was also evident when measured with R_{ST} (=0.0142, P<0.001) primarily due to the influence of the Gmo132 and Gmo145 loci and to a lesser extent Gmo120 and Gmo151 (**Table** 4).

Estimates of pairwise genetic distances: 8 regional pools

The D_A measure of genetic distance (**Table** 5a) indicates that the structure identified above using the F_{ST} and R_{ST} measures is due primarily to genetic differences between cod collected in the Gulf of St. Lawrence and Sydney Bight areas (Divs. 4T, 4R and 4Vn) and those collected near the Gulf entrance in Divisions 4Vs, 3Pn and 3Ps, as well as among the samples in the latter group. There is markedly less heterogeneity among samples from the Gulf and Sydney Bight relative to the others (**Table** 5a; but see below for a discussion of the potential origin of two of the three samples from the Sydney Bight area; Div. 4Vn, regional group 3 in **Table** 1). The pattern of differences and similarities among these populations can be visualised with a multidimensional scaling analysis applied to the matrix of D_A genetic distances (**Figure** 3). A plot of dimensions 1 vs. 2 (explaining approximately 37 % and 35 % of the total variance respectively) indicates that the two samples from inshore Newfoundland (i.e. Fortune and Placentia Bays) differ the most; Fortune Bay along dimension 1 and Placentia Bay along dimension 2 (**Figure** 3a). A plot of dimension 1 vs. 3 (17 % of the variance) shows that the sample from 3Pn and that from offshore 3Ps differ from the rest and from each other along dimension 3 (**Figure** 3b). A plot of dimension 1 vs. 4 (11 % of variance) shows a spread of samples along dimension 4, with cod from Division 4Vs at the extreme opposite of the two samples from north of the Laurentian Channel (3Pn and 3Ps offshore, **Figure** 3c). Finally, cod from 4T, 4R, and 4Vn are never very far apart from each other suggesting greater similarity among these samples than between and among the rest (**Figure** 3a-d). The genetic similarity between cod caught within the Gulf (Divs. 4T and 4R) and those caught in Div. 4Vn may, however, be caused by the fact that two of the three samples within the latter regional group (Div. 4Vn) may contain migratory cod of presumed 4T origin as suggested by the evidence described below.

Cod tag recovery data suggest that southern Gulf (Div. 4T) cod can be caught in the Sydney Bight region in early May during their presumed spawning migration into the Gulf (D. Gascon, unpublished report; Department of Fisheries and Oceans, Institut Maurice-Lamontagne, C.P 1000, Mont-Joli, PQ, G5H 3Z4, Canada; see also Templeman 1979 and Taggart et al. 1995) and two of our samples from this region (local groups 9 and 10; **Table** 1) were collected in early May of 1996 and 1997. Based on the tagging evidence and on the near absence or low proportions of spawning fish within both collections (**Table** 1), we suspect these two samples likely contain migratory cod mixed with resident cod. Reanalysis after excluding these two samples from the pool of 4Vn cod (regional group 4) is consistent with this hypothesis: the genetic distance (D_A) between the remaining 4Vn cod (local group 11; N=48) and all other samples (including those from the southern and northern Gulf) increase in magnitude and significance (see **Table** 5a values within square brackets). This explanation is consistent with the fact that neither sample appears genetically ($P>0.40$) nor phenotypically (otolith elemental fingerprints and vertebra number) distinguishable from southern Gulf cod (for otolith and vertebrae, respectively: S. Campana and K. Frank, personal communication, Marine Fish Division, Bedford Institute of Oceanography, Halifax, NS, B2Y 4A2, Canada). The maximum likelihood estimate of contribution to the Winter mixed aggregations by cod from this region (Div. 4Vn) was thus obtained using only the late May 1997 sample.

We also conducted the analyses detailed above for the 1996 and 1997 data separately. The results provided a similar pattern of differentiation in each of the two years with the possible exception of the cod from the Sydney Bight area (4Vn) which were not distinguishable from 4T cod in 1996 but were distinguishable in 1997 (**Table** 5b,c).

In summary, we detected genetic differences ($\alpha<0.002$ after sequential Bonferroni correction) in 22 out of 28 comparisons between cod samples (regional groups) collected on or near their respective spawning grounds. Four additional pairwise comparisons indicated genetic differences at $\alpha<0.01$ or 0.05 (**Table** 5a). Cod collected on the spawning grounds within the northern and southern Gulf of St. Lawrence (Div. 4T and 4R) were different from the sample of (mostly spawning) cod presumed to be resident in the Sydney Bight region (Div. 4Vn). These three regional collections (northern and southern Gulf, and Sydney Bight) were genetically distinguishable from Scotian Shelf cod (Div. 4Vs), and all four collections were largely distinguishable from cod collected on the northern flanks of the Laurentian Channel in Divisions 3Pn and 3Ps. Along the northern flank of the Laurentian Channel the regional group from Div. 3Pn was distinguishable from the offshore and both inshore samples from the neighbouring Division to the east (3Ps, **Table** 5a). The three collections within Division 3Ps, including the two inshore samples from Placentia and Fortune Bays, were also largely genetically distinguishable from each other (**Table** 5a). Interestingly, with the samples we analysed in the present study the comparison between northern and southern Gulf cod did not approach statistical significance (see below) and neither did the comparison between northern Gulf (4R) cod and cod collected offshore in the 3Ps region of south Newfoundland.

(ii) Mixed-stock samples:

Estimates of genetic structure among the 7 management Divisions:

The analysis of F_{ST} among the samples collected from the seven NAFO Divisions during the mixed-stock periods of 1995 to 1997 (**Table** 2) revealed evidence of some (weak) structure

(**Table** 6). Although this weak structure was due primarily to locus Gmo2, all six single locus F_{ST} estimates were positive (**Table** 6). When analysed separately for the 1996 and 1997 winter periods, there was evidence for weak structure among the 1996 samples (F_{ST}=0.0012, P=0.023) but not among those collected in 1997 (F_{ST}=00007, P=0.067). No single locus F_{ST} estimate was significant in the 1996 samples indicating that the weak structure detected overall was due to the combined effect of all six loci. When estimated with R_{ST} there was no evidence of structure among the samples from the 7 Divisions collected between 1995 and 1997 or among those collected exclusively in 1996 (R_{ST}= - 0.0015) or in 1997 (R_{ST}=0.0014, P=0.07).

Estimates of pairwise genetic distances among the 7 management Divisions:

Analysis of the 1995 to 1997 pooled data using the D_A measure of genetic distance indicated that three out of 21 pairwise comparisons, each involving a different pair of samples, differed from each other. There was little or no evidence to reject the null hypothesis of no genetic differentiation among the remaining samples collected during the winter mixed period (**Table** 7). When data were analysed by year there was no evidence for genetic differentiation among the 1996 samples (P>0.033, α=0.05/10=0.005 after sequential Bonferroni correction for 10 comparisons). Two of the samples collected during the 1997 winter mixed period, one from management Division 4Vn-Vs (N=115) and the other from management Division 4R (N=98) differed genetically from each other (D_A = 0.068, P = 0.001, α=0.05/15=0.003 after sequential Bonferroni correction for 15 comparisons).

(3) Maximum likelihood analysis of stock contribution to the winter mixed-stock samples from the approaches to the Gulf of St. Lawrence.

Estimates of the proportions contributed by the various regional spawning components to the winter mixed aggregations are provided in Figs. 4 and 5. We used the entire stock-separated data set (1994 to 1997) to examine ***(i)*** the various stock component contributions to the pool of mixed-stock (Winter) samples, including whether contributions changed between the years 1996 and 1997 (Fig 4). We then ***(ii)*** examined whether the contributions by the various stock components varied regionally among the four management Divisions where mixed-stock samples were collected in the Winters of 1995, 1996, and 1997 combined (**Figure** 5). In both of these analyses we assessed the contributions by cod from the northern and southern Gulf of St. Lawrence separately. The estimated proportional contributions to the overwintering mixtures by cod from these two regions may, however, be strongly influenced by the fact that the northern and southern Gulf stock components were marginally distinguishable at best, perhaps as a result of the limited geographic coverage of the northern Gulf region in our samples (see Div. 4R, Fig 1).

(i) Temporal variation in stock contribution (1996 vs. 1997).

Figure 5 shows the estimated proportional contribution to the pooled mixed-stock samples by cod from the eight regional pools sampled in the stock-separated period (1995, 1996 and 1997 pooled). The proportional contributions to the mixed samples did not vary significantly between 1996 and 1997 (Figs. 4a and 4b). We therefore re-estimated the expected contributions and their associated 95% confidence intervals after pooling the data from three consecutive Winters [January 1995 (limited coverage), January 1996 and January 1997; **Figure** 4c]. The contribution of cod from the southern Gulf of St. Lawrence to the mixed-stock samples was 51 % (CI: 38-55%). The cod population resident in the northern Gulf (Div. 4R) contributed approximately 13% (CI: 8-20%). Cod resident in the Sydney Bight (Div. 4Vn) appear to have contributed only about 3% (CI: 0-6 %) and those resident on the eastern Scotian Shelf (Div. 4Vs) appear to have contributed 15 % (CI: 12-24%). The contributions to the mixed-stock samples by cod from Divisions 3Pn and 3Ps north of the Laurentian Channel appear to have been small (i.e., 3Pn: 6 % CI: 3-11%; 3Ps offshore: 7%, CI: 3-10%) to negligible (i.e., the inshore populations from Placentia Bay: 3% CI: 0-5 %, and Fortune Bay: 3 %, CI: 1-6%).

(ii) Geographic variation in contribution

The distribution of cod in the Cabot Strait during the Winter is generally not homogeneous. A number of concentrated aggregations were found in similar locations in 1996 and 1997 along the slopes on both sides of the Laurentian Channel (Campana et al. 1998; G. A. Chouinard, Canada Department of Fisheries and Oceans, Marine Fish Division, Gulf Fisheries Centre, Moncton, N.B. E1C 9B6, unpublished data). We therefore estimated the proportions contributed by the eight cod regional pools to each of these winter aggregations (see **Figure** 2 and Fig 5). For example, cod from the southern Gulf of St. Lawrence (Div. 4T) contributed from as much as 66% [CI: 34-68%] to the mixed-stock aggregation in Div. 4R, to as low as 48% [33-53%] to the aggregation in Div. 3Pn (**Figure** 5). Similarly, cod from the northern Gulf of St. Lawrence (Div. 4R) contributed the most to the mixed aggregations in Divisions 4R, 3Ps and 4Vn-4Vs (~14-15%) and less so to the aggregation found in Div. 3Pn (~9%; **Figure** 5). Cod from Sydney Bight (Div. 4Vn, off Cape Breton) contributed less than 8% to any of the Winter aggregations. Cod from Div. 4Vs contributed between 13 and 16% to most aggregations, except that found in Div. 4R where their contribution dropped to 5% or less. Finally, 3Pn cod contributed to a very modest degree (<8%) to the mixed-stock aggregations in Divisions 3Pn and 3Ps, and little elsewhere. The contribution by cod from Div. 3Ps and in particular by the inshore collections of Placentia and Fortune Bay was generally low or minimal everywhere (**Figure** 5). However, it should be noted that because of the relatively small sample sizes of the Winter collections when considered individually, most of the estimates presented in **Figure** 5 have broad 95% confidence intervals, some that include zero. The broad confidence intervals limit our ability to examine the question of whether or not the contributions by the various stock components varied regionally.

Discussion

A suite of genetic analyses, including structure (F_{ST}, R_{ST}) and distance (D_A) measures reveal that there are significant differences among cod populations sampled in the Gulf of St. Lawrence and its approaches during the stock-separated periods of 1995 to 1997. The cod we examined were presumed (based on historical information and the observed spawning state of cod collected for this study) to represent distinct spawning components on or near their spawning grounds in the northern and southern regions of the Gulf of St. Lawrence, on both sides of the Cabot Strait (Laurentian Channel), and in regions to the east and southeast of Cape Breton Island. Though significant, the degree of genetic structure is relatively weak (but see below) and is due primarily to genetic differences among cod from the Gulf of St. Lawrence (Divs. 4T and 4R), from the Sydney Bight area off Cape Breton Island (Div. 4Vn), from a number of genetically distinguishable collections in south Newfoundland (Divs. 3Pn and 3Ps), and from the areas to the southeast of Cape Breton Island on the Scotian Shelf (Div. 4Vs). Thus, regarding the first question we posed in the **Introduction**, it appears that different cod populations can be identified in the Gulf region and its approaches at a spatial scale that generally corresponds to the existing management Divisions and at a temporal scale that corresponds to seasonal spawning migrations.

Our results from the independent analyses of 1996 and 1997 stock-separated data provided similar patterns of differentiation, despite the forced reduction in sample sizes. Thus, regarding the second question posed, the resolved population structure appears to be temporally stable, at least over a two- to three-year period. We acknowledge that this may not always be the case given the exception provided by analyses related to cod from the Sydney Bight area for which there is evidence that some samples may have been contaminated by transient cod.

Given the documented seasonal migration of cod from the Gulf of St. Lawrence in Autumn and early Winter to overwintering grounds in the Cabot Strait, and their subsequent spring and summer return migration (Jean 1964, Templeman 1974, 1979, Halliday and Pinhorn 1982, Taggart et al. 1995), there is a reasonable expectation that the aggregations characteristic of the region in Winter represent a mixture of the stock-separated populations identified in this study. The evidence of weak genetic heterogeneity among the cod aggregations observed during the winter mixed-stock period is consistent with this expectation. Thus, regarding our third question, the structured components we distinguished genetically are more or less separated during the spring and summer spawning periods but during the overwintering period they are mixed in the approaches to the Gulf, though there is compelling evidence that the mixtures are not homogeneous.

Out application of Millar's (1987) maximum likelihood algorithm, based on reference data from the stock-separated period, allowed us to address the fourth question - can the components of the mixtures be proportionally estimated? And has there been temporal variation in these proportions? Our results indicate that cod from the southern Gulf of St. Lawrence (Div. 4T) and possibly the northern Gulf as well (Div. 4R) dominate the composition of overwintering aggregations on both sides of the Laurentian Channel, including those cod collected to the east and southeast of Cape Breton Island in Divs. 3Ps and 4Vs. When considered overall, the contribution of the southern Gulf of St. Lawrence cod to the mixed Winter aggregations is estimated at ~51% (**Figure** 4). However, when analysed separately for each of the four management Divisions represented by our winter collections, the contribution from southern Gulf cod ranged from as high as 66% (CI: 34 - 68%) on the northern side of the Laurentian Channel in Div. 4R, to as low as 47% (CI: ~ 32 - 57 %) in both the 3Pn and 3Ps Divisions of southern Newfoundland. Cod from the northern Gulf

of St. Lawrence in region 4R contributed approximately 13% to all winter aggregations (**Figure** 5). Cod presumed to be a resident stock in the Sydney Bight region off Cape Breton (Div. 4Vn) contributed very little (~3%) to the mixed-fishery region, consistent with the resident stock hypothesis. Cod from the eastern Scotian Shelf (Division 4Vs) contributed approximately 15% to the mixed-stock aggregations.

Has there been significant temporal variation in these contributions between the years 1996 and 1997? The answer is almost certainly no. The contributions from the various reference stocks to the mixed-stock aggregations changed little between the years 1996 and 1997 (**Figure** 4) when all samples collected during each Winter in the mixed fishery region were pooled. A more detailed analysis of temporal variation in contribution to the overwintering mixtures in each management Division was, however, not possible due to the broad confidence intervals resulting from the forced reductions in sample size.

When analysed separately among the four management Divisions represented by the overwintering mixtures, the contributions from the various reference stock components varied somewhat among Divisions (**Figure** 5). However, here again, our ability to examine the question of whether or not important regional differences exist among stock components in their contribution to the overwintering aggregations is limited by the broad confidence intervals associated with the maximum likelihood estimates of contribution in this analysis.

In summary, cod from the southern Gulf of St. Lawrence in Div. 4T dominated the composition of the winter mixed aggregations in every Division sampled including those north and south of the Laurentian Channel, though, as stated earlier, these estimates may be affected by the near absence of genetic differentiation between cod from the northern and southern regions of the Gulf of St. Lawrence (see **Table** 5a). Cod from the northern Gulf (Div. 4R) and from Sydney Bight off Cape Breton (Div. 4Vn) also appeared in the winter mixed aggregations on both sides of the Laurentian Channel, although the contributions by 4Vn cod were minimal or almost nil (Figs. 4 and 5). Cod from Divisions 3Pn and 3Ps contributed very little to the mixed-stock samples.

The results that suggest a lack of strong genetic differentiation between cod from the northern and southern Gulf regions (Div. 4R and 4T, respectively) are not entirely consistent with the hypotheses of little or no mixing of these stock components during the overwintering period on either side of the Laurentian Channel in the Cabot Strait area. The apparent inconsistency between the genetic and tagging results may be a consequence of the very limited geographic coverage of the northern Gulf of St. Lawrence region. It should be pointed out, however, that a lack of differentiation between cod aggregations north and south of the Laurentian Channel was also found in a comparison of cod from a related location on the northern Scotian Shelf around Scatarie Bank (collected June 1994) and cod from Placentia Bay in southern Newfoundland (collected between February and April 1994; Ruzzante et al. 1998, reviewed in Ruzzante et al. 1999). It is possible that the samples of northern Gulf cod collected from region 4R off western Newfoundland are not a complete representation of the genetic composition of cod from the northern Gulf region and that they represent only part of that stock component (note we had no samples from Div. 4S, which represents a significant proportion of the northern Gulf; see **Figure** 1). Also, although the samples were collected off southwestern Newfoundland in region 4R where northern Gulf cod are known to spawn, they are not representative of the entire spawning cycle of northern Gulf cod (Ouellet et al. 1997). We suspect that a more representative sample of

northern Gulf cod may allow for a more comprehensive examination of genetic differentiation relative to southern Gulf cod.

The magnitude of the genetic structure detected among the stock-separated components inside and outside the Gulf St. Lawrence, though significant, was low (see **Table** 4, F_{ST} and R_{ST} estimates). A subdivided population or a group of populations among which gene flow is restricted (but not nil) will show a deficiency of heterozygotes, and this deficiency will be proportional to the magnitude of genetic subdivision. Standard estimates of population subdivision such as F_{ST}, G_{ST}, and R_{ST} in essence measure the proportional heterozygote deficiency in the total population (Chakraborti and Jin 1992). It is also known, however, that the effect of population substructuring is inversely related to the number of alleles and thus to the level of heterozygosity (Jin and Chakraborti 1995). For a given level of gene flow, measures of population structure such as F_{ST} and G_{ST} are expected to be relatively low (approximately an order of magnitude lower) for hypervariable microsatellite loci, than for blood group and protein loci (Jin and Chakraborti 1995). Fish microsatellite loci in general, and cod microsatellite in particular, are among the most variable microsatellite loci described thus far (Brooker et al. 1994, Ruzzante et al. 1996a, 1998, reviewed Ruzzante et al. 1999). The numbers of alleles per locus in our samples ranged between 21 (Gmo132) and 94 (Gmo151; **Table** 2). It is therefore not surprising that the levels of population substructuring detected among the eight separate components is low (**Table** 4) and of the same magnitude as that estimated among four population components of northern cod on the northeast Newfoundland Shelf off Newfoundland and Labrador (see **Table** 2 in Ruzzante et al. 1998). In the latter case there is considerable non-genetic evidence (i.e. tagging; Taggart 1997) consistent with the structure reported in Ruzzante et al. 1998 and elsewhere (Bentzen et al. 1996). Although departures from Hardy-Weinberg equilibrium can result from factors other than population subdivision, such as selection, inbreeding, phenotypic assortative mating, and/or the presence of null alleles (Devlin et al. 1990, Chakraborti and Jin 1992), population subdivision is thought to be the most important of these factors for microsatellite loci (Lander 1989).

We found no evidence of genetic structure between cod collected from the western and eastern sides of the southern Gulf of St. Lawrence. This is consistent with our understanding of the seasonal patterns of distribution of southern Gulf cod as a function of age (Tremblay and Sinclair 1985, Hanson and Chouinard 1992, Hanson 1996) as well as a function of population concentration (Swain and Wade 1993, Swain and Kramer 1995). Population size and range for cod in the southern Gulf of St. Lawrence are positively correlated and the region of greatest concentration shifts with changes in population size (Swain and Wade 1993) suggesting the existence of an interaction between density dependent benefits associated with food resources and density independent costs associated with temperature (Swain and Kramer 1995).

To a large extent, it can be argued that the winter fishery in the approaches to the Gulf of St. Lawrence has been historically managed (exploited) under an assumption of panmixia. The results provided above indicate that the assumption is invalid. Thus, there has been the ability to overexploit and erode genetic resources via the depletion of the constituent components that are neither panmixic nor temporally and spatially represented in a proportionally uniform manner. However, the results equally indicate that exploitation patterns can be modified and/or designed around the spatial and temporal scale of the stock structure identified here during the stock-separated period and around the spatially varying contributions to the overwintering mixed-stock fishery. We acknowledge that such a management scheme might be complex, but without it

comes the risk that the most readily exploited components will become eliminated (Larkin 1977, Iles and Sinclair 1982, Clark 1990) and it would be functionally inconsistent with the principles of resource conservation and the maintenance of biodiversity.

Acknowledgments

For their efforts in securing the cod samples, we are grateful to G. Chouinard (Institute Maurice Lamontangue), M. Hanson (Gulf Fisheries Centre), J. Brattey (NW Atlantic Fisheries Centre), S. Campana (Bedford Institute of Oceanography) and to their colleagues and support staff within the Canada Department of Fisheries and Oceans (DFO), as well as to the crews of the research trawlers *Wilfred Templeman*, *Alfred Needler* and *Teleost* and to the participants in the "Sentinel Fishery Programme". We thank A. Pickle, J. McPhail, L. Bussey, V. Bishop, and K. Spence for assistance in the laboratory. We thank S. Campana, G. Chouinard, K. Frank, and D. Swain (Gulf Fisheries Centre, DFO) for insights related to the research, and M. Hansen (Danish Institute for Fisheries Research) for comments on the manuscript. We especially thank N. Barrowman (Department of Mathematics and Statistics at Dalhousie University) for statistical advice and for programming the MLE function in Splus, and D. Hamilton and T. Moorhead of the same Department for statistical advice. Funding in support of DER, DC and research operations was provided by DFO ("high priority" research contract F5245-5-0161 to MGPL and the Oceanography Department at Dalhousie University).

References

Beacham, T. D., R. E. Withler, and A. P. Gould. 1985. Biochemical genetic stock identification of chum salmon (*Oncorhynchus keta*) in southern British Columbia. Canadian Journal of Fisheries and Aquatic Sciences **42**: 437-448.

Beacham, T. D., R. E. Withler, and T. A. Stevens. 1996. Stock identification of chinook salmon (*Oncorhynchus tsawytsha*) using minisatellite DNA variation. Canadian Journal of Fisheries and Aquatic Sciences **53**: 380-394.

Bentzen, P., C. T. Taggart , D. E. Ruzzante, and D. Cook. 1996. Microsatellite polymorphism and the population structure of cod (*Gadus morhua*) in the North West Atlantic. Canadian Journal of Fisheries and Aquatic Sciences **53**: 2706-2721.

Brooker, A.L., D. Cook, P. Bentzen, J. M. Wright, and R. W. Doyle. 1994. Organization of microsatellites differs between mammals and cold-water teleost fish. Canadian Journal of Fisheries and Aquatic Sciences **51**: 1958-1966.

Brown, B. L. J. M. Epifanio, P. E. Smouse, and C. J. Kobak. 1996. Temporal stability of mtDNA haplotype frequencies in American shad stocks: to pool or not to pool across years? Canadian Journal of Fisheries and Aquatic Sciences **53**: 2274-2283.

Campana, S. E., G. Chouinard, M. Hanson, A. Fréchet, and J. Brattey. 1998. Stock composition of cod aggregations near the mouth of the Gulf of St. Lawrence in January 1996 based on an analysis of otolith elemental fingerprints. DFO Canadian Stock Assessment Secretariat. Research Document **98/55**. 15 p.

Chakraborty, R. and L. Jin. 1992. Heterozygote deficiency, population substructure and their implications in DNA fingerprinting. Human Genetics **88**: 267-272.

Chouinard, G. A. 1994. Distribution of groundfish and herring during the 1994 Cabot Strait survey. DFO Atlantic Fisheries Research Document. 94/68. 24p.

Clark, C. W. 1990. Mathematical Bioeconomics. 2nd edition. Wiley Interscience, New York.

Devlin, B., N. Risch, and K. Roeder. 1990. No excess of homozygosity at loci used for DNA fingerprinting. Science **249**: 1416-1420.

Epifanio, J. M., P. E. Smouse, C. J. Kobak, and B. L. Brown. 1995. Mitochondrial DNA divergence among populations of American shad (*Alosa sapidissima*): how much variation is enough for mixed-stock analysis? Canadian Journal of Fisheries and Aquatic Sciences **52**: 1688-1702.

Ferguson, M.M and R.G. Danzmann. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? Canadian Journal of Fisheries and Aquatic Sciences **55**:1553-1563.

Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Bussack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Canadian Journal of Fisheries and Aquatic Sciences **41**: 400-408.

Galvin, P., S. McKinnell, J. B. Taggart, A. Ferguson, M. O'Farrell, and T. F. Cross. 1995. Genetic stock identification of Atlantic salmon (*Salmo salar* L.) using single locus minisatellite DNA profiles. Journal of Fish Biology **47**(Suppl. A): 667-676.

Goodman, S. J. 1997. R_{ST} CALC: A collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Molecular Ecology **6:** 881-885.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. 1980. Use of biochemical genetic variants for identification of sockeye salmon (*Oncorhynchus nerka*) stocks in Cook Inlet. Canadian Journal of Fisheries and Aquatic Sciences **37**: 1236-1247.

Halliday, R. G., and A. T. Pinhorn. 1982. The groundfish resource in the Gulf of St. Lawrence. Canadian Technical Report Fisheries and Aquatic Sciences **1086**: 16 p.

Hanson, J. M. 1996. Seasonal distribution of juvenile Atlantic cod in the southern Gulf of St. Lawrence. Journal of Fish Biology **49**: 1138-1152.

Hanson, J. M. and G. A. Chouinard. 1992. Distribution and feeding of juvenile cod (*Gadus morhua*) in the principal nursery area of the southern Gulf of St. Lawrence. In: de Lafontaine, Y., T. Lambert, G. R. Lilly, W. D. McKone, and R. J. Miller (eds.). Juvenile stages: the missing link in fisheries research. Canadian Technical Report of Fisheries and Aquatic Sciences **1890**: 93-103.

Hedrick, P. W. and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications **2**: 30-46.

Herbinger, C.M., R.W. Doyle, E. R. Pitman, D. Paquet, K. A. Mesa, D. B. Morris, J. M. Wright, and D. Cook. 1995. DNA fingerprint based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. Aquaculture **137**: 245-256.

Iles, T.D., and M. Sinclair. 1982. Atlantic herring: stock discreteness and abundance. Science, **215**: 627-633.

Jean, Y. 1964. Seasonal distribution of cod (*Gadus morhua* L.) along the Canadian Atlantic coast in relation to water temperature. Journal of the Fisheries Research Board of Canada **21**: 429-460.

Jin, L., and Chakraborty. 1995. Population structure, stepwise mutations, heterozygote deficiency and their implications in DNA forensics. Heredity **74**:274-285.

Lander, E. S. 1989. DNA fingerprinting on trial. Nature 339: 501-505.

Larkin, P. A. 1977. An epitaph for the concept of maximum sustainable yield. Transactions of the American Fisheries Society **106:** 3-11.

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

Martin, W. R. and Y. Jean. 1964. Winter cod tagging off Cape Breton and on offshore Nova Scotia Banks, 1959-1962. Journal of the Fisheries Research Board of Canada **21**: 215-238.

MathSoft, Inc. 1996. S-PLUS Version 3.4 Release 1 for Sun SPARC, SunOS 5.3. Statistical Sciences Inc, Seattle.

McConnell, S.; L. Hamilton; D. Morris; D. Cook; D. Paquet; P. Bentzen; and J. Wright. 1995. Isolation of salmonid microsatellite loci and their application to the population genetics of Canadian east coast stocks of Atlantic salmon. Aquaculture **137**:19-30.

Michalakis, Y., and L. Excoffier. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics **142**:1061-1064.

Millar, R. B. 1987. Maximum likelihood estimation of mixed fishery composition. Canadian Journal of Fisheries and Aquatic Sciences **44**: 583-590.

Millar, R. B. 1990. Comparison of methods for estimating mixed stock fishery composition. Canadian Journal of Fisheries and Aquatic Sciences **47**: 2235-2241.

Miller, K. M., R. E. Withler, and T. D. Beacham. 1996. Stock identification of coho salmon (*Oncorhynchus kisutch*) using minisatellite DNA variation. Canadian Journal of Fisheries and Aquatic Sciences **53**: 181-195.

Miller , S.A., D.D. Dykes and H.F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research **16**: 1215.

Milner, G. B., D. J. Teel, F. M. Utter, and C. L. Burley. 1981. Columbia river stock identification study: validation of genetic method. Annual report of research (FY80). NWAFC, NOAA, Seattle, WA 98112.

Myers, R. A., J. A. Hutchings, and N. J. Barrowman. 1996. Hypotheses for the decline of cod in the North Atlantic. Marine Ecology Progress Series **138**: 293-308.

Myers, R. A., J. A. Hutchings, and N. J. Barrowman. 1997. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecological Applications **7**: 91-106.

Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution **19**:153-170.

Ouellet, P., Y. Lambert, and M. Castonguay. 1997. Spawning of Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence: a study of adult and egg distributions and characteristics. Canadian Journal of Fisheries and Aquatic Sciences **54**: 198-210.

Pella, J. J. and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. P. 247-276 *In:* N. Ryman and F. M. Utter [eds.] Population genetics and applications to fisheries management. University of Washington Press, Seattle, WA.

Policansky, D. and J. J. Magnuson. 1998. Genetics, metapopulations, and ecosystem management of fisheries. Ecological Applications **8(1) Supplement**: S119-S123.

Rice, J. R. [ed.], 1997. Proceedings of the workshop on cod stock components, March 3-5, 1997 St. John's Newfoundland. Canadian Stock Assessment Proceedings. Series. **97/06**.

Rice, W. R., 1989. Analyzing tables of statistical tests. Evolution **43**: 223-225.

Ruzzante, D. E. 1998. A comparison of several measures of genetic distances and population structure with microsatellite data: bias and sampling variance. Canadian Journal of Fisheries and Aquatic Sciences **55**:1-14.

Ruzzante, D. E., C. T. Taggart, and D. Cook 1996a. Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. Canadian Journal of Fisheries and Aquatic Sciences **53**: 2695-2705.

Ruzzante, D. E., C. T. Taggart, and D. Cook. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in NW Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. Molecular Ecology **7**: 1663-1680.

Ruzzante, D. E., C. T. Taggart, and D. Cook. 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the Northwest Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fisheries Research, accepted manuscript.

Ruzzante, D. E., C. T. Taggart, D. Cook, and S. V. Goddard. 1996b. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level. Canadian Journal of Fisheries and Aquatic Sciences **53**: 634-645.

Ruzzante, D. E., C. T. Taggart, D. Cook, and S. V. Goddard. 1997. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: a test and evidence of temporal stability. Canadian Journal of Fisheries and Aquatic Sciences **54**: 2700-2708.

Ryman, N., F. Utter, and L. Laikre. 1995. Protection of intraspecific biodiversity of exploited fishes. Reviews in Fish Biology and Fisheries **5**: 417-446.

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

Smouse, P. E., R. S. Waples, and J. A. Tworek. 1990. A genetic mixture analysis for use with incomplete source population data. Canadian Journal of Fisheries and Aquatic Sciences **47**: 620-634.

Swain, D. P. and D. L. Kramer. 1995. Annual variation in temperature selection by Atlantic cod *Gadus morhua* in the southern Gulf of St. Lawrence, Canada, and its relation to population size. Marine Ecology Progress Series **116**: 11-23.

Swain, D. P. and E. J. Wade. 1993. Density-dependent geographic distribution of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences **50**: 725-733.

Taggart, C. T. 1997. Bank-scale migration patterns in northern cod. North Atlantic Fisheries Organization Scientific Council Studies **29**: 51-60.

Taggart, C. T., J. Anderson, C. Bishop, E. Colbourne, J. Hutchings, G. Lillly, J. Morgan, E. Murphy, R. Myers, G. Rose, and P. Shelton. 1994. Overview of cod stocks, biology, and environment in the Northwest Atlantic region of Newfoundland, with emphasis on northern cod. International Council for the Exploration of the Seas (ICES) Marine Science Symposia, **198**: 140-157.

Taggart, C. T., P. Penney, N. Barrowman, and C. George. 1995. The 1954-1993 Newfoundland cod-tagging database: statistical summaries and spatial-temporal distributions. Canadian Technical Report Fisheries and Aquatic Sciences, **2042**. 441 p.

Taggart, C. T., D. E. Ruzzante, and D. Cook. 1998. Localised stocks of cod (*Gadus morhua)* in the Northwest Atlantic: the genetic evidence and otherwise. In: *The implications of localized fishery stocks* (eds. Hunt von Herbing I, Kornfield I, Tupper M, Wilson J), pp. 65-90. NRAES (Natural Resource, Agriculture, and Engineering Service) Ithaca, New York, 200p.

Takezaki, N., and M. Nei. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics **144**: 389-399.

Templeman, W. 1974. Migrations and intermingling of Atlantic cod (*Gadus morhua*) stocks of the Newfoundland area. Journal of the Fisheries Research Board of Canada **31**:1073-1092.

Templeman, W. 1979. Migration and intermingling of stocks of Atlantic cod, *Gadus morhua*, of the Newfoundland and adjacent areas from tagging in 1962-66. International Council for North Atlantic Fisheries (ICNAF) Research Bulletin **14**. 50p.

Tremblay, M. J. and M Sinclair. 1985. Gulf of St. Lawrence cod: age-specific geographic distributions and environmental occurrences from 1971 to 1981. Canadian Technical Report of Fisheries and Aquatic Sciences **1387**, 43 p.

Utter, F. and N. Ryman. 1993. Genetic markers and mixed stock fisheries. Fisheries (Bethesda) **18**: 11-21.

Waples, R. S. 1990. Temporal changes in allele frequency in Pacific salmon: implications for mixed-stock fishery analysis. Canadian Journal of Fisheries and Aquatic Sciences **47**: 968-976.

Waples, R. S. and D. J. Teel. 1990. Conservation genetics of Pacific Salmon. I. Temporal changes in allele frequency. Conservation Biology **4**: 144-156.

Ward, R. D., M. Woodwark, and D. O. F. Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. Journal of Fish Biology **44**: 213-232.

Weir, B. S. 1996. Genetic data analysis II. Sinauer Associates, Inc., Sunderland, MA

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

Wirgin, I., B. Jessop, S. Courtenay, M. Pedersen, S. Maceda, and J. R. Waldman. 1995. Mixed-stock analysis of striped bass in two rivers of the Bay of Fundy as revealed by mitochondrial DNA. Canadian Journal of Fisheries and Aquatic Sciences **52**: 961-970.

Wirgin, I. I., J. R. Waldman, L. Maceda, J. Stabile, and V. J. Vecchio. 1997. Mixed stock analysis of Atlantic coast striped bass (*Morone saxatilis*) using nuclear DNA and mitochondrial DNA markers. Canadian Journal of Fisheries and Aquatic Sciences **54**: 2814-2826.

Wood, C. C., S. McKinnell, T. J. Mulligan, and D. A. Fournier. 1987. Stock identification with the Maximum Likelihood mixture model: sensitivity analysis and application to complex problems. Canadian Journal of Fisheries and Aquatic Sciences **44**: 866-881.

Wright, J. M. 1993. DNA fingerprinting of fishes. In: *Biochemistry and molecular biology of fishes. Vol. 2* (ed. Hochachka PW, Mommsen T), pp. 57-91. Elsevier Science Publishers B. V. Amsterdam, The Netherlands.

Wright, S. 1951. The genetical structure of populations. Annals Eugenics **15**:323-354.

Xu, S., C. J. Kobak, and P. E. Smouse. 1994. Constrained least squares estimation of mixed population stock composition from mtDNA haplotype frequency data. Canadian Journal of Fisheries and Aquatic Sciences **51**: 417-425.

Table 1. Summary statistics for cod samples collected during the stock-separated period. Fishing sets (column 5) are pooled into geographically related sub-groups (column 4) collected on or near the same date (columns 3). Sub-groups are pooled within collection trips to local groups (column 2). Geographically related local groups collected in different years are pooled within management Divisions to regional groups (column 1).

Regional group and management Division	Local group	Sample date	Sub-Group & N	Fishing set	Average latitude (degree)	Average longitude (degree)	Depth range (m)	Median length (cm)	Length range (cm)	Median age (yr.)	Age range (yr.)	% immature, pre-, post-, spawning
(1a) Southern Gulf 4T West, N=242	**(1)** J095 (N=60)	01-02 Jul 95	**(1)** 30	23-27	47.09	-64.20	032-042	46	41-48	07	05-08	00, 27, 30, 43
		02-05 Jul 95	**(2)** 30	28-33	47.21	-64.44	032-044	44	40-50	06	05-09	00, 67, 03, 30
	(2) 4TVn-WR(N=100)	13 Jun 96	**(3)** 52	1	47.57	-64.20	064	50	36-70	07	05-09	00, 42, 08, 50
		13 Jun 96	**(4)** 48	2	47.65	-64.17	068	49	40-72	08	05-13	00, 44, 00, 56
	(3) TR44 (N=32)	04 Jul 96	**(5)** 32	44	48.92	-64.18	139	46	42-53	-	-	-
	(4) MB06 (N=50)	13 Jun 97	**(6)** 26	1	47.35	-64.27	054	51	48-63	08	06-12	00, 12, 69, 19
		13 Jun 97	**(7)** 24	3	47.37	-64.28	055	52	48-55	07	04-10	04, 08, 54, 33
(1b) Southern Gulf 4T East, N=149	**(5)** 4TVn -NP (N=100)	15 Jun 96	**(8)** 33	1	46.57	-61.33	095	48	41-72	07	04-10	00, 30, 09, 61
		15 Jun 96	**(9)** 33	2	46.59	-61.35	090	52	43-67	07	04-09	00, 33, 00, 66
		15 Jun 96	**(10)** 34	3	46.68	-61.26	102	55	43-84	07	05-12	00, 35, 03, 62
	(6) NP06 (N=49)	11 Jun 97	**(11)** 18	1	46.58	-61.17	080	54	48-57	08	05-09	00, 32, 11, 58
		11 Jun 97	**(12)** 31	2	46.67	-61.27	104	52	48-56	07	04-10	00, 10, 16, 74
(2) Northern Gulf 4R, N=148	**(7)** N242 (N=100)	30 Apr 96	**(13)** 50	1	47.94	-59.55	195	62	43-83	08	05-15	00, 56, 08, 36
		30 Apr 96	**(14)** 50	2	47.98	-59.52	175	57	38-84	08	05-12	08, 38, 20, 34
	(8) N9705 (N=48)	30 Apr 97	**(15)** 30	1	47.90	-59.53	104	47	43-53	06	04-09	00, 50, 13, 37
		30 Apr 97	**(16)** 18	2	48.00	-59.52	108	51	47-53	07	05-08	00, 67, 06, 28
(3) Sydney Bight 4Vn, N=196	**(9)** N242 (N=100)	01 May 96	**(17)** 50	3	47.02	-60.10	187	48	35-84	07	04-10	06, 60, 30, 04
		01 May 96	**(18)** 50	4	46.94	-60.12	125	52	42-89	08	05-12	00, 76, 18, 06
	(10) N9705 (N=48)	01 May 97	**(19)** 48	3	46.47	-59.52	055	49	38-67	05	04-10	00, 42, 19, 40
	(11) KG97 (N=48)	18 May 97	**(20)** 48	2	46.57	-60.20	114	53	40-81	-	-	02, 06, 13, 79
(4) Eastern Scotian Shelf 4Vs, N=243	**(12)** N242 (N=99)	01 May 96	**(21)** 49	5	44.29	-59.01	142	50	42-70	06	03-09	00, 32, 68, 00
		01 May 96	**(22)** 50	6	44.25	-59.03	142	54	48-72	07	04-09	00, 34, 64, 02
	(13) N9705 (N=48)	01 May 97	**(23)** 48	5-6	45.08	-57.75	063-070	41	38-62	04	03-07	04, 38, 54, 04
	(14) N9705 (N=48)	01 May 97	**(24)** 48	10-11	44.15	-58.95	055-078	42	40-53	04	03-08	00, 46, 29, 25
	(15) N222 (N=48)	21-26 Jul 94	**(25)** 48	30-32,84-87,89	44.31	-59.02	062-223	39	23-77	04	02-08	30, 26, 45, 00
(5) South Nfld 3Pn, N=107	**(16)** WT186 (N=61)	13-14 Apr 96	**(26)** 61	32-35	47.25	-58.43	-	49	35-88	06	03-12	-
	(17) WT202 (N=46)	05-06 Apr 97	**(27)** 46	41,42,44,46-49	47.46	-58.93	209-449	47	31-78	06	04-09	-
(6) South Nfld offshore 3Ps, N=194	**(18)** WT187 (N=87)	24 Apr 96	**(28)** 37	48	45.32	-55.42	-	65	50-89	07	06-09	-
		29 Apr 96	**(29)** 25	58	45.04	-55.42	-	60	36-76	06	03-09	-
		29 Apr 96	**(30)** 25	63	45.05	-54.95	-	63	48-74	07	04-08	-
(7) Placentia Bay, 3Ps, N=61	**(19)** SentPBay (N=61)	26 Jun 97	**(31)** 61	1	47.23	-55.02	19	57	45-74	-	-	-
(8) Fortune Bay, 3Ps, N=46	**(20)** SentFBay (N=46)	19 Jun 97	**(32)** 46	1	47.38	-55.73	71	66	36-105			14, 41, 38, 08

Table 2. Summary statistics for cod samples collected during the mixed-stock period. Fishing sets (column 5) are pooled into geographically related groups (column 4) collected on or near the same date (columns 3). Groups are pooled within collection trips by year and management Divisions (columns 1 and 2).

NAFO Division	Collection trip and (N)	Sample date	Group	Fishing set	N	Average latitude (degree)	Average longitude (degree)	Depth range (m)	Median length (cm)	Length range (cm)	Median age (yr.)	Age range (yr.)	% immature, pre-, post-, spawning
(1) 3Pn	N214 (N=49)	Jan 95	1	42	49	47.42	-59.45	445	44	34-55	06	05-07	00,18,55,27
	WT182 (N=96)	Jan 96	2	34	06	47.55	-59.47						
			3	36	30	47.40	-59.3						
			4	48	45	47.33	-59.22						
			5	49	15	47.37	-59.13	421-440	47	42-58	06	05-10	00,18,79,03
	T201 (N=144)	Jan 97	6	36	48	47.57	-59.50						
			7	16	09	47.53	-58.17						
			8	22	39	47.32	-58.43						
			9	12	32	47.40	-59.30						
			10	24	04	47.33	-58.97						
			11	26	12	47.33	-59.13	186-429	40	35-59	05	03-09	25,14,53,08
(2) 4Vn	N214 (N=49)	Jan 95	12	108	49	46.45	-59.20	224	43	38-58	06	05-07	06,20,55,18
	WT182 (N=96)	Jan 96	13	08	09	46.92	-59.87						
			14	39	50	47.33	-60.00						
			15	42	01	47.13	-60.57						
			16	43	36	47.13	-60.17	154-434	48	41-72	07	05-11	00,06,71,23
(3) 4Vn south	WT182 (N=72)	Jan 96	17	72	18	46.33	-59.08						
			18	74	01	46.52	-59.22						
			19	75	50	46.53	-59.35						
			20	76	03	47.73	-59.22	190-429	41	35-57	05	03-09	25, 11,64, 00
(4) 4Vn-4Vs	N255 (N=115)	Mar 97	21	35	19	45.77	-58.05						
			22	36	48	45.77	-58.00						
			23	37	48	45.87	-58.07	181-202	48	34-62	07	02-12	-
	T201 (N=130)	Jan 97	24	40	31	47.33	-60.25						
			25	56	20	47.13	-60.30						
			26	57	45	47.13	-60.13						
			27	66	02	46.75	-59.23						
			28	68	32	46.53	-59.38	191-434	41	34-63	05	03-11	10,25,61,05
(5) 3Ps	WT182 (N=96)	Jan 96	29	118	07	46.55	-57.77						
			30	122	01	46.57	-58.15						
			31	130	45	46.73	-57.87						
			32	132	13	46.73	-57.63						
			33	134	02	46.93	-57.90						
			34	136	28	46.93	-58.17	114-464	49	43-75	06	04-10	00,17,76,03
	T201 (N=96)	Jan 97	35	80	29	46.75	-57.90						
			36	97	35	46.75	-57.75						
			37	98	32	46.72	-57.73	305-430	45	37-50	05	03-09	42,31,27,00
(6) 4Vs	WT182 (N=47)	Jan 96	38	92-94	26	45.53	-57.90						
			39	98	14	45.33	-57.90						
			40	100	03	45.33	-57.35						
			41	102	04	45.33	-57.62	235-461	46	37-67	05	04-10	00,13,74,13
(7) 4R	T201 (N=95)	Jan 97	42	08	14	48.13	-60.02						
			43	49	34	47.95	-60.00						
			44	52	48	47.75	-59.78	474-519	46	38-61	06	04-10	00,12,62,21

Table 3. Single locus statistics for all cod samples collected during the stock-separated and mixed-stock periods in the Gulf of St. Lawrence and approaches providing: N(ind.), the number of individuals sampled per locus; n(alleles), the number of alleles per locus; range (bp), allele size range in basepairs; Het_{obs} and Het_{exp}, the observed and expected heterozygosities; and D [(Het_{obs} - Het_{exp}) / Het_{exp}.], heterozygote deficiency.

Locus	N(ind.)	n(alleles)	range (bp)	Het_{obs}	Het_{exp}	D
Gmo2	2333	26	97-148	0.793	0.802	-0.012
Gmo4	2328	68	111-295	0.974	0.956	0.019
Gmo120	2282	53	110-288	0.982	0.951	0.033
Gmo132	2258	21	101-155	0.729	0.711	0.023
Gmo145	2242	65	135-227	0.971	0.946	0.026
Gmo151	2290	94	87-216	0.913	0.934	-0.023

Table 4. Single locus and overall estimates of F_{ST} and R_{ST} among the 8 regional groups of stock-separated cod samples from the Gulf of St. Lawrence and approaches: (1) southern Gulf of St. Lawrence (4T), N=392; (2) northern Gulf of St. Lawrence (4R), N=148; (3) Sydney Bight (4Vn), N=196; (4) eastern Scotian Shelf (4Vs), N=243; (5) south Newfoundland (3Pn), N=108; (6) south Newfoundland (3Ps) offshore, N=87; (7) south Newfoundland (3Ps) Placentia Bay, N=61; and (8) south Newfoundland (3Ps) Fortune Bay, N=46. See Table 1 for sample details.

	Locus						
Measure	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Gmo151	Overall
F_{ST}	0.0005	0.0007*	**0.0012**	**0.0044**	**0.0013**	**0.0024**	**0.0017**
R_{ST}	-0.0031	-0.0029	**0.0125**	**0.0414**	**0.0304**	**0.0144**	**0.0142**

α=0.05/6=0.0083; **Bold: P<0.001;** *: P<0.05

Table 5. Pairwise estimates of genetic distance D_A based on polymorphism at six microsatellite DNA loci among cod representing the 8 regional groups of stock-separated samples from the Gulf of St. Lawrence and approaches: (a) data from 1994, 1995, 1996 and 1997 combined; (b) data from 1996 only; and (c) data from 1997 only. Estimates within [square brackets] exclude local-groups 9 and 10 (see Table 1) from the Sydney Bight (4Vn) reference samples that were presumed to contain transient cod of 4T origin collected in early May 1996 and 1997.

5a. D_A estimates for the combined (1994 to 1997) data.

	Southern Gulf (4T)	Northern Gulf (4R)	Sydney Bight (4Vn)	Eastern Scotian Shelf (4Vs)	South Nfld offshore (3Pn)	South Nfld offshore (3Ps)	South Nfld Placentia Bay (3Ps)	South Nfld Fortune Bay (3Ps)
Southern Gulf (4T) N=392	-	0.037 [0.037]#	0.030 **[0.097]**	**0.037**	**0.054**	**0.061**	**0.092**	**0.091**
Northern Gulf (4R) N=148		-	0.043 **[0.108]**	0.046**	**0.070**	0.061	**0.106**	**0.107**
Sydney Bight (4Vn) N=196 [N=96]			-	**0.045** **[0.104]**	**0.061** **[0.111]**	0.063* [0.115]**	**0.092** **[0.130]**	0.090# **[0.142]**
Eastern Scotian Shelf (4Vs) N=243				-	**0.069**	**0.071**	**0.094**	**0.104**
South Nfld offshore (3Pn) N=108					-	**0.085**	**0.105**	**0.113**
South Nfld offshore (3Ps) N=87						-	**0.111**	0.112*
South Nfld (3Ps) Placentia Bay N=61							-	0.125*
South Nfld (3Ps) Fortune Bay N=46								-

Initial K(number of pairwise comparisons)=28; $\alpha = 0.05/28 \cong 0.002$; **Bold: P<0.002;** **: P<0.01; *: P<0.05; #: P<0.10

5b. D_A estimates for the 1996 data only.

	Southern Gulf (4T)	Northern Gulf (4R)	Sydney Bight (4Vn)	Eastern Scotian Shelf (4Vs)	South Nfld offshore (3Pn)	South Nfld offshore (3Ps)
Southern Gulf (4T) N=232	-	0.051#	0.050	**0.063**	**0.076**	**0.068**
Northern Gulf (4R) N=100		-	0.061	**0.078**	**0.092**	0.075*
Sydney Bight (4Vn) N=100			-	**0.081**	0.085#	0.076*
Eastern Scotian Shelf (4Vs) N=99				-	**0.105**	**0.099**
South Nfld offshore (3Pn) N=61					-	0.090*
South Nfld offshore (3Ps) N=87						-

$\alpha = 0.05/15 = 0.003$; **Bold: P<0.003**; **: P<0.01; *: P<0.05; #: P<0.10

5c. D_A estimates for the 1997 data only.

	Southern Gulf (4T)	Northern Gulf (4R)	Sydney Bight 1 May 97 (4Vn)	Sydney Bight 18 May 97 (4Vn)	Eastern Scotian Shelf (4Vs)	South Nfld (3Pn)	South Nfld Placentia Bay (3Ps)	South Nfld Fortune Bay (3Ps)
Southern Gulf (4T) N=100	-	0.090	0.104*	**0.106**	0.071*	**0.135**	**0.121**	**0.125**
Northern Gulf (4R) N=48		-	0.104	0.128*	0.072	**0.146**	**0.130**	**0.146**
Sydney Bight (4Vn) N=48, 01 May 97			-	0.116	0.085	**0.143**	0.125**	0.139**
Sydney Bight (4Vn) N=48, 18 May 97					0.106*	0.122	**0.130**	**0.142**
Eastern Scotian Shelf (4Vs) N=96					-	**0.122**	0.091**	0.109**
South Nfld (3Pn) N=46						-	**0.138**	**0.152**
South Nfld (3Ps) Placentia Bay N=61							-	0.125*
South Nfld. (3Ps) N=46								-

$\alpha = 0.05/21 \cong 0.002$; **Bold: P<0.004;** **: P<0.01; *: P<0.05; #: P<0.10

Table 6. Single locus and overall estimates of F_{ST} and R_{ST} among 7 management Division groups of mixed-stock cod samples (1995, 1996 and 1997 combined) from the approaches to the Gulf of St. Lawrence: (1) 3Pn, N=289; (2) 4Vn north, N=192; (3) 4Vn south, N=155; (4) 4Vn-Vs, N=115; (5) 3Ps, N=192; (6) 4Vs, N=47; (7) 4R, N=96. Total N=1086. See Table 2 for sample details.

	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Gmo151	Overall
F_{ST}	**0.0034**	0.0008	0.0008	0.0012	0.0006	0.0005	**0.0011**
R_{ST}	-0.0001	0.0021	-0.0010	-0.0002	0.0009	-0.0001	0.0002

α = 0.05/6=0.0083. **Bold:P<0.0083**

Table 7. Pairwise estimates of genetic distance D_A (Nei et al. 1983) based on polymorphism at six microsatellite DNA loci among cod representing 7 management Division groups of mixed-stock samples from the approaches to the Gulf of St. Lawrence: (a) data from 1995, 1996 and 1997 combined. Total N: 1086. See Table 2 for sample details.

management Division groups	1. 3Pn (N=289)	2. 4Vn north (N=192)	3. 4Vn south (N=155)	4. 4Vn-Vs (N=115)	5. 3Ps (N=192)	6. 4Vs (N=47)	7. 4R (N=96)
1. 3Pn (N=289)	-	0.033	0.037	0.048*	0.034*	0.085*	0.048
2. 4Vn north (N=192)		-	0.041	0.055	0.035	0.088	0.051
3. 4Vn south (N=155)			-	0.057**	0.046	0.101	0.053
4. 4Vn-Vs (N=115)				-	0.050*	0.088	0.068**
5. 3Ps (N=192)					-	0.082	0.042
6. 4Vs (N=47)						-	0.110
7. 4R (N=96)							-

Sequential Bonferroni correction initial K=21; α=0.05/21=0.002. **Bold: P<0.002**; **: P<0.01; *: P<0.05)

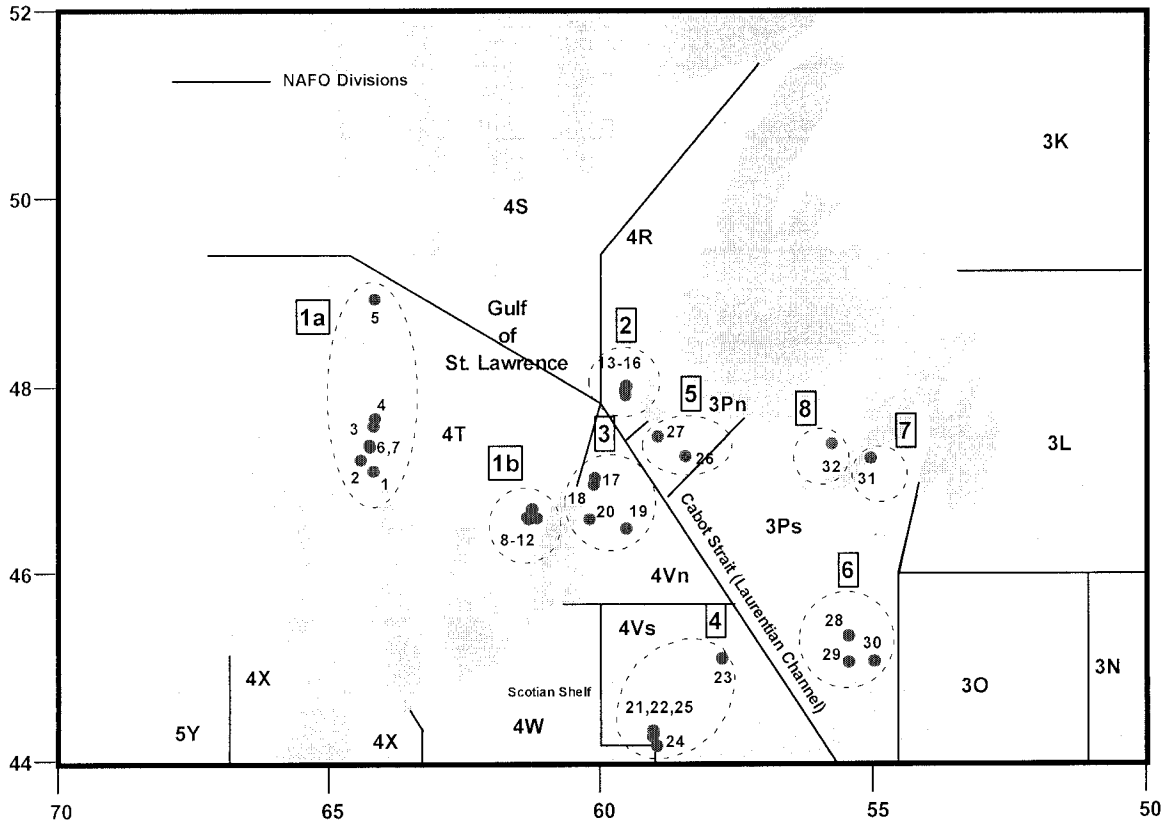

Figure 1. Chart of the North Atlantic Fisheries Organization (NAFO) statistical management Divisions showing locations of cod sample collections during the stock-separated period. Regional groups (1-8) are numbered according to local groups (1-32) as listed in Table 1.

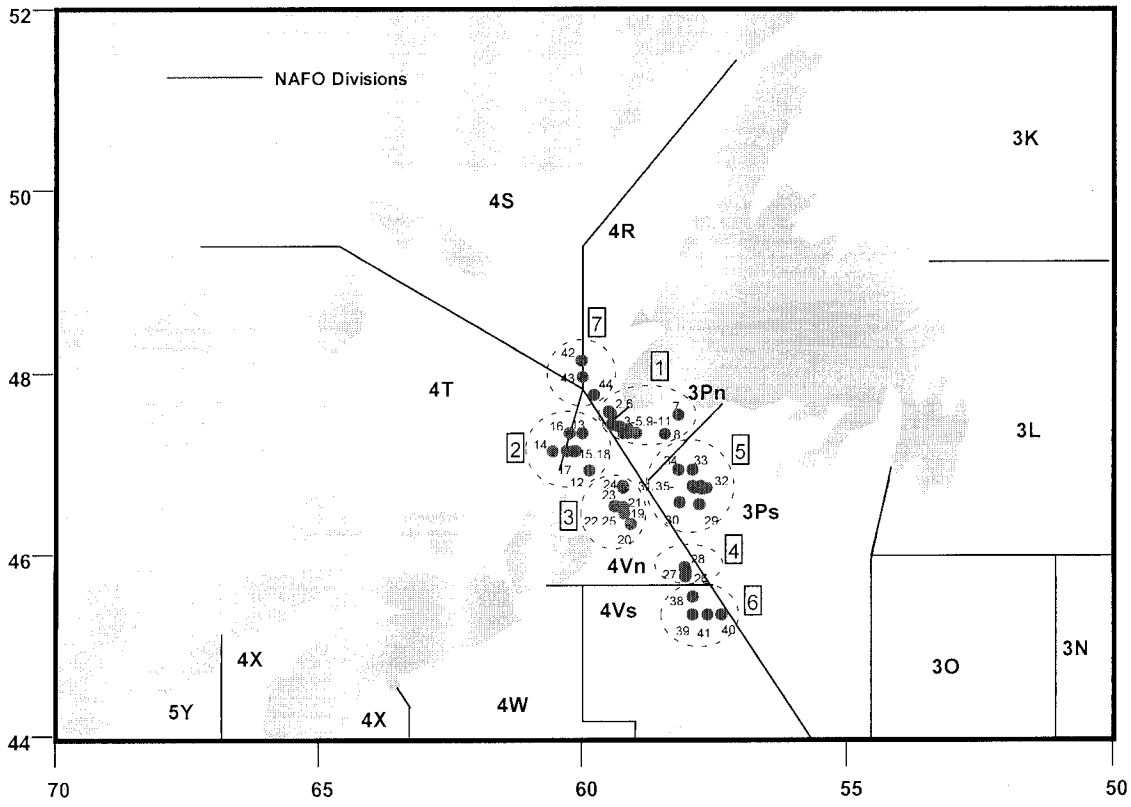

Figure 2. Chart of the North Atlantic Fisheries Organization (NAFO) statistical management Divisions showing locations of cod sample collections during the mixed-stock period. Numbered groups (1-44) are pooled by management Division groups (1-7) as in Table 2.

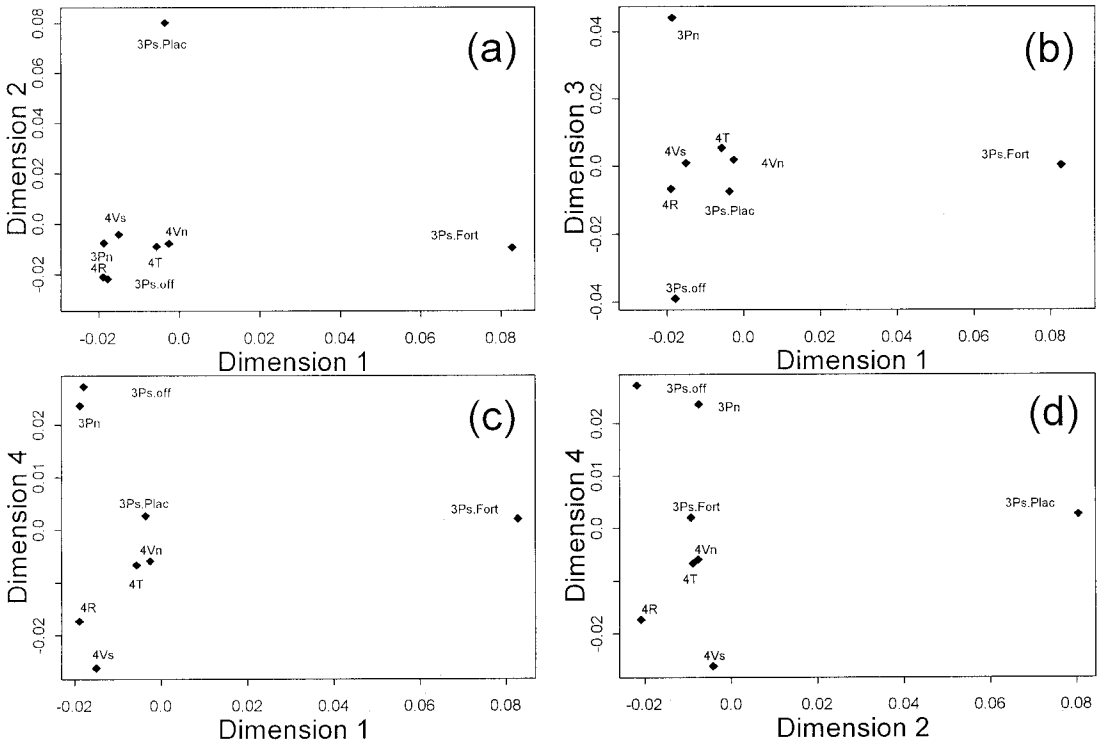

Figure 3. Scattergram of a multidimensional scaling analysis applied to the D_A matrix of genetic distances among 8 regional groups of cod samples collected during the stock-separated period. (a) Dimension 1 vs. 2; (b) dimension 1 vs. 3; (c) dimension 1 vs. 4; (d) dimension 2 vs. 4.

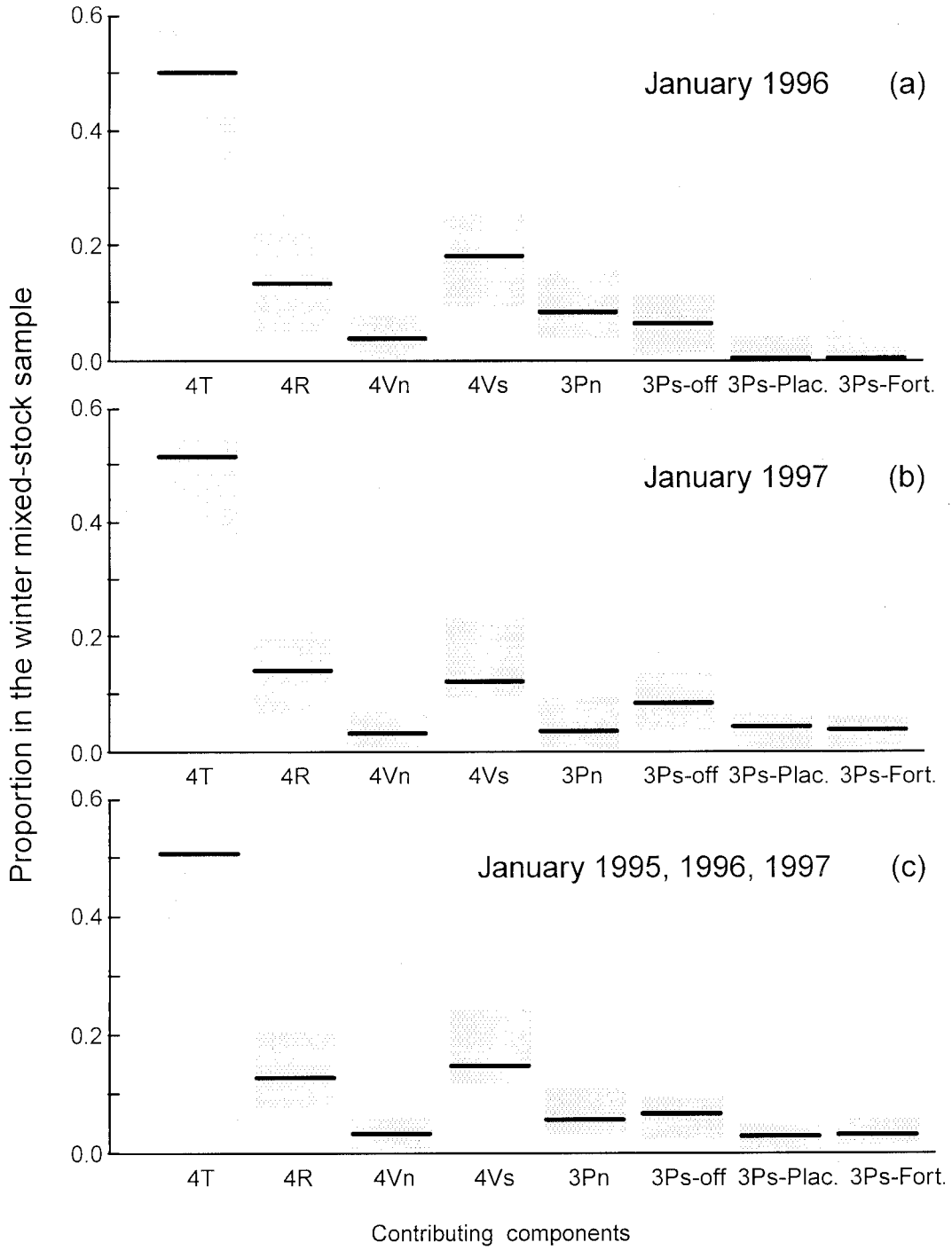

Figure 4. Expected maximum likelihood estimates (heavy horizontal lines) and 95% bootstrap confidence intervals (shaded regions) of contributions (proportions) by cod from eight regional groups to all (pooled) mixed-stock (Winter) samples. Estimates reflect the proportions of cod from each regional group that can explain the allele frequency distribution found in the mixed-stock samples and are based on the genetic composition of reference samples collected during the stock-separated periods between April and July of 1994 to 1997. Estimates are provided for the mixed-stock samples of: **(a)** January 1996; **(b)** January 1997; and **(c)** January 1995, 1996, and 1997 combined. The results indicate that there was no significant temporal change (1996 to 1997) in each regional group's contribution to the mixed-stock samples.

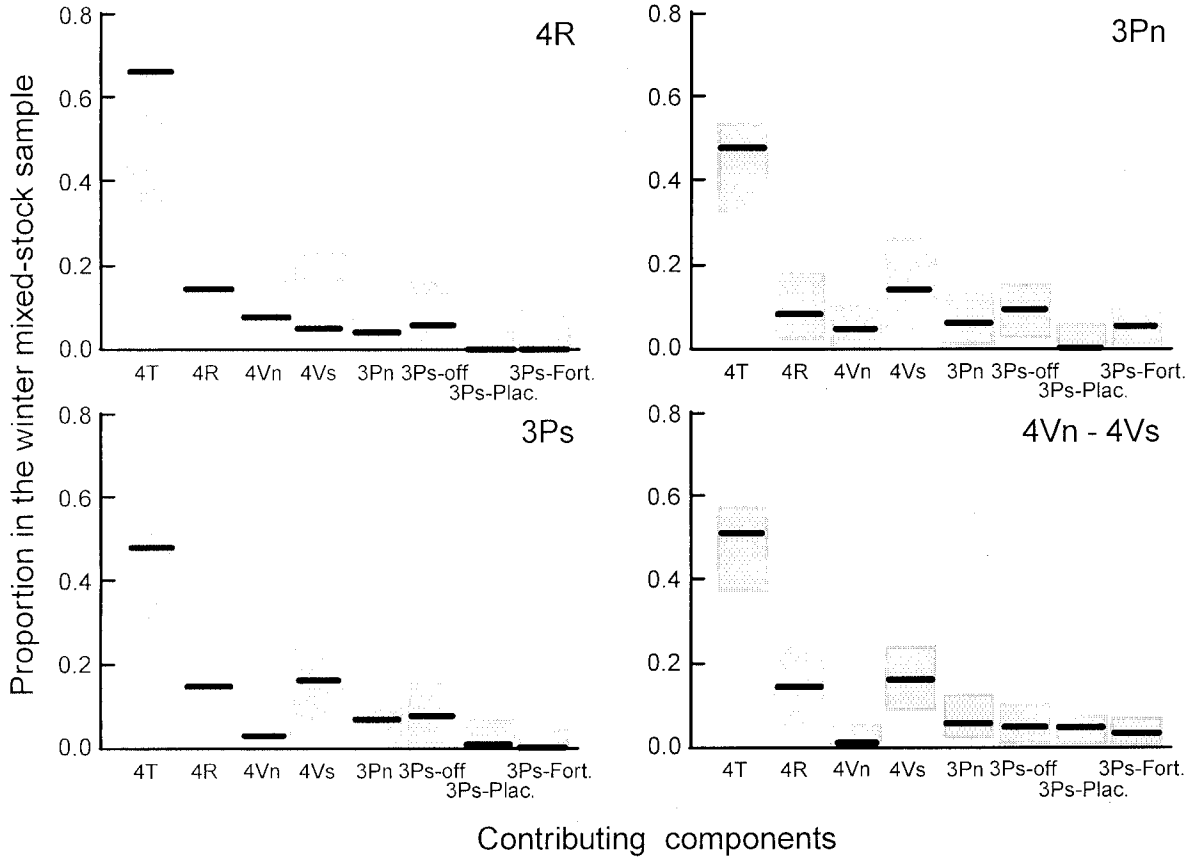

Figure 5. Expected maximum likelihood estimates (heavy horizontal lines) and 95% bootstrap confidence intervals (shaded regions) of contributions (proportions) by cod from eight regional groups to all (pooled Winter 1996 and 1997) samples collected in each of four management Divisions (4R, 3Pn, 3Ps, 4Vn and 4Vs pooled). Estimates reflect the proportions of cod from each regional group that can explain the allele frequency distribution found in the mixed-stock samples and are based on the genetic composition of reference samples collected during the stock-separated periods between April and July of 1994 to 1997.