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MIXED-STOCK ANALYSIS OF ATLANTIC COD NEAR THE GULF OF ST. LAWRENCE BASED ON MICROSATELLITE DNA

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Abstract. The collapse of various stock complexes of cod (*Gadus morhua*) in the northwest Atlantic has 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 whether: (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) component contributions to mixtures can be estimated. We use polymorphism at six 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. Multidimensional scaling analysis of 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 are 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 management 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.

Key words: cod; F_{ST}; Gadus morhua*; genetic distance; genetic structure; Gulf of St. Lawrence; maximum likelihood estimation; microsatellite; mixed-stock analysis.*

INTRODUCTION

Management 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. 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 con-

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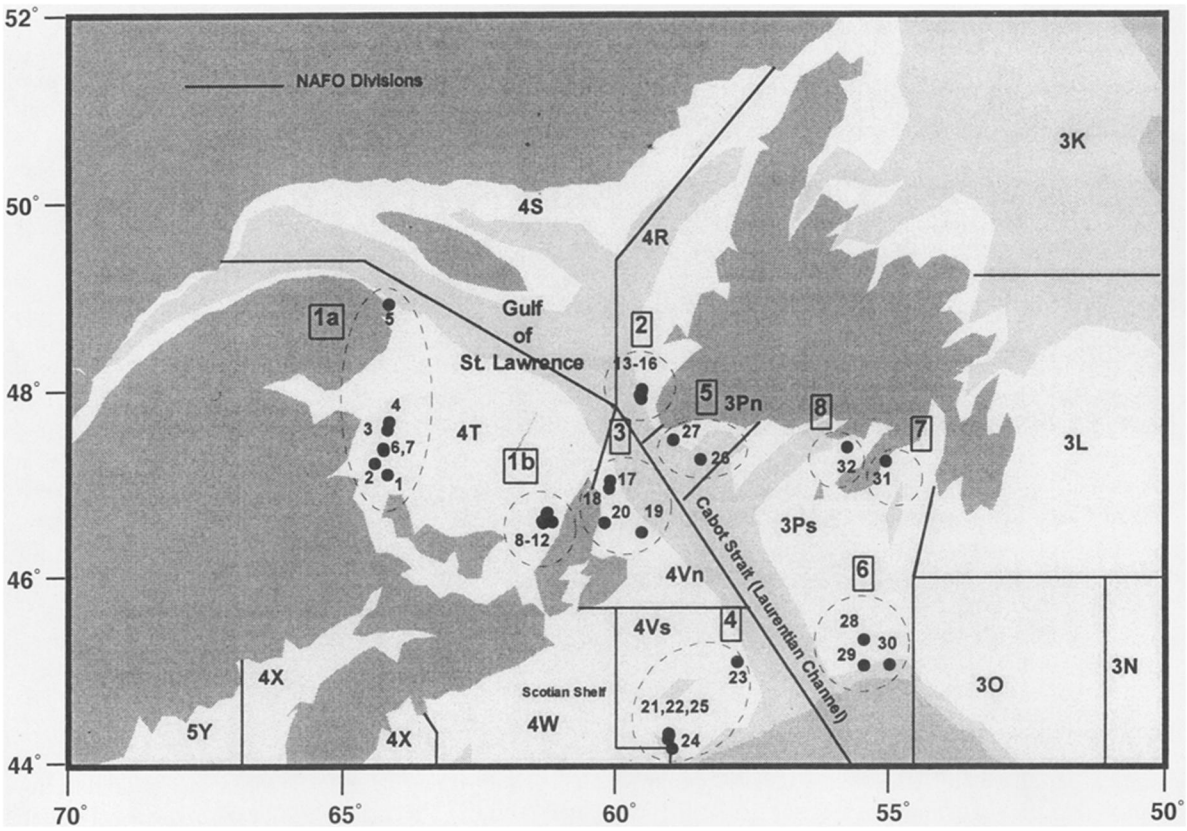

FIG. 1. Chart of the North Atlantic Fisheries Organization (NAFO) statistical management divisions showing locations of cod sample collections during the stock-separated period. Regional groups (1–8) are numbered according to local subgroups (1–32) as listed in Table 1.

servation strategies and management policy. In general, management 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 (Fig. 1). There exist within this large region (~300 000 km^2) 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 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: 88, 136, 338, and Fig. 11). 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: 160, 194, 196, and Fig. 11). 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 distribution range of cod in the northwest 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 1994–1997 (the stock-separated period) which allows the determination of the short term (2–3 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 are small; and/or (e) 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, Koljonen 1995, Koljonen and McKinnell 1996, Koljonen and Pella 1997), 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 mitochondrial DNA (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.

Materials and Methods

Sampling

We collected samples for genetic analyses from >2300 cod over four yr between 1994 and 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 and Fig. 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 and Fig. 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 January 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 handlines and/or gillnets at depths as shallow as ~20 m deployed in the coastal (Sentinel) fishery (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 (Tables 1 and 2). However, considerable effort was made 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. 1996*a, b*, 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 and alcohol equivalent to ~75 μL of blood was washed in high TE (100 mmol/L Tris-HCl pH 8.0, 40 mmol/L 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 μL TE.

Polymerase chain reaction (PCR) amplification of six microsatellite loci, Gmo2, Gmo132, Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993), and Gmo120 (Ruzzante et al. 1996*a*) 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 genotypic 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 genotypic 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, for comparative purposes, 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: Eq. 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 F_{ST} or R_{ST} estimates over loci. The difficulties associated with interpreting F_{ST} or F_{ST}-related analyses have often been highlighted (e.g., Slatkin 1985); they relate to the fact that many of the method's assumptions are generally violated in studies dealing with natural populations. The method has also recently been described as antiquated and criticized for providing ambiguous results that can be ". . . open to multiple interpretations" (Bossart and Prowell 1998). We, however, share alternative views provided by Bohonak et al. (1998), and believe the method is useful when properly applied and its limitations recognized. The main problem arises when F_{ST} is used to estimate gene flow (N_em) and population size and, most critically, when these estimates are interpreted at face value, something that appears to be rarely done (Bohonak et al. 1998). In this paper we simply used F_{ST} to describe the genetic composition of our samples and the extent of allele frequency differences among populations and did not estimate N_em.

We estimated pairwise genetic distances among populations using D_A (Nei et al. 1983), a nonSMM (stepwise mutation model) estimate of genetic distance with low variance relative to other nonSMM 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 applied multidimensional scaling (MDS) analysis to the D_A matrix. This multivariate method simplifies data with minimum loss of information and is likely to describe data more truthfully than trees when there is considerable genetic exchange between close geographic neighbors (Cavalli-Sforza et al. 1994), as is likely to be the case in our study. Using MDS we illustrate relationships among populations in more than two orthogonal dimensions; dimensions that represent the effect of historical and recurrent mixing and migration on observed gene frequencies (Menozzi et al. 1978, Cavalli-Sforza et al. 1993, 1994). This information is fundamentally different from that obtained from an F_{ST} analysis and other equilibrium measures. In all cases significance levels were adjusted for multiple comparisons using the sequential Bonferroni approach (Rice 1989). All these statistical tests and analyses of genetic distances and population structure were conducted using S-PLUS (MathSoft 1996) standard code or functions written by D. Ruzzante.

TABLE 1. Summary statistics for cod samples collected during the stock-separated period.

Regional group, NAFO Division (*N*) Local group (*N*)	Subgroup (*N*)	Sample date	Fishing set	Mean latitude (°N)	Mean longitude (°W)	Depth range (m)
1a) Southern Gulf, 4T West (242)						
1) J095 (60)	1 (30)	1–2 Jul 1995	23–27	47.09	−64.20	32–42
	2 (30)	2–5 Jul 1995	28–33	47.21	−64.44	32–44
2) 4TVn–WR (100)	3 (52)	13 Jun 1996	1	47.57	−64.20	64
	4 (48)	13 Jun 1996	2	47.65	−64.17	68
3) TR44 (32)	5 (32)	4 Jul 1996	44	48.92	−64.18	139
4) MB06 (50)	6 (26)	13 Jun 1997	1	47.35	−64.27	54
	7 (24)	13 Jun 1997	3	47.37	−64.28	55
1b) Southern Gulf, 4T East (149)						
5) 4TVn–NP (100)	8 (33)	15 Jun 1996	1	46.57	−61.33	95
	9 (33)	15 Jun 1996	2	46.59	−61.35	90
	10 (34)	15 Jun 1996	3	46.68	−61.26	102
6) NP06 (49)	11 (18)	11 Jun 1997	1	46.58	−61.17	80
	12 (31)	11 Jun 1997	2	46.67	−61.27	104
2) Northern Gulf, 4R (148)						
7) N242 (100)	13 (50)	30 Apr 1996	1	47.94	−59.55	195
	14 (50)	30 Apr 1996	2	47.98	−59.52	175
8) N9705 (48)	15 (30)	30 Apr 1997	1	47.90	−59.53	104
	16 (18)	30 Apr 1997	2	48.00	−59.52	108
3) Sydney Bight, 4Vn (196)						
	17 (50)	1 May 1996	3	47.02	−60.10	187
9) N242 (100)	18 (50)	1 May 1996	4	46.94	−60.12	125
10) N9705 (48)	19 (48)	1 May 1997	3	46.47	−59.52	55
11) KG97 (48)	20 (48)	18 May 1997	2	46.57	−60.20	114
4) Eastern Scotian Shelf, 4Vs (243)						
12) N242 (99)	21 (49)	1 May 1996	5	44.29	−59.01	142
	22 (50)	1 May 1996	6	44.25	−59.03	142
13) N9705 (48)	23 (48)	1 May 1997	5–6	45.08	−57.75	63–70
14) N9705 (48)	24 (48)	1 May 1997	10–11	44.15	−58.95	55–78
15) N222 (48)	25 (48)	21–26 Jul 1994	30–32, 84–87, 89	44.31	−59.02	62–223
5) South Newfoundland, 3Pn (107)						
16) WT186 (61)	26 (61)	13–14 Apr 1996	32–35	47.25	−58.43	...
17) WT202 (46)	27 (46)	5–6 Apr 1997	41, 42, 44, 46–49	47.46	−58.93	209–449
6) South Newfoundland offshore, 3Ps (194)						
18) WT187 (87)	28 (37)	24 Apr 1996	48	45.32	−55.42	...
	29 (25)	29 Apr 1996	58	45.04	−55.42	...
	30 (25)	29 Apr 1996	63	45.05	−54.95	...
7) Placentia Bay, 3Ps (61)						
19) SentPBay (61)	31 (61)	26 Jun 1997	1	47.23	−55.02	19
8) Fortune Bay, 3Ps (46)						
20) SentFBay (46)	32 (46)	19 Jun 1997	1	47.38	−55.73	71

Notes: Fishing sets (column 4) are pooled into geographically related subgroups (column 2) collected on or near the same date (column 3). Subgroups are pooled within collection trips to local groups (column 1). Geographically related local groups collected in different years are pooled within management divisions to regional groups (column 1).

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). The maximum likelihood analysis was conducted with functions written within S-PLUS (MathSoft 1996). In this analysis, cod in the samples from the mixed-stock periods exhibiting single-locus genotypes with alleles not found jointly in any of the eight regional collections from the stock-separated period were ignored. This procedure typically eliminated <2% of the total number of individuals and the individuals ignored were often those with at least one allele at very low frequency. 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.

TABLE 1. Extended.

Median length (cm)	Length range (cm)	Median age (yr)	Age range (yr)	Immature, pre-, post-, spawning (% of total)
46	41–48	7	5–8	0, 27, 30, 43
44	40–50	6	5–9	0, 67, 3, 30
50	36–70	7	5–9	0, 42, 8, 50
49	40–72	8	5–13	0, 44, 0, 56
46	42–53	...	...	...
51	48–63	8	6–12	0, 12, 69, 19
52	48–55	7	4–10	4, 8, 54, 33
48	41–72	7	4–10	0, 30, 9, 61
52	43–67	7	4–9	0, 33, 0, 66
55	43–84	7	5–12	0, 35, 3, 62
54	48–57	8	5–9	0, 32, 11, 58
52	48–56	7	4–10	0, 10, 16, 74
62	43–83	8	5–15	0, 56, 8, 36
57	38–84	8	5–12	8, 38, 20, 34
47	43–53	6	4–9	0, 50, 13, 37
51	47–53	7	5–8	0, 67, 6, 28
48	35–84	7	4–10	6, 60, 30, 4
52	42–89	8	5–12	0, 76, 18, 6
49	38–67	5	4–10	0, 42, 19, 40
53	40–81	...	...	2, 6, 13, 79
50	42–70	6	3–9	0, 32, 68, 0
54	48–72	7	4–9	0, 34, 64, 2
41	38–62	4	3–7	4, 38, 54, 4
42	40–53	4	3–8	0, 46, 29, 25
39	23–77	4	2–8	30, 26, 45, 0
49	35–88	6	3–12	...
47	31–78	6	4–9	...
65	50–89	7	6–9	...
60	36–76	6	3–9	...
63	48–74	7	4–8	...
57	45–74	...	...	...
66	36–105	...	...	14, 41, 38, 8

RESULTS: SINGLE LOCUS STATISTICS

The total number of cod analyzed 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 genotypic 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).

RESULTS: VARIATION WITHIN AND AMONG SAMPLES

We next examine the variation within and among the stock-separated samples and the mixed-stock samples.

Stock-separated samples

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

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 in the previous paragraph (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, and 20; Table 1) were from inshore and offshore locations in southern Newfoundland (Div. 3Ps) and were not pooled. In the following paragraphs we detail the results of these analyses for each region, and in each case we discuss why samples were, or were not, pooled for the maximum likelihood analysis:

1. *Southern Gulf of St. Lawrence (Div. 4T).*—Chi-squared pseudoprobability tests indicated marginal evidence of heterogeneity in allele frequency distribution among the six samples (local groups 1–6; Table 1, column 1) collected in 1995, 1996, and 1997 ($P < 0.05$ for four of six loci, although none was significant after sequential Bonferroni correction, $\alpha = 0.05/6 = 0.0083$). These six samples also showed evidence of structure with F_{ST} ($F_{ST} = 0.0028$, $P < 0.001$) and R_{ST}

TABLE 2. Summary statistics for cod samples collected during the mixed-stock period.

Regional group, NAFO Div.	Collection trip (*N*)	Sub-group (*N*)	Sample date	Fishing set	Mean latitude (°N)	Mean longitude (°W)	Depth range (m)	Median length (cm)	Length range (cm)	Median age (yr)	Age range (yr)	Immature, pre-, post-, and spawning fish (% of total)
1) 3Pn	N214 (49)	1 (49)	Jan 1995	42	47.42	−59.45	445	44	34–55	6	5–7	0, 18, 55, 27
	WT 182 (96)	2 (6)	Jan 1996	34	47.55	−59.47	...	...	...	...	...	...
		3 (30)		36	47.40	−59.3	...	...	...	...	...	...
		4 (45)		48	47.33	−59.22	...	...	...	...	...	...
		5 (15)		49	47.37	−59.13	421–440	47	42–58	6	5–10	0, 18, 79, 3
	T201 (144)	6 (48)	Jan 1997	36	47.57	−59.50	...	...	...	...	...	...
		7 (9)		16	47.53	−58.17	...	...	...	...	...	...
		8 (39)		22	47.32	−58.43	...	...	...	...	...	...
		9 (32)		12	47.40	−59.30	...	...	...	...	...	...
		10 (4)		24	47.33	−58.97	...	...	...	...	...	...
		11 (12)		26	47.33	−59.13	186–429	40	35–59	5	3–9	25, 14, 53, 8
2) 4Vn	N214 (49)	12 (49)	Jan 1995	108	46.45	−59.20	224	43	38–58	6	5–7	6, 20, 55, 18
	WT182 (96)	13 (9)	Jan 1996	8	46.92	−59.87	...	...	...	...	...	...
		14 (50)		39	47.33	−60.00	...	...	...	...	...	...
		15 (1)		42	47.13	−60.57	...	...	...	...	...	...
		16 (36)		43	47.13	−60.17	154–434	48	41–72	7	5–11	0, 6, 71, 23
3) 4Vn south	WT182 (72)	17 (18)	Jan 1996	72	46.33	−59.08	...	...	...	...	...	...
		18 (1)		74	46.52	−59.22	...	...	...	...	...	...
		19 (50)		75	46.53	−59.35	...	...	...	...	...	...
		20 (3)		76	47.73	−59.22	190–429	41	35–57	5	3–9	25, 11, 64, 0
4) 4Vn–4Vs	N255 (115)	21 (19)	Mar 1997	35	45.77	−58.05	...	...	...	...	...	...
		22 (48)		36	45.77	−58.00	...	...	...	...	...	...
		23 (48)		37	45.87	−58.07	181–202	48	34–62	7	2–12	...
	T201 (130)	24 (31)	Jan 1997	40	47.33	−60.25	...	...	...	...	...	...
		25 (20)		56	47.13	−60.30	...	...	...	...	...	...
		26 (45)		57	47.13	−60.13	...	...	...	...	...	...
		27 (2)		66	46.75	−59.23	...	...	...	...	...	...
		28 (32)		68	46.53	−59.38	191–434	41	34–63	5	3–11	10, 25, 61, 5
5) 3Ps	WT182 (96)	29 (7)	Jan 1996	118	46.55	−57.77	...	...	...	...	...	...
		30 (1)		122	46.57	−58.15	...	...	...	...	...	...
		31 (45)		130	46.73	−57.87	...	...	...	...	...	...
		32 (13)		132	46.73	−57.63	...	...	...	...	...	...
		33 (2)		134	46.93	−57.90	...	...	...	...	...	...
		34 (28)		136	46.93	−58.17	114–464	49	43–75	6	4–10	0, 17, 76, 3
	T201 (96)	35 (29)	Jan 1997	80	46.75	−57.90	...	...	...	...	...	...
		36 (35)		97	46.75	−57.75	...	...	...	...	...	...
		37 (32)		98	46.72	−57.73	305–430	45	37–50	5	3–9	42, 31, 27, 0
6) 4Vs	WT182 (47)	38 (26)	Jan 1996	92–94	45.53	−57.90	...	...	...	...	...	...
		39 (14)		98	45.33	−57.90	...	...	...	...	...	...
		40 (3)		100	45.33	−57.35	...	...	...	...	...	...
		41 (4)		102	45.33	−57.62	235–461	46	37–67	5	4–10	0, 13, 74, 13
7) 4R	T201 (95)	42 (14)	Jan 1997	8	48.13	−60.02	...	...	...	...	...	...
		43 (34)		49	47.95	−60.00	...	...	...	...	...	...
		44 (48)		52	47.75	−59.78	474–519	46	38–61	6	4–10	0, 12, 62, 21

Notes: Fishing sets (column 5) are pooled into geographically related subgroups (column 3) collected on or near the same date (column 4). Groups are pooled within collection trips by year and management divisions (columns 1 and 2).

(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 ($P \geq 0.004$; $\alpha = 0.05/15 = 0.003$). 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 ($P \geq 0.050$). However, when these same data were pooled within years there was evidence of temporal change across years with both F_{ST} (F_{ST} = 0.0053, $P < 0.001$) and R_{ST} (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} (F_{ST} = −0.0002), R_{ST} (R_{ST} = 0.0023, P = 0.120), or D_A (D_A = 0.036, P = 0.110) when the pool

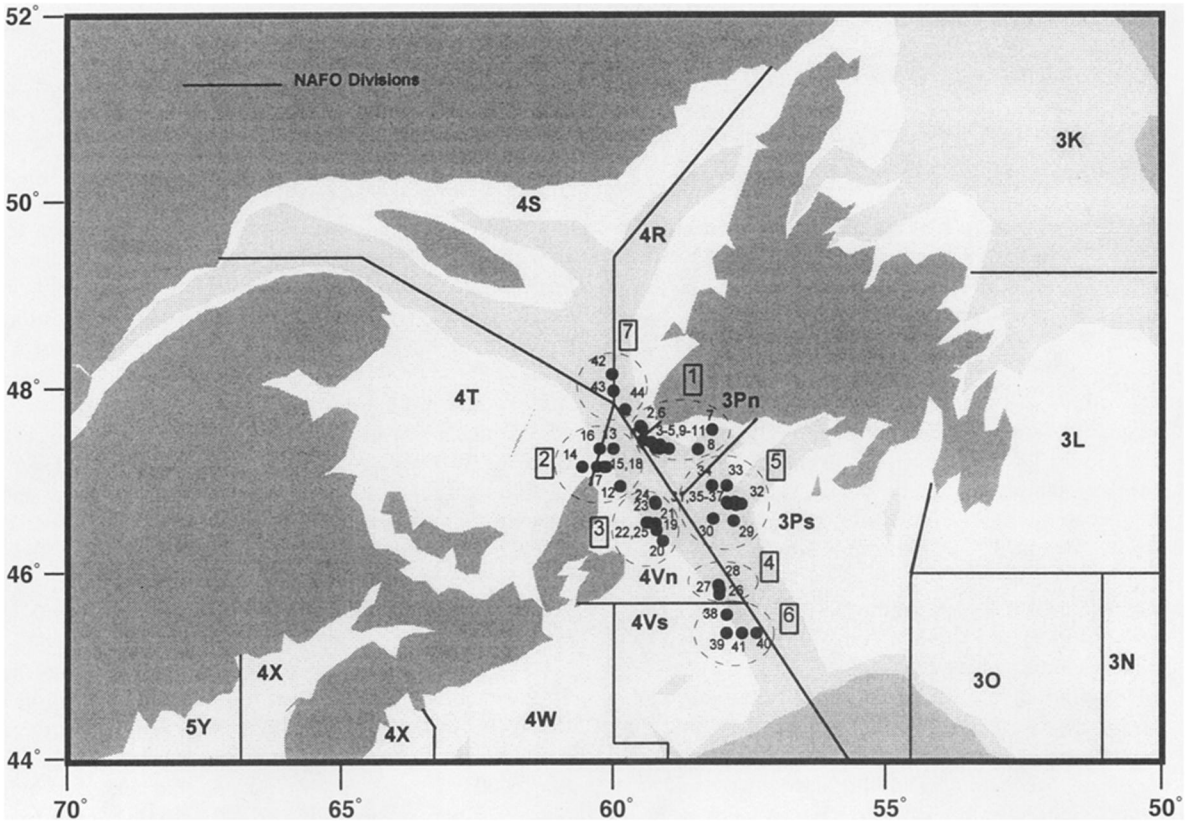

FIG. 2. Chart of the North Atlantic Fisheries Organization (NAFO) statistical management divisions showing locations of cod sample collections during the mixed-stock period. Numbered subgroups (1–44) are pooled by management division groups (1–7) as listed in Table 2.

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 six 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).

2. *Northern Gulf of St. Lawrence (Div. 4R).*—Chi-squared 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 five of the six loci ($P \geq 0.019$; $\alpha = 0.05/6 = 0.0083$); the one exception being Gmo145 ($P = 0.006$). No genetic structure was detected using either F_{ST} (F_{ST} = 0.0018, $P = 0.084$) or R_{ST} (R_{ST} = 0.0049, $P = 0.138$),

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.

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

Notes: N (ind.) is the number of individuals sampled per locus; n (alleles), the number of alleles per locus; Range (bp), allele size range in base pairs; Het_{obs} and Het_{exp}, the observed and expected heterozygosities; and D ($[Het_{obs} - Het_{exp}]/Het_{exp}$), heterozygote deficiency.

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).

3. *Sydney Bight (Div. 4Vn).*—Three of the six loci examined showed either significant (Gmo151, $P < 0.001$, $\alpha = 0.008$) or marginal (Gmo2 and Gmo145; $P \leq 0.038$) evidence of heterogeneity in allele frequency distribution among the three samples collected in 1996 and 1997 (local groups 9, 10, and 11; Table 1). There was evidence of genetic structure with both F_{ST} ($F_{ST} = 0.0032$, $P = 0.009$, $N_1 = 100$, $N_2 = 48$, $N_3 = 48$) and R_{ST} ($R_{ST} = 0.025$, $P < 0.001$). 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 ($P \cong 0.036$). 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 prespawning 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 prespawners (~68% and ~42%) relative to local group 11 (showing ~79% spawners and ~6% prespawners; 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 second paragraph in *Results*: *Estimates of pairwise genetic distances* and Table 5a).

4. *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} ($F_{ST} = 0.001$, $P = 0.145$) whereas there was evidence when measured with R_{ST} ($R_{ST} = 0.011$, $P = 0.002$). 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, which are likely to be marked in populations with overlapping generations (see Jorde and Ryman 1995, Ryman 1997). We pooled the three samples into a single regional pool (regional group 4; $N = 243$).

5. *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 three of the six 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} ($F_{ST} = 0.0005$, $P = 0.354$) but some evidence of heterogeneity was suggested by R_{ST} ($R_{ST} = 0.042$, $P < 0.001$) 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 nonmissing 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; $N = 107$).

6. *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} ($F_{ST} = 0.0019$, $P = 0.049$) and evident when measured with R_{ST} ($R_{ST} = 0.045$, $P < 0.001$). 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. 1996*b*, 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 five regional pools comprising regional groups 1a and 1b (Southern Gulf, $N = 392$), 2 (North-

TABLE 4. Single-locus and overall estimates of F_{ST} and R_{ST} among the eight regional groups of stock-separated cod samples from the Gulf of St. Lawrence and approaches.

Measure	Locus						
	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***

Note: See Table 1 for sample details. $\alpha = 0.05/6 = 0.0083$.
* $P < 0.05$, *** $P < 0.001$.

ern Gulf, $N = 148$), 3 (Sydney Bight, $N = 196$), 4 (Eastern Scotian Shelf, $N = 243$), and 5 (South Newfoundland, 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, in particular for those of populations with overlapping generations (see Waples and Teel 1990, Jorde and Ryman 1995, Ryman 1997). 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.

7. *Estimates of genetic structure among the eight 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} ($R_{ST} = 0.0142$, $P < 0.001$) primarily due to the influence of the Gmo132 and Gmo145 loci and to a lesser extent Gmo120 and Gmo151 (Table 4).

8. *Estimates of pairwise genetic distances: eight 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 Divs. 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 next paragraph 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 visualized with a multidimensional scaling analysis applied to the matrix of D_A genetic distances (Fig. 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 (Fig. 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 (Fig. 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, Fig. 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 (Fig. 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 data*; see also Templeman 1979, 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). This explanation is consistent with the fact that neither sample appears genetically ($P > 0.40$) nor phenotypically (otolith elemental fingerprints and

TABLE 5. Pairwise estimates of genetic distance D_A based on polymorphism at six microsatellite DNA loci among cod representing the eight 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.

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

Regional group; N	Southern Gulf (4T); 392	Northern Gulf (4R); 148	Sydney Bight (4Vn); 196	[Sydney Bight (4Vn); 48]	Eastern Scotian Shelf (4Vs); 243	South Nfld offshore (3Pn); 108	South Nfld offshore (3Ps); 87	South Nfld Placentia Bay (3Ps); 61	South Nfld Fortune Bay (3Ps); 46
Southern Gulf (4T); 392		0.037 (0.037)‡	0.030	**0.097**	**0.037**	**0.054**	**0.061**	**0.092**	**0.091**
Northern Gulf (4R); 148			0.043	**0.108**	0.046**	**0.070**	0.061	**0.106**	**0.107**
Sydney Bight (4Vn); 196					**0.045**	**0.061**	0.063*	**0.092**	0.090†
[Sydney Bight (4Vn); 48]					**0.104**	**0.111**	0.115**	**0.130**	**0.142**
Eastern Scotian Shelf (4Vs); 243						**0.069**	**0.071**	**0.094**	**0.104**
South Nfld offshore (3Pn); 108							**0.085**	**0.105**	**0.113**
South Nfld offshore (3Ps); 87								**0.111**	0.112*
South Nfld (3Ps) Placentia Bay; 61									0.125*
South Nfld (3Ps) Fortune Bay; 46									

Notes: Estimates in the row and column labeled [Sydney Bight (4Vn); $N = 96$)] exclude local groups 9 and 10 (see Table 1) from the reference samples that were presumed to contain transient cod of 4T origin collected in early May 1996 and 1997. Initial K (number of pairwise comparisons) = 28; $\alpha = 0.05/28 \simeq 0.002$.

† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$; bold face type indicates $P < 0.002$.

‡ $P < 0.10$ the significance of this value relates only to the case with the smaller data set from 4VN (48 fish).

b) D_A estimates for the 1996 data only.

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

Note: $\alpha = 0.05/15 = 0.003$.

† $P < 0.10$, * $P < 0.05$; bold type indicates $P < 0.003$.

c) D_A estimates for the 1997 data only.

Regional group, date; N	Southern Gulf (4T)	Northern Gulf (4R)	Sydney Bight (4Vn) 1 May 1997	Sydney Bight (4Vn) 18 May 1997	Eastern Scotian Shelf (4Vs)	South Nfld (3Pn)	South Nfld Placentia Bay (3Ps)	South Nfld Fortune Bay (3Ps)
Southern Gulf (4T); 100		0.090	0.104*	**0.106**	0.071*	**0.135**	**0.121**	**0.125**
Northern Gulf (4R); 48			0.104	0.128*	0.072	**0.146**	**0.130**	**0.146**
Sydney Bight (4Vn), 1 May 1997; 48				0.116	0.085	**0.143**	0.125**	0.139**
Sydney Bight (4Vn), 18 May 1997; 48					0.106*	0.122	**0.130**	**0.142**
Eastern Scotian Shelf (4Vs); 96						**0.122**	0.091**	0.109**
South Nfld (3Pn); 46							**0.138**	**0.152**
South Nfld (3Ps) Placentia Bay; 61								0.125*
South Nfld (3Ps); 46								

Note: $\alpha = 0.05/21 \cong 0.002$.

* $P < 0.05$, ** $P < 0.01$; bold type indicates $P < 0.004$.

vertebra number) distinguishable from southern Gulf cod (for otolith and vertebrae, respectively: S. Campana and K. Frank, *personal communication*). 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

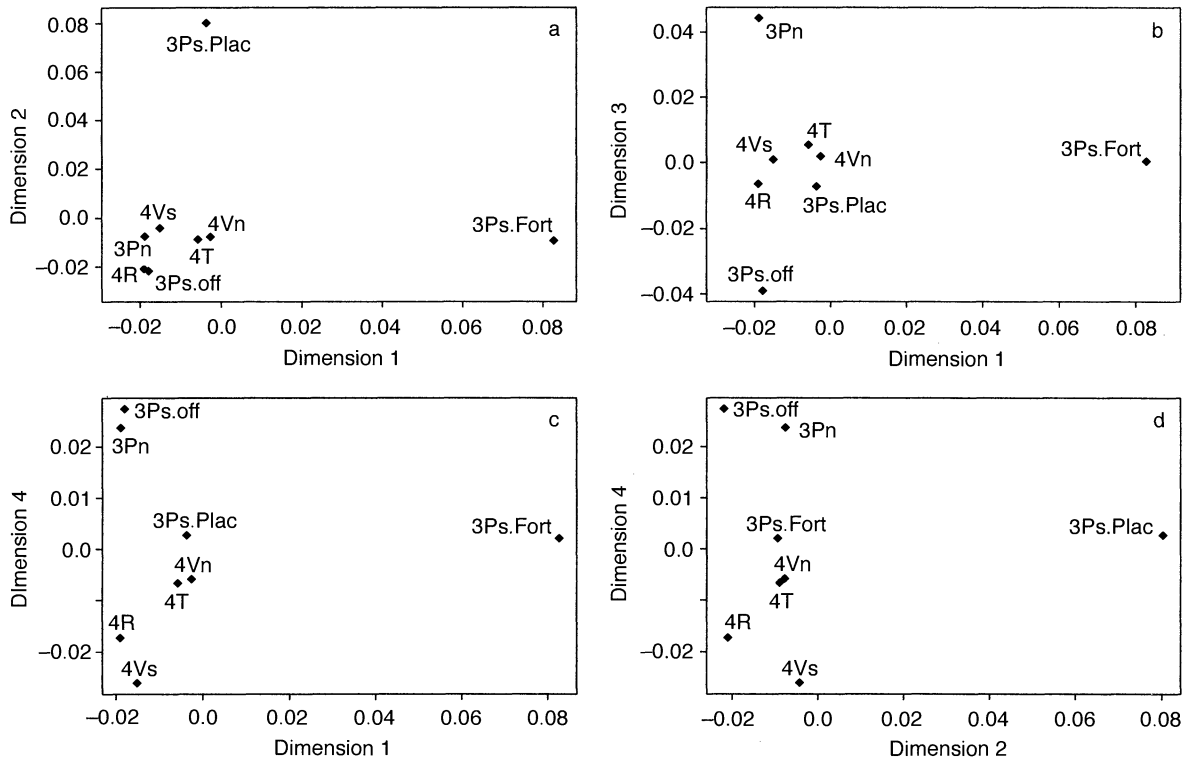

FIG. 3. Scattergram of a multidimensional scaling analysis applied to the D_A matrix of genetic distances among eight regional groups of cod samples collected during the stock-separated period. (a) Dimension 1 vs. 2; (b) dimension 1 vs. 3; (c) dimension 1 vs. 4; (d) dimension 2 vs. 4.

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 (Tables 5b and 5c).

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 neighboring 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 analyzed in the present study the comparison between northern and southern Gulf cod did not approach statistical significance (see paragraph 7 of *Discussion*) and neither did the comparison between northern Gulf (4R) cod and cod collected offshore in the 3Ps region of south Newfoundland.

Mixed-stock samples

1. *Estimates of genetic structure among the seven 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 analyzed 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 seven divisions collected between 1995 and 1997 or

TABLE 6. Single-locus and overall estimates of F_{ST} and R_{ST} among the seven management division groups of mixed-stock cod samples from the approaches to the Gulf of St. Lawrence.

	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

Notes: Data are from 1995, 1996, and 1997 combined. Total N = 1086. See Table 2 for sample details; α = 0.05/6 = 0.0083. Bold type indicates $P < 0.0083$.

among those collected exclusively in 1996 (R_{ST} = −0.0015) or in 1997 (R_{ST} = 0.0014, P = 0.07).

2. *Estimates of pairwise genetic distances among the seven 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 analyzed 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).

RESULTS: MAXIMUM LIKELIHOOD ANALYSIS

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 (1) 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 (2) 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 (Fig. 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).

Temporal variation in stock contribution (1996 vs. 1997).—Fig. 4 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; Fig. 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-

TABLE 7. Pairwise estimates of genetic distance D_A (Nei et al. 1983) based on polymorphism at six microsatellite DNA loci among cod representing seven management division groups of mixed-stock samples from the approaches to the Gulf of St. Lawrence.

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

Notes: Data are from 1995, 1996, and 1997 combined. Total N = 1086. See Table 2 for sample details. Sequential Bonferroni correction initial K = 21; α = 0.05/21 = 0.002.

* $P < 0.05$, ** $P < 0.01$.

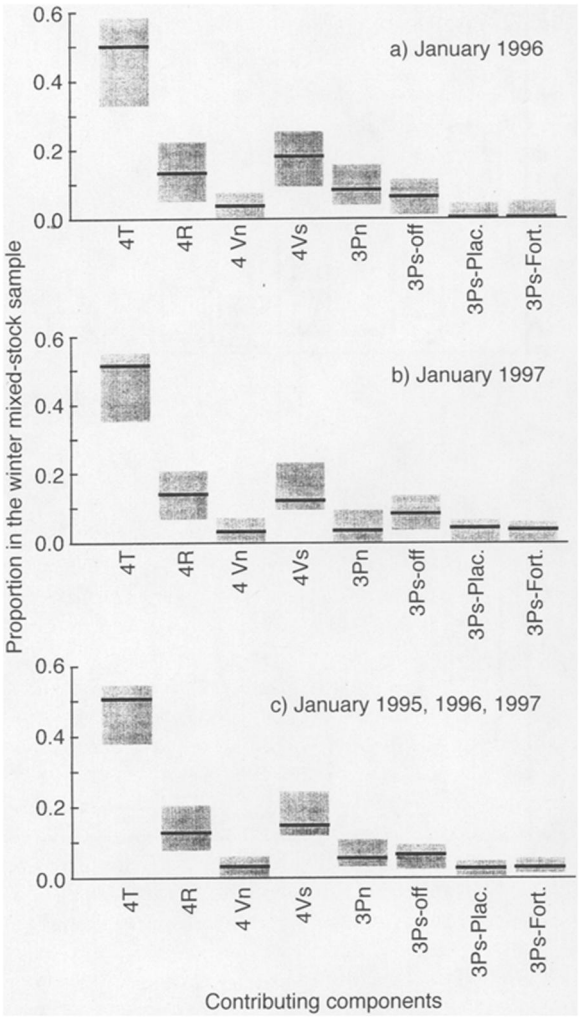

FIG. 4. Expected maximum likelihood estimates (heavy horizontal lines) and 95% bootstrap confidence intervals (shaded regions) of contributions (proportions) by cod from eight regional groups to all (pooled) mixed-stock (winter) samples. Estimates reflect the proportions of cod from each regional group 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 1994–1997. 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 (1996–1997) in each regional group's contribution to the mixed-stock samples.

stock samples by cod from Divs. 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%).

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, *unpublished data*). We therefore estimated the proportions contributed by the eight cod regional pools to each of these winter aggregations (see Fig. 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% (CI: 33–53%) to the aggregation in Div. 3Pn (Fig. 5). Similarly, cod from the northern Gulf of St. Lawrence (Div. 4R) contributed the most to the mixed aggregations in Divs. 4R, 3Ps, and 4Vn–4Vs (~14–15%) and less so to the aggregation found in Div. 3Pn (~9%; Fig. 5). Cod from Sydney Bight (Div. 4Vn, off Cape Breton) contributed <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%. Finally, 3Pn cod contributed to a very modest degree (<8%) to the mixed-stock aggregations in Divs. 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 (Fig. 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 Fig. 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 paragraph 9 of *Discussion*) 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 Abstract, it appears that different cod populations can be identified in the Gulf region and its approaches at a spatial scale that generally corresponds

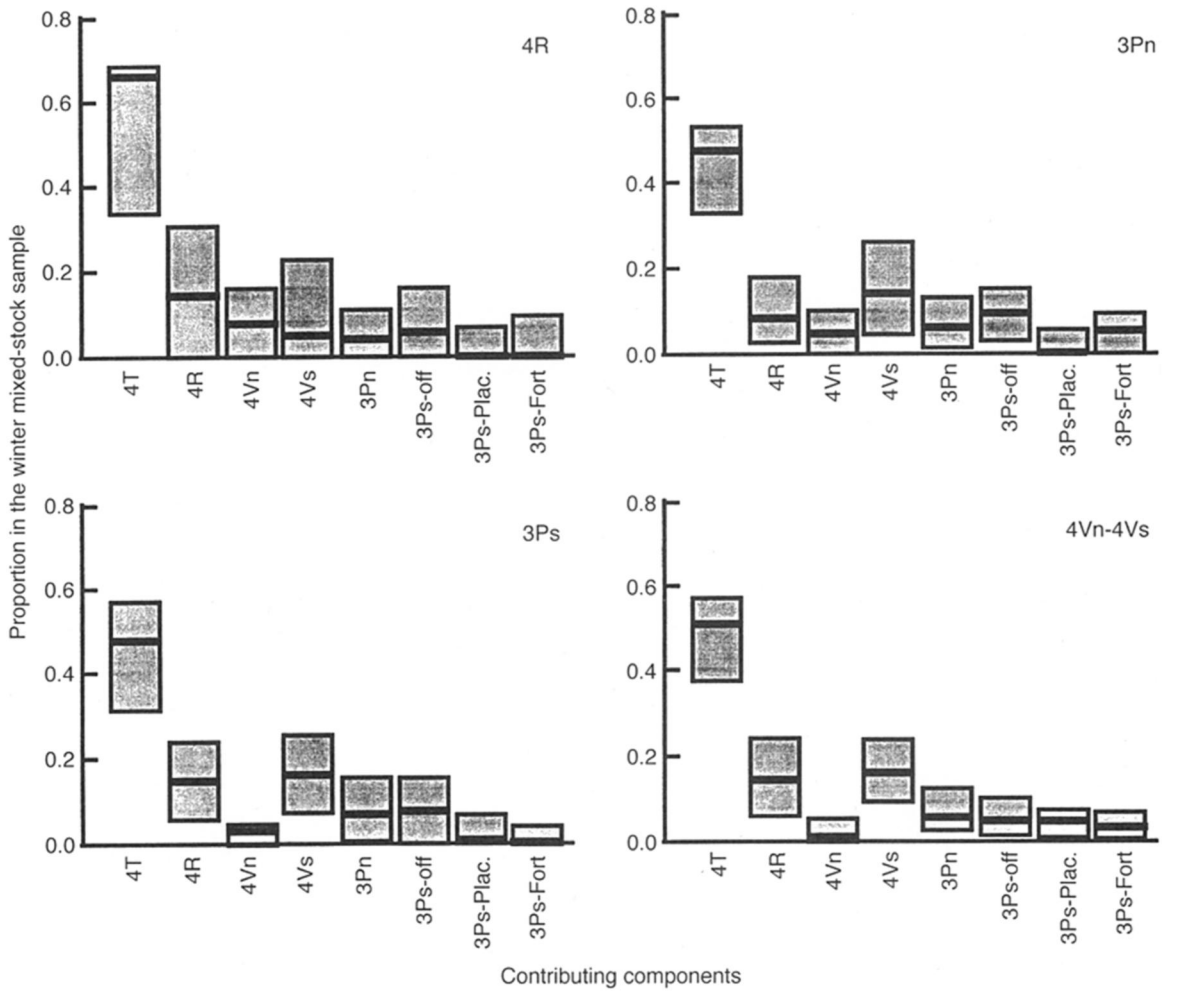

FIG. 5. Expected maximum likelihood estimates (heavy horizontal lines) and 95% bootstrap confidence intervals (shaded regions) of contributions (proportions) by cod from eight regional groups to all (pooled winter 1996 and 1997) samples collected in each of four management divisions (4R, 3Pn, 3Ps, and pooled 4Vn and 4Vs). 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 1994–1997.

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 observed among the winter mixed-stock cod aggregations 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.

Our 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% (Fig. 4). However, when analyzed 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 Divs. 3Pn and 3Ps of southern Newfoundland. Cod from the northern Gulf of St. Lawrence in region 4R contributed ~13% to all winter aggregations (Fig. 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 (Div. 4Vs) contributed ~15% to the mixed-stock aggregations.

Has there been significant temporal variation in these contributions between the years 1996 and 1997? The answer almost certainly is no. The contributions from the various reference stocks to the mixed-stock aggregations changed little between the years 1996 and 1997 (Fig. 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 analyzed separately among the four management divisions represented by the overwintering mixtures, the contributions from the various reference stock components varied somewhat among divisions (Fig. 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 Divs. 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, see Ruzzante et al. 1998, a study that describes the genetic structure of cod aggregations throughout the species' range [southern Labrador to Georges Bank, cod from the Gulf of St. Lawrence are not included] in relation to oceanographic features, bathymetric structure and temporal differences in spawning behavior, all of which represent potential barriers to gene flow; reviewed in Ruzzante et al. 1999, a review of our work on cod population genetics up to, but excluding the present paper). 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 Fig. 1). Also, although the samples were collected off southwestern Newfoundland in region 4R where northern Gulf cod are known to spawn, they are not representatives 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.

We described the genetic composition of our samples and the relationship among them using F_{ST} (and for comparative purposes, R_{ST}) and multidimensional scaling plots of the D_A (Nei et al. 1983) measure of genetic distance. The difficulties associated with interpreting F_{ST} or F_{ST}-related analyses have often been highlighted (e.g., Slatkin 1985); they relate to the fact that many assumptions of the method are almost certainly never valid in studies dealing with natural populations. The main problem, however, lies in using F_{ST} for the pur-

pose of estimating gene flow (i.e., the effective migrants per generation, N_em) and other demographic parameters such as population sizes, and we have refrained from taking this step in this manuscript. We simply used F_{ST} to describe the genetic composition of our samples and the extent of allele frequency differences among populations. To examine the relationships among populations more closely we used multidimensional scaling analysis of the matrix of pairwise D_A (Nei et al. 1983) genetic distances. The various orthogonal dimensions in this analysis represent the effect of historical and recurrent mixing and migration on observed gene frequencies (Menozzi et al. 1978, Cavalli-Sforza et al. 1993, 1994), information that is fundamentally different from that obtained from an F_{ST} analysis and other equilibrium measures. New statistical methods for estimating population parameters under nonequilibrium conditions are increasing (e.g., Davies et al. 1999, Luikart and England 1999), but it appears that they have yet to show that they can produce accurate estimates (Neigel 1997, Beerli 1998).

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 (Chakraborty 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 Chakraborty 1995, see also Hedrick 1999). 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 Chakraborty 1995, see also Hedrick 1999). 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. 1996*a*, 1998, reviewed Ruzzante et al. 1999). The numbers of alleles per locus in our samples ranged between 21 (Gmo 132) 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 nongenetic 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, Chakraborty 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 management 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) which would be functionally inconsistent with the principles of resource conservation and the maintenance of biodiversity.

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