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DFO Atlantic Fisheries
Research Document 95/23

MPO Pêches de l'Atlantique
Document de recherche 95/23

Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level.

by

Daniel E. Ruzzante,[*] Christopher T. Taggart,[†,‡] Doug Cook,[§] and Sally Goddard[¶]

[*]Department of Biology, Dalhousie University, Halifax, N.S., B3H 4J1

[†]Department of Oceanography, Dalhousie University, Halifax, N.S., B3H 4J1

[‡] Science Branch, Canada Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, PO Box 5667, St. John's, NF, A1C 5X1

[§] Marine Gene Probe Laboratory, Department of Biology, Dalhousie University, Halifax, N.S. B3H 4J1

[¶] Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF, A1C 5S7

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ABSTRACT

Microsatellite DNA provided evidence that cod populations overwintering in inshore Newfoundland are genetically distinguishable from cod overwintering offshore. We compared variation in five loci in samples collected over three years from five inshore locations around Trinity Bay, Newfoundland, and from an offshore region on the northern Grand Banks (North Cape). Cod collected inshore were divided into two groups based on the level of antifreeze in the blood, those that show high antifreeze levels and are presumed to have overwintered in cold (<0°C) inshore waters and those that show low antifreeze levels and are presumed to have overwintered offshore in warmer (>2°C) waters. Fish overwintering inshore (high antifreeze) differed significantly from offshore fish in allele-sharing distance, while fish with low antifreeze levels did not. Subpopulation structure (Rst) was detected when offshore cod were compared to inshore cod with high levels of antifreeze but not when compared to those with low levels of antifreeze. Both results are consistent with the hypothesis that cod overwintering inshore constitute a population that is genetically distinct from offshore cod. We suggest, therefore that inshore and offshore cod from the areas studied remain genetically distinct despite the fact that individuals from both populations intermingle inshore during the summer and fall feeding migration. Our study has thus found evidence of population structure at a finer geographical scale than has been shown to date for this species.

RÉSUMÉ

L'ADN microsatellite a fourni la preuve que les populations de morue qui hivernent sur la côte de Terre-Neuve présentent des caractéristiques génétiques distinguables de celles des morues qui hivernent au large. Nous avons comparé les différences sur cinq loci dans des échantillons prélevés sur une période de trois ans en cinq points de la côte, le long de la baie Trinity (Terre-Neuve), et en un point du large situé au nord des Grands-Bancs de Terre-Neuve (North Cape). La morue provenant de la région côtière a été divisée en deux groupes, selon le taux d'antigel dans son sang, soit un groupe comprenant des morues à fort taux d'antigel, dont on présumait qu'elles avaient hiverné dans les eaux côtières froides (<0 °C) et un groupe à faible taux d'antigel, dont on présumait qu'il avait hiverné dans les eaux plus chaudes (>2 °C) du large. Il s'est avéré que les morues qui avaient hiverné dans les eaux côtières (taux d'antigel élevé) différaient considérablement des morues du large quant à la distance de partage des allèles, ce qui n'était pas le cas chez les poissons présentant un faible taux d'antigel. On a décelé une structure de sous-population (Rst) quand on comparait des morues du large à des morues des eaux côtières présentant un fort taux d'antigel, mais non lorsqu'on les comparait à des morues ayant un faible taux d'antigel. Ces deux résultats concordent avec l'hypothèse selon laquelle la morue hivernant dans les eaux côtières constitue une population distincte du point de vue génétique de la morue du large. Nous estimons donc que les morues des zones côtières et extracôtières étudiées conservent cette distinction génétique malgré le fait que les deux populations se mélangent dans les eaux côtières durant les migrations trophiques d'été et d'automne. Notre étude apporte la preuve de l'existence d'une structure de population à une échelle géographique plus réduite que celle qui avait été déterminée jusqu'ici pour l'espèce considérée.

INTRODUCTION

Knowledge of population structure is essential for the conservation of genetic resources and for an adequate analysis of population dynamics. For natural populations under exploitation, an incorrect interpretation of the genetic structure can lead to overexploitation and to erosion of genetic resources via depletion of some or all of the populations' spawning components. This problem is particularly relevant to marine fish species which, for political or admninistrative convenience, are often managed under assumptions of single, homogeneous, and large breeding populations. When these assumptions are not met, and there are a number of discrete or semidiscrete spawning components within management units, those components most easily captured are likely to be eliminated with often detrimental effects on the stock as a whole.

Atlantic cod (*Gadus morhua*) are distributed in the western Atlantic from Labrador (~63° N) to Cape Hatteras (~35° N). Cod populations inhabiting the region off Labrador and Newfoundland, referred to as Northern cod, exhibit an annual pattern of inshore-offshore migration (Lear 1984, 1986) with most mature individuals overwintering in warm (>2°C) slope water along the margins of the continental shelf. Spawning is protracted (3-4 months from late winter through early summer), generally beginning earlier in the north than in the south (Myers *et al.* 1993). Evidence to date shows spawning to be most concentrated offshore along the edge of the continental shelf (see review in Taggart *et al.* 1994) though a recent study by Hutchings et al. (1993) suggests that inshore spawning may be more pronounced than previously thought. Following offshore spawning, adult cod migrate inshore to the summer feeding grounds. They then return offshore in late autumn-early winter (Templeman 1966), and are joined in this migration by cod maturing for the first time (reviewed in Lear and Green 1984). By age-4 cod are beginning to adopt the distributional and migratory behavior of the older age classes (Rose 1993).

While the majority of the cod show the above pattern of migration, it has long been known that some fish remain inshore through the winter (Fletcher *et al.* 1987). The extent to which fish overwintering inshore versus offshore constitute distinct stocks remains largely unknown (Lear 1984; Hutchings *et al.* 1993; Angel *et al.* 1994). Distinct offshore spawning components do appear to exist, based on analysis of variation in vertebral complement (Templeman 1981; Lear and Wells 1984), on the geographic distribution of spawning (Hutchings *et al.* 1993), and on tag recovery data (Templeman 1974, 1979; Lear 1984; reviewed in Lear and Green 1984 and in Taggart *et al.* 1995). The regular occurrence of juvenile and adult cod overwintering in cold (<0°C), coastal waters (Fletcher *et al.* 1987; Valerio *et al.* 1992, Goddard *et al.* 1992, 1994; Wroblewski *et al.* 1994) coupled with evidence of inshore spawning (Wroblewski *et al.* In press; Smedbol and Wroblewski In press) suggest that inshore population(s), distinct from the offshore population(s) may exist (Templeman 1966). However, to date there is no published evidence that these populations differ genetically.

Different genetic techniques (i.e., allozymes, mitochondrial or nuclear DNA, etc.) vary in their ability to detect population structure. Previous studies on the genetic structure of Atlantic cod populations have used several techniques. Those based on allozyme loci have produced ambiguous results. They tend to show significant differences among neighbouring populations when a limited number of blood protein loci are examined (Jamieson 1975, Cross and Payne 1978, Jamieson and Otterlind 1971, Møller 1968, Dahle and Jørstad 1993), but these differences disappear when a larger number of conventional electrophoretic loci are examined over the distributional range of the species (Mork *et al.* 1982, 1985; see also Grant *et al.* (1987) for similar results on Pacific cod, *Gadus macrocephalus*). More recent studies based on mitochondrial DNA variation have shown limited or no differentiation of populations throughout most of the species' range (Smith *et al.* 1989; Carr and Marshall 1991a; Árnason and Rand 1992; but see Dahle 1991) or within smaller geographic areas such as around the coast of Iceland (Árnason et al. 1992) or among management divisions within the range of northern cod (Carr and Marshall 1991a,b; Pepin and Carr 1993). Most recently, in a study comparing cod populations across the Atlantic Ocean, Pogson *et al.* (1995) demonstrated that nuclear DNA restriction fragment length polymorphism (RFLP) loci are capable of

detecting genetic population structure at ocean basin scales where allozyme loci do not.

We make use of the fact that adult northern cod make antifreeze glycoproteins in response to the cold winter temperatures characteristic of coastal Newfoundland and Labrador (Fletcher *et al.* 1987) to identify fish that overwinter in the cold inshore waters (Goddard *et al.* 1994). As unprotected blood and the extracellular fluids of cod will freeze at temperatures between -0.5°C and -0.8°C (Fletcher *et al.* 1982), the ability of cod to produce antifreeze glycoproteins appears to be essential for overwintering in coastal waters where temperatures can approach the freezing point of sea-water (near -1.8°C) for several months each year. Antifreeze appears in cod plasma as temperatures fall below 0°C, reaches a maximum level after approximately 75 days, and remains high until water temperatures rise above 0°C (Fletcher *et al.* 1987; Goddard *et al.* 1994). In adult cod antifreeze levels are thus a function of length of continuous exposure to sub-zero temperatures and can be used as a physiological time-tag to infer the recent thermal history of the fish. High protein levels are characteristic of individuals that overwinter in relatively cold (<0°C) coastal water masses. Conversely, low or nonexistent glycoprotein levels are consistent with overwintering in warmer (>0°C) offshore water masses (Goddard *et al.* 1994).

In this paper we show that cod populations overwintering in inshore Newfoundland are genetically distinguishable from cod populations overwintering offshore along the edge of the continental shelf of the Grand Banks. We employ nuclear DNA microsatellite loci as genetic markers and antifreeze protein levels (expressed as blood plasma thermal hysteresis) and time of sampling as indicators of inshore overwintering. We distinguish populations genetically based on evidence of departures from Hardy-Weinberg equilibrium, heterogeneity of allele frequency distributions, allele sharing distances (Bowcock *et al.* 1994), and population structure (Rst) (Slatkin 1995).

MATERIAL AND METHODS

Cod Sample Collections

Cod were collected between January 1992 and June 1994 and were drawn from 14 different sample populations (Table 1). Four of these sample collections are broadly categorized as being from offshore (North Cape region of the northern Grand Bank) winter and early summer aggregations while 10 are from winter and early summer aggregations found inshore in the immediate vicinity of the Random Island region of Trinity Bay (Southwest Arm, Northwest Arm, Smith Sound, Heart's Ease Ledge), elsewhere in Trinity Bay (Bellevue), and Bonavista Bay (Table 1, Fig. 1). Water temperatures at all collection depths were recorded using a calibrated trawl-mounted or wire-mounted profiling conductivity, temperature, depth probe (CTD).

Offshore (North Cape) collections of cod were made using an otter trawl deployed at depths of between 400 m and 1000 m where average bottom temperature among collection dates, locations and depths ranged between 2.5°C and 3.4°C and where average lengths of the cod collected for genetic and antifreeze analysis ranged between 33 cm and 54 cm (Table 1). The offshore collections made in January and February were from what are presumed to be pre-spawning aggregations while the June collections are from assumed post-spawning aggregations. Of the offshore collections, only those cod collected during January 1993 and June 1994 were assessed for blood plasma thermal hysteresis.

Inshore collections of cod were made using a combination of gear (otter trawl, cod trap, gillnet and jigger) deployed at depths of between 140 m and 200 m in the deeper channel regions of SW Arm and at shallower depths of between 14 m and 150 m elsewhere inshore (Table 1). Average water temperatures at depth associated with collections in SW Arm were consistently sub-zero and ranged between -0.5°C and -1.4°C while collections from other locations, depths, and dates were associated with warmer water temperatures that averaged between 0.6°C and 5.5°C (Table 1). The average lengths of cod collected among dates and locations in the vicinity of Random Island ranged between 38 cm and 62 cm while

collections made at Bellevue and Bonavista Bay ranged in length between 27 cm and 34 cm (Table 1). Cod collected from Heart's Ease Ledge in July 1993 were from an aggregation that was known to be spawning at that location (Wroblewski *et al.* In press; Smedbol and Wroblewski In press). Blood plasma thermal hysteresis was determined for 7 of the 10 inshore sample populations (Table 1).

Cod Tissue Collections

Blood samples were collected from live cod (which were measured for total length and weight) using a sterile 2 ml syringe and hypodermic needle (21 gauge) inserted ventrally into the blood vessels that run in the haemal arches of the vertebrae between the anal fin and the caudal peduncle (Nielsen and Johnson 1983) and ~1 ml of blood was withdrawn. If the sample was destined entirely for genetic analysis, it was preserved immediately in 5 ml of 95% ethanol and stored. Alternatively, approximately one half was preserved in this manner and the remainder, to be used for antifreeze analysis, was injected directly into Vacutainers (Becton Dickinson™) containing sodium heparin, mixed gently, and held on ice until centrifuged. Samples were centrifuged for 10 minutes at 4000g, the blood plasma was removed, placed in 1.5 ml Eppendorf tubes, and stored until analysed. Muscle tissue samples for genetic analysis were taken from the anterior of the tongue of dead cod (also measured for length and weight).

Genetic Analysis

DNA extraction from alcohol preserved tissue was as described in Bentzen *et al.* (1990). Briefly, a sample of approximately 100 mg muscle or 100 ul of blood in enthanol, was washed in high TE (100 mM Tris-HCl, 40 mM EDTA pH 8.0) to remove ethanol. The samples were then placed in 250 µl of lysis buffer (10 mM Tris-HCl, pH 8.3, 1 mM EDTA, 400 mM NaCl containing 0.8% sodium dodecyl sulfate (SDS) and 400 µg/ml Proteinase K). Samples were digested at 65°C for 18 hours and transferred to an SST tube (Becton Dickinson™). The samples were extracted twice with 2.5 ml chloroform. The supernatant containing the purified nucleic acids was transferred to a 1.5 ml Eppendorf tube and precipitated with 0.2 M NaCl and 1 volume of isopropanol. The DNA pellet was resuspended in 100 µl of TE (10 mM Tris, 0.1 mM EDTA ph 8.0) and adjusted to 25 ng/µl as a PCR template. PCR analysis was as previously described by Brooker *et al.* (1994) using 5 sets of cod microsatellite primers: Gmo2, Gmo132, and Gmo145 (Brooker *et al.* 1994), Gmo4 (Wright 1993) and Gmo120 [Primer sequences (5'->3'): GAGCAAACATGCTCAGAGTG; GACTGATCTCCATGAGAGG]. Gmo2, Gmo4, Gmo120, and Gmo132 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). The PCR products were resolved on 6.5% sequencing gels and the alleles sized relative to a sequence ladder generated from M13mp18.

Blood Plasma Antifreeze Analysis

Antifreeze activity within each plasma sample was determined using a nanolitre osmometer (Clifton Technical Physics, Hartford, New York) and the protocol described in Goddard *et al.* (1994). In brief, the blood plasma freezing and melting points were determined by microscopic observation of the freezing and melting behavior of a single ice crystal within the plasma. The antifreeze glycoproteins act in a non-colligative manner to inhibit ice propogation by binding to embryonic ice crystals and preventing the addition of water molecules to the ice lattice (DeVries 1983). However, the effect on the melting point is purely colligative. This results in a difference between the freezing and melting points of a solution containing antifreeze, proportional to the amount of antifreeze present. The difference (measured in °C) is termed thermal hysteresis and is commonly used as a direct measure of antifreeze activity (Kao *et al.* 1986).

Antifreeze activity, as measured by thermal hysteresis in the individual plasma samples, was used to infer the overwintering behavior of the individual cod. High antifreeze levels (≥0.2°C thermal hysteresis) are indicative of cold-water (inshore) overwintering, while low or non-existent winter antifreeze levels (≤0.09°C thermal hysteresis) are indicative of warm-water (offshore) overwintering.

Data Analysis

Tests for Hardy-Weinberg equilibrium (HWE) were conducted using the goodness of fit, and the log-likelihood ratio tests following Weir (1990, p. 84-85). We estimated Rogers', allele sharing (Bowcock *et al.* 1994), and average squared genetic distances (Goldstein *et al.* 1995). Estimates of subpopulation structure were obtained using Rst (Slatkin 1995). For comparative purposes we also calulated Fst values following Weir and Cockerham (1984). Significance values for all tests (i.e., HWE, homogeneity of allele frequency distributions, genetic distances and estimates of sub-population structure) were determined by Montecarlo simulations and the bootstrap method (minimum 1000 resampling trials per individual comparison; Manly 1991). In all cases, bootstrapping was conducted across individuals and populations for each locus separately. Bootstrapping across individuals and populations enables estimation of significance values for the test under consideration (e.g., HWE, Rst, etc.). Bootstrapping each locus independently enables comparison of estimates across loci. Estimates of Rst and Fst combined over loci were calculated by first averaging numerators and denominators across loci and then taking ratios, as suggested by Weir and Cockerham (1984), Slatkin (1995) and Goldstein *et al.* (1995).

RESULTS

Microsatellite DNA variation among locations and sampling dates

We examined variation at five microsatellite loci (i.e., Gmo2, Gmo4, Gmo120, Gmo132, and Gmo145) in 448 of the 543 cod collected from among the 14 sample populations. We classified alleles as "private", if present in a single sample population, as "rare", if present in between two and 13 of the populations, and as "common", if present in all 14 sample populations. The number of private alleles per sample population ranged between zero and 6 with a mean ± SD of 2.6 ± 1.9 (Table 2). Private alleles represented 22.7% of all alleles present (mean frequency: 0.001). Three of the private alleles were present in two individuals, and each of the remaining was present only once. The mean ± SD number of rare alleles per sample was 80.5 ± 7.6 and rare alleles represented 62% of all alleles present (mean frequency = 0.021, range: 0.002-0.073). The mean ± SD number of common alleles per sample was 5 ± 1.87, representing 15.3% of all alleles present (mean frequency = 0.11, range: 0.04-0.43). The sum of the frequencies of common alleles ranged from 0.178 for Gmo120 to 0.896 for Gmo132. The mean (over loci) sum of frequencies of common alleles was 0.56. Considering all allele types, the mean number of alleles per locus per sample was 21.6. On average, each sample population contained 66.4% of all alleles detected.

When sample populations were pooled according to whether they originated from inshore or offshore locations, 26% (i.e., 39 alleles) of the alleles present inshore were absent offshore (i.e., they are private to the inshore locations) while this figure was only 9.6% (i.e., 13 alleles) for offshore samples. In general, however, inshore and offshore private alleles were at very low frequencies. Twenty four (i.e., 62%) of the inshore private alleles and 10 (i.e., 77%) of the offshore private alleles were represented only once (mean ± SD frequency of private alleles in inshore locations: 0.0022 ± 0.0017, and in offshore locations: 0.0015 ± 0.0007).

Table 2 summarizes the observed and expected heterozygosities, as well as disequilibrium coefficients [i.e., $D = (H_{obs} - H_{exp})/H_{exp}$] for the 14 sample populations. Analysis of Hardy-Weinberg equilibrium using the goodness of fit and the log-likelihood ratio tests indicated departure from equilibrium ($\alpha = 0.0007$ with Bonferroni adjustment given 14 groups and 5 loci) for three of the 10 inshore sample populations in at least one of the five loci examined (BoB-Jun 92: Gmo145; SmS-Jul 93: Gmo120; and SWA-Jun 94: Gmo120). In addition, 10 of the 14 sample populations (of which seven were from inshore locations) showed very low ($p \leq 0.005$) significance values with at least one of the tests for at least one locus.

Microsatellite DNA variation among loci

Table 3 summarizes the level of genetic variability among loci and the probability values for tests of homogeneity of allelic frequency distributions. The mean ± SD number of alleles per locus was 32.6 ± 12.8 with a minimum of 14 for Gmo132 and a maximum of 45 for Gmo4 (Fig. 3). These were also the loci with the lowest and the highest observed and expected heterozygosities, respectively (Table 3).

Allele frequency distributions were heterogeneous for four of the five loci examined (i.e., Gmo2, Gmo4, Gmo120, and Gmo145) when all 14 sample populations were compared (Table 3a). For Gmo2 and Gmo145, the heterogeneity of allele frequency distributions was likely due, at least in part, to differences among the three and four inshore locations sampled in 1992 and 1993 respectively (Table 3b and 3c). No heterogeneity of allele frequency distributions was detected among inshore locations sampled in 1994 (Table 3d). Similarly, there was no evidence for lack of homogeneity in allele frequency distribution among years for inshore or offshore sample populations when they were pooled within year (Table 3e and 3f).

Allele frequency distributions differed between inshore samples (pooled) and offshore samples (pooled) for Gmo120 but not for the other loci (Table 3g). This was true also when the inshore sample was restricted to individuals with high ($\geq$0.20°C) thermal hysteresis (Table 3h), but not when it was restricted to those with low or near zero ($<$0.09°C) measures of thermal hysteresis (Table 3i, and also see below).

Genetic distances among locations and sampling dates

We calculated Rogers', allele sharing, and average squared distances among all 14 sample populations (Fig. 4) and then estimated their significance by bootstrapping. The mean (± SD, range) Rogers' distance was 0.1221 (± 0.0006, 0.09-0.17; Fig. 4a) and none of the pairwise comparisons differed significantly from zero (α = 0.0005 after Bonferroni adjustment for 91 simultaneous tests, $p \geq 0.003$ and $p > 0.05$ in 84 comparisons). The mean (± SD, range) allele sharing distance was 0.40 (± 0.0017, 0.28-0.53) and four of the 91 pairwise comparisons differed significantly from zero (p=0.000). Three of these four comparisons were between inshore and offshore samples from 1992 and 1993. The fourth comparison involved two of the offshore samples. Note, however, that four is approximately the number of comparisons out of 91 that might be expected to differ from zero by chance alone. The mean (± SD, range) average squared distance was 220.48 (± 16.80, 188.76-261.38) and none of the pairwise comparisons differed significantly from zero ($p \geq 0.063$; Fig. 4c).

To summarize thus far, 12 of the 14 sample populations contained private alleles. Twenty six percent of the alleles present inshore were absent offshore, and the corresponding figure for offshore samples was less than 10%. Although the number of inshore and offshore private alleles can be expected to decrease with increasing sample sizes, evidence from a related study in which approximately 1300 cod were assayed for the same microsatellite loci (Ruzzante, D. E., C. T. Taggart, and D. Cook unpublished) suggests the sample sizes in the inshore-offshore comparison (i.e., $N_{inshore}$ = 308, $N_{offshore}$ = 140) were sufficiently large for this decrease to be negligible.

Three of the 10 inshore sample populations showed evidence of departure from Hardy-Weinberg equilibrium for one of the five loci examined. Allele frequency distributions were, with the exception of Gmo132, heterogeneous when comparing all 14 sample populations (Table 3). The allele frequency distribution for Gmo120 was heterogeneous when comparing offshore cod with all cod sampled inshore or only with those showing high antifreeze levels. Genetic distances appeared to be significant for only four out of 91 pairwise comparisons when measured with the allele sharing method and are expected to occur at this frequency by chance alone. None of the genetic distances between pairs of sample populations differed from zero when measured with either Rogers' or the average squared distance.

Genetic distances and population structure between inshore and offshore cod

We pooled individuals according to whether they were caught at inshore or offshore locations. For individuals collected inshore we used antifreeze level in the plasma, which in cod is correlated with length of exposure to sub-zero temperature (Goddard and Fletcher 1994; Goddard et al. 1994), as an indicator of overwintering grounds.

Overwintering grounds as inferred from measures of thermal hysteresis

High antifreeze levels are typical of fish overwintering in cold (<0°C) inshore water, while near zero or low levels are typical of fish overwintering in warmer (>2°C) water masses, characteristic of the deep offshore area. Individuals with thermal hysteresis (≥0.20°C) are known to have spent at least 20 days in sub-zero waters and higher levels are indicative of longer periods in sub-zero waters (Goddard et al. 1994). Consistent with this evidence, we found that average measures of thermal hysteresis among the nine sample populations for which scores were available were significantly correlated (r^2=0.60, p<0.01) with water temperatures at the time and location of cod sample collections and declined exponentially with increasing temperature (Fig. 2). High population average measures of thermal hysteresis (>0.2°C) were associated with the sub-zero temperatures found at collection depths in the SW Arm of Trinity Bay between April and June. Relatively low average levels (<0.2°C) were consistently found in offshore sample collections (Table 1). However, the North Cape collection (offshore) in January 1993 differed from all other collections in that the mode (0.07°C) and median (0.07°C) values of thermal hysteresis were strikingly dissimilar from the mean (0.18°C), reflecting a strongly skewed distribution driven by five of the 19 individuals in the sample with thermal hysteresis values >0.2°C (Fig.2). These individuals were all less than 40 cm in length, and were considered to be juveniles. The physiology of antifreeze production is different in adult and juvenile cod, juveniles being capable of producing antifreeze at temperatures as high as 3°C during the winter. This accounts for the high antifreeze levels present in cod overwintering offshore.

Cod collected inshore were thus separated into two groups: (1) individuals with high thermal hysteresis (≥0.20°C) indicating inshore overwintering, and (2) individuals with near-zero thermal hysteresis (≤ 0.09°C), the majority of which are expected to be inshore migrants recently arrived from offshore. However, the possibility that this last group also includes inshore overwintering individuals that have been exposed to a warmer (near surface) water mass in the inshore for a period long enough to loose antifreeze (See Fletcher et al. 1987) cannot be eliminated. We excluded from the analysis individual cod with values of thermal hysteresis between 0.09°C and <0.20°C because of the uncertainty regarding their overwintering grounds.

Genetic distances

Rogers' genetic distances between inshore and offshore sample pools range between 0.055 and 0.077 and none of them was significant (p ≥ 0.060, Table 4) regardless of whether offshore cod were compared to all inshore cod, or to those with known high (≥0.20°C) or near-zero (≤0.09°C) thermal hysteresis. Similarly, Rogers' genetic distance between inshore cod with high thermal hysteresis and those with near-zero thermal hysteresis was not significant (p = 0.077, Table 4). Average squared distances ranged between 202.26 and 225.83 and none was significant (p ≥ 0.404).

Allele sharing distances between inshore and offshore sample pools ranged between 0.292 and 0.319 and were significantly different from zero in two cases: when offshore cod were compared with all inshore cod (p = 0.000), or with inshore cod with known high (≥0.20°C) thermal hysteresis (p=0.000; Table 4). Of the 39 inshore private alleles (discussed above), 37 were found among the 228 cod (i.e. 74% of all cod caught inshore) with high thermal hysteresis (≥0.20°C) or for which no thermal hysteresis score was available. Allele sharing distances did not differ significantly from zero for inshore cod with near-zero thermal hysteresis versus offshore cod, or for inshore cod with high thermal hysteresis versus those with near-zero thermal hysteresis (Table 4)

When years were analyzed separately, allele sharing distances between all inshore cod and offshore cod, or between inshore cod with high thermal hysteresis and offshore cod remained significant or highly significant for 1992 and 1993. For 1994, p-values were low but not significant at $\alpha = 0.05$ ($p = 0.061$; and $p = 0.097$; Table 4).

We also compared the group of cod collected from an inshore spawning aggregation on Heart's Ease Ledge (HEL) in July 1993, but for which no antifreeze records were available (See Table 1), with offshore cod sampled in 1993, and with the group of inshore cod with high (≥0.20°C) thermal hysteresis sampled in 1993. The cod from the inshore spawning aggregation differed significantly in allele sharing distance from offshore cod ($D = 0.528$, $p = 0.000$; Table 4). They were also marginally different from inshore cod known to have high thermal hysteresis ($D = 0.425$, $p = 0.036$, Table 4).

The low p-value for this last comparison (HEL vs inshore cod with high thermal hysteresis) prompted us to test whether pairs of random samples from the inshore-high-antifreeze group would be different from each other. We found that, on average (test repeated 10 times with different random collections), the two random samples were not different ($p = 0.168$). Thus, these results indicate that individuals from the inshore spawning aggregation (HEL) are clearly different from offshore cod, but they also suggest they may be somewhat different from other inshore cod aggregations. We do not have sufficient data at present to test this hypothesis further.

Population structure:

Table 5 summarizes the evidence for population structure as measured by Rst (Slatkin 1995) exhibited by the five loci separately and combined. There was no evidence for significant population structure when all 14 sample populations were considered (Table 5a), or when the pooled offshore cod were compared to all pooled inshore cod regardless of thermal hysteresis (Table 5b). However, when only individuals with high thermal hysteresis were considered in the inshore sample, inshore and offshore cod appear to have come from genetically distinct populations, i.e., Rst differed significantly from zero for Gmo120, Gmo145, and for the five loci combined (Table 5c). Note that Gmo120, and Gmo145 (and also Gmo4) exhibited marked differences in allele frequencies among the three groups (i.e., inshore-high-antifreeze, inshore-low-antifreeze, and offshore), even for relatively common alleles (Fig. 5). Furthermore, Gmo120 and Gmo145 both exhibited significant departures from HWE in some of the inshore sample populations (Discussed above). There was no evidence that inshore cod with near-zero thermal hysteresis and offshore cod come from separate populations, whether each locus was considered separately or all loci were combined (Table 5d). Inshore cod with high thermal hysteresis did not differ significantly from those with near-zero thermal hysteresis, reflecting the likelihood that the group of inshore cod with near-zero thermal hysteresis can include inshore migrants recently arrived from offshore as well as cod that overwintered in sub-zero inshore water and were later exposed to warmer near-surface water masses.

Table 5 also shows the level of population structure as determined by Fst. Contrary to Rst, Fst was not significantly different from zero in any of the comparisons tested indicating the Fst measure fails to detect evidence of population structure with this data.

We also calculated the number of migrants among subpopulations expected under the Rst and Fst models according to Slatkin (1995, equations 15a and 15b, p. 459). Although estimates of the number of migrants may be meaningless when the estimates of population structure (from which the number of migrants are derived) are not different from zero, they are included in Table 5 for comparative purposes. The expected number of migrants were considerably higher (up to two orders of magnitude) when derived under the Fst than under the Rst model (Table 5).

DISCUSSION

We have provided evidence, based on microsatellite DNA variation, that cod populations overwintering in inshore Newfoundland, and identified as such by their high antifreeze levels, are genetically distinguishable from cod populations overwintering offshore along the edge of the continental shelf on the Grand Banks.

Correlation of genetic distance with overwintering grounds as determined by thermal hysteresis

We were able to use blood plasma antifreeze levels to categorize individuals into inshore or offshore overwintering aggregations because adult cod produce antifreeze during the winter if they are continuously exposed to sub-zero temperatures for a period of weeks - months (Goddard *et al.* 1994) but not if they remain in warm (>2°C) water during the winter (Fletcher *et al.* 1987). The inshore and offshore overwintering environments are generally sub-zero and >2°C, respectively. Therefore overwintering in these two different thermal environments results in the antifreeze categories used in this study.

Typically, cod move to their offshore overwintering grounds in early winter and begin to form spawning aggregations on the outer slopes of the continental shelf (Lear and Green 1984). The landward feeding migration of cod that have overwintered and spawned offshore begins in spring, with the majority of adults arriving inshore in June-July (Lear and Green 1984). In this study, samples were collected from offshore areas in January, February and June, and from inshore areas during April, June and July. Knowledge of northern cod migration patterns coupled with the sampling dates suggests that while the samples collected offshore were from offshore overwintering individuals, the inshore samples could have come from inshore overwintering cod or from individuals newly arrived from offshore in the spring. However, since high antifreeze levels are only seen in cod residing for weeks-months in sub-zero temperatures, thermal hysteresis was used as a valid method of discriminating between the two overwintering groups. From May onwards, cod that have overwintered inshore move from cold and deep water to warmer surface waters, and gradually lose their plasma antifreeze (Fletcher *et al.* 1987; Goddard *et al.* 1994). For this reason, cod caught inshore with thermal hysteresis values between 0.09°C and 0.2°C could not be assigned to either group with certainty and were, therefore, excluded in some of our analyses.

Five individual cod from samples collected offshore and classified as juveniles on the basis of length, showed elevated antifreeze activity (Fig. 2). This is the result of a difference in physiology between adult and juvenile cod. During the winter when exposed to the same seasonal conditions of temperature and photoperiod, juvenile cod produce significantly more antifreeze than adults and can produce antifreeze at temperatures as high as 3°C (Goddard *et al.* 1992). It is extremely unlikely that some of these offshore juveniles with high thermal hysteresis would migrate inshore during the winter. Nevertheless, if such juveniles were present in our inshore samples, they would be indistinguishable from the group of inshore cod on the basis of thermal hysteresis alone. Despite this possibility, we detected genetic differences between inshore and offshore cod which suggests that our estimates of sub-population structure may be conservative. Clearly, age, length, physiology, time of year, and available thermal environment must all be considered when using antifreeze levels to draw conclusions not only about recent thermal history but also about the genetic basis for population structure.

The genetic evidence suggesting population subdivision between inshore and offshore cod described here is consistent with the morphometric differences detected for these groups by Pepin and Carr (1993) and with the concept of local residency or fidelity at coastal bay scales discussed by Wroblewski *et al.* (In press). Cod tagged inshore (Trinity Bay) during winter (January to March) exhibit a high probability of being captured in the local area (68% within 30 nautical miles and 83% within 60 nautical miles) even 3 or more years subsequent to release (Taggart *et al.* 1995; Wroblewski *et al.* In press). Our findings are also consistent with a general pattern of population differentiation exhibited among distinct offshore cod aggregations within the Northern cod complex (Bentzen, P., D. E. Ruzzante, C. T. Taggart, and D. Cook unpublished) which reflect Lear's (1984, p.157) observation of cod "homing to specific offshore overwintering

and spawning areas, although with some straying".

Comparison among different genetic measures

The sub-population structure between inshore and offshore cod was detected with the allele sharing distance (Bowcock *et al. 1994*), and Rst estimate (Slatkin, 1995), but not with Rogers' distance, average squared distance, or the Fst estimate (Tables 4 and 5). The discrepancy between methods is not unexpected. The allele sharing distance is based on the proportion of alleles that are shared between populations regardless of their frequencies, and is thus more heavily influenced by private and rare alleles than either Rogers' or the average squared distance estimates. However, Rst and the average squared distance generated contrasting results despite the fact that both methods are based on a stepwise mutation model which assumes the mutation process has a "memory" of the prior allelic state (Goldstein *et al.* 1995; Slatkin, 1995; see also Valdes *et al.* 1993; Shriver *et al.* 1993; Di Rienzo *et al.* 1994, and references therein). Furthermore, both methods were specifically developed for microsatellite loci and thus, account for their high mutation rates. We suggest that the discrepancy is due to the fact that Rst is a measure of the variance in allele lengths that remains between populations once the variance within populations has been accounted for, whereas the average squared distance is a measure only of the variance between populations. The discrepancy thus implies that Rst is a more sensitive measure of sub-population structure and may be more appropriate for estimating demographic parameters (e.g., migration rates) among sub-populations of a single species than the average squared distance. Goldstein *et al.* (1995) also suggested that the average squared distance is appropriate for more distantly related groups, i.e., is expected to reconstruct phylogenies more accurately than the allele sharing distance only after approximately 500 generations from the time of divergence.

Finally, the genetic difference between inshore overwintering and offshore overwintering cod was detected with Rst but not with Fst. Slatkin (1995) concluded that under the assumptions of a generalized stepwise mutation model at microsatellite loci, "Rst will generally provide less biased estimates of demographic parameters ... than ... Fst". This suggests that, if the generalized stepwise mutation model accurately mimics the mutation process at microsatellite loci, infering high levels of migration or historical association between populations from the lack of detectable variation with the Fst measure would be erroneous.

Contrast between microsatellite and mtDNA evidence

Our results contrast sharply with those derived from the analysis of DNA sequence variation in the cytochrome b region of mitochondrial DNA, which failed to detect sub-population structure within the northern cod complex (Carr and Marshall, 1991a,b; Pepin and Carr, 1993). Carr *et al.* (1995), in particular, compared inshore "bay" cod (also from Trinity Bay) with offshore cod and found no evidence that cod overwintering in Trinity Bay are genetically distinct from offshore cod. In their study, a single common genotype occurred at a frequency greater than 80% and no alternative genotype occurred at frequencies higher than 3%. The contrast between Carr *et al.'s* (1995) results and ours is all the more remarkable when one considers that individual cod assayed for mitochondrial DNA variation in their study and those assayed for nuclear DNA variation in the present paper were all drawn from the same spawning aggregation at Heart's Ease Ledge during July 1993, and those used in this study show significant allele sharing distances with offshore samples (Table 4). The fact that sub-population structure between inshore and offshore cod off Newfoundland can be detected with microsatellite DNA allele length variation but not with sequence variation in the cytochrome b region of mitochondrial DNA suggests the latter technique might not be sufficiently sensitive to address questions of stock structure in cod at small spatial and perhaps temporal scales. There may not be enough variation in mtDNA to distinguish populations; for example almost no interspecific variation was observed in the cytochrome b region of mtDNA among East African cichlid fish from Lake Victoria (Meyer *et al.* 1990; reviewed in Meyer 1993). Our results support Park and Moran's (1994) assertion that the "potential for detecting variation is much greater" in nuclear DNA (e.g., vntr's) than in mtDNA studies, and that if genetic differences exist, they are more likely to be detected by nuclear than

by mitochondrial DNA studies (see also Carvalho and Hauser 1994; and Ward and Grewe 1994). The contrast between mtDNA and microsatellite DNA analysis in resolving population structure may be related to the fact that, due to the lack of recombination, mtDNA behaves as a single locus, whereas many independent loci can be analyzed using microsatellite DNA probes (Wright and Bentzen 1994). In addition, microsatellite DNA loci can show extremely high levels of allelic variation, even higher than levels shown by mtDNA. For example, Carr *et al.* (1995) found a maximum of 17 mtDNA alleles, while the two most variable microsatellite loci in the present study, Gmo4 and Gmo120, had 45 and 43 allelic variants, respectively (Table 3). Another advantage of using microsatellite loci is that they are codominant markers inherited in a Mendelian fashion, in contrast to the haploid mtDNA which is predominantly maternally inherited (see Gyllensten *et al.* 1991, and Margoulas and Zouros 1993, for exceptions). The former can be tested for Hardy-Weinberg expectations and can thus provide additional information about population structure. The difference in the mode of inheritance between mitochondrial and microsatellite DNA suggests the combination of both techniques may be very powerful in resolving questions of population structure for species with differential migration or dispersal rates between sexes (see e.g., Morin *et al.* 1994 for a study based single sequence repeat nuclear loci and mtDNA on chimpanzees).

In summary, we have demonstrated the existence of significant sub-population structure between inshore overwintering cod from Trinity Bay, Newfoundland, and offshore overwintering cod on the Grand Banks. These two sub-populations remain genetically distinct despite the fact that individuals from both regions intermingle during most of summer and fall as a result of the inshore feeding migration by offshore individuals. It is not known if there is any mixing during the formation of spawning aggregations, but our results suggest that there is little genetic mixing. Our results provide evidence of genetic differences among populations within the northern cod complex at a smaller spatial scale than the current management divisions. Future efforts to rebuild the (currently collapsed) cod fishery should consider the possibility of differential contribution to recruitment by inshore and offshore stock components.

ACKNOWLEDGMENTS

We are grateful to C. George, W. Eddison, K. Smedbol, G. Rose and the Captains, crews and technical staff of the vessels SHAMOOK, WILFRED TEMPLEMAN, GADUS ATLANTICA and NORTHERN QUEST for assistance in securing cod samples and in making hydrographic measurements. We thank M. H. Kao for assistance in the blood plasma analysis and S. Neale for assistance in microsatellite analysis. We thank W. Blanchard (Statistical Computing Service, Dalhousie University) for his valuable help with programming in Splus, and W. Blanchard and D. Hamilton for statistical advice. We also thank M. Ball, R. Doyle, G. Fletcher, S. McConnell, S. Walde, and E. Zouros, for discussions throughout the development of this paper and/or comments on a draft. Funding for this research was provided by the Ocean Production Enhancement Network (OPEN) through the Interim Funding Research Programme (IFRP) which is supported by the Government of Canada and by the Canada/Newfoundland and Canada/Nova Scotia COOPERATION agreements, and by the Department of Fisheries and Oceans Northern Cod Science Programme.

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Table 1. Summary statistics for cod populations analyzed using microsatellite probes.

Sample Identifier	Date	Location	Lat.	Long.	Depth range (m)	Temperature (°C) Mean	Range	Length (cm) N	Mean (SD)	Range	Thermal hysteresis (°C) N	Mean (SD)	Range
NCp	Jan-92	North Cape, Grand Bank	48.50	-49.46	439 - 482	3.1	2.8 - 3.8	82	53.9 (6.5)	44 - 75			
NCp	Jan-93	North Cape, Grand Bank	48.50	-49.50	380 - 380	2.8	2.8 - 2.8	30	34.3 (7.1)	21 - 47	19	0.18 (0.18)	0.07 - 0.63
NCp	Feb-93	North Cape, Grand Bank	48.10	-47.40	935 - 935	3.4	3.4 - 3.4	30	49.0 (3.4)	42 - 57			
NCp	Jun-94	North Cape, Grand Bank	48.30	-49.10	436 - 436	2.5	2.5 - 2.5	50	33.3 (7.2)	21 - 48	41	0.07 (0.01)	0.06 - 0.11
BoB	Jun-92	Bonavista Bay	48.58	-53.90	31 -37	3.2	0.8 - 4.8	17	34.2 (6.9)	26 - 53	7	0.09 (0.09)	0.04 - 0.28
Bvu	Jul-92	Bellevue, Trinity Bay	47.65	-53.74	20 - 20	5.3	5.3 - 5.3	26	27.1 (3.9)	24 - 36			
SWA	Jun-92	SW Arm, Trinity Bay	48.02	-53.90	152 - 154	-1.4	-1.4-(-1.4)	24	48.1 (9.9)	32 - 67	17	0.43 (0.11)	0.24 - 0.63
SWA	Apr-93	SW Arm, Trinity Bay	48.02	-53.93	15 - 32	-0.7	-0.7-(-0.7)	63	61.7 (10.4)	39 - 106	61	0.31 (0.14)	0.07 - 0.65
SWA	Jun-93	SW Arm, Trinity Bay	48.02	-53.90	139 - 216	-0.9	-1.2-(-0.6)	61	38.5 (6.3)	27 - 52	59	0.44 (0.13)	0.09 - 0.76
SmS	Jul-93	Smith Sound, Trinity Bay	48.17	-53.67	14 - 45	1.1	1.1 - 1.1	34	45.1 (8.9)	33 - 74			
HEL	Jul-93	Hearts Ease Ledge,	48.06	-53.59	40 - 40	2.4	2.4 - 2.4	25	49.8 (6.5)	38 - 64			
SWA	Jun-94	SW Arm, Trinity Bay	48.02	-53.87	163 - 275	-1.1	-1.3-(-0.5)	34	42.1 (8.4)	24 - 58	34	0.34 (0.11)	0.07 - 0.61
NWA	Jun-94	NW Arm, Trinity Bay	48.09	-53.74	18 - 150	0.6	-1.0 - 0.9	28	48.5 (7.6)	30 - 72	28	0.14 (0.09)	0.06 - 0.43
SmS	Jun-94	Smith Sound, Trinity Bay	48.22	-53.56	25 - 25	5.5	5.5 - 5.5	39	50.1 (5.0)	43 - 67	39	0.08 (0.01)	0.06 - 0.13

Legend to Table 1: Data provided by date and location (geographic name and decimal degrees of N latitude (Lat) and W longitude (Long)) of sample collection. Depth range of sample collections; water temperature at collection depths (mean and range); sample size (number of fish collected) and mean (SD) and range of length. Thermal hysteresis: N: number of fish assayed, mean (SD) and range of thermal hysteresis Data on thermal hysteresis were available for individuals in 9 (7 inshore + 2 offshore) out of the 14 sample populations.

Table 2. Levels of genetic variation in 14 sample populations of Gadus morhua at 5 microsatellite loci.

sample (population)	Mean sample size per locus	No of alleles private	No of alleles rare	No of alleles total	Mean No of alleles per locus	mean H_{obs}	mean H_{exp}	mean D^*
SWA june 92	23	2	73	100	20.0	.888	.850	.042
BoB june 92	17.6	0	66	91	18.2	.908	.845	.081
Bvu june 92	25	2	75	102	20.4	.822	.837	-.027
SWA apr 93	40	2	83	110	22.0	.875	.854	.021
SWA june 93	40	5	92	123	24.6	.880	.875	.001
SmS july 93	32	6	80	111	22.2	.951	.863	.106
HEL july 93	23.6	3	75	103	20.6	.889	.855	.039
NWA june 94	27.2	1	75	101	20.2	.880	.862	.023
SmS june 94	36.3	1	87	113	22.6	.856	.866	-.018
SWA june 94	30.6	4	85	114	22.8	.853	.872	-.031
NCp jan 92	40	5	84	114	22.8	.850	.873	-.025
NCp jan 93	29.8	2	88	115	23.0	.887	.861	.033
NCp feb 93	24.6	4	74	103	20.6	.925	.853	.088
NCp jan 94	40	0	90	115	23.0	.850	.870	-.021

(*) calculated by averaging over D's for individual loci

Average disequilibrium coefficient=0.022

Table 3. Single locus statistics. N: Number of individuals. n: number of alleles. H_{obs}: observed heterozygosity; H_{exp}: expected heterozygosity. Probability of homogeneity of allelic frequency distributions estimated by Montecarlo simulations (1000 bootstrap samples per comparison). Comparison: (a) 14 sample populations. (b) Inshore 1992, 3 sample populations. (c) Inshore 1993, 4 sample populations. (d) Inshore 1994, 3 sample populations. (e) Inshore 1992, 1993, 1994, samples pooled within years. (f) Offshore 1992, 1993, 1994. (g) Between inshore and offshore samples. (h) Between inshore high antifreeze and offshore samples. (i) Between inshore low antifreeze and offshore samples. (*: $p \leq 0.01$; **: $p \leq 0.002$ with Bonferroni adjustment assuming 5 simultaneous tests.)

						WITHIN YEAR AMONG INSHORE LOCATIONS			AMONG YEARS INSHORE		OFFSHORE	INSHORE VS OFFSHORE ALL	ANTIFREEZE HIGH	ANTIFREEZE LOW
	N	n	Allele size range (bp)	H_{obs}	H_{exp}	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
Gmo2	430	26	92 - 208	0.747	0.804	0.000**	0.006*	0.070	0.363	0.068	0.040	0.488	0.498	0.238
Gmo4	430	45	153 - 267	0.986	0.957	0.008*	0.031	0.483	0.579	0.089	0.034	0.106	0.417	0.321
Gmo120	422	43	134 - 238	0.955	0.954	0.003*	0.516	0.286	0.039	0.108	0.058	0.010*	0.015	0.175
Gmo132	438	14	97 - 131	0.744	0.724	0.339	0.865	0.251	0.137	0.241	0.469	0.166	0.352	0.800
Gmo145	429	35	137 - 215	0.953	0.940	0.002**	0.478	0.000**	0.042	0.011	0.066	0.123	0.042	0.251
Overall	448	163		0.877	0.876									

Table 4. Genetic distances. Rogers', allele sharing, and average squared genetic distances between inshore and offshore sample populations for all three years combined and for each year separately. INSHORE: all inshore individuals; IN-HIGH: inshore individuals with thermal hysteresis ≥ 0.20 °CC; IN-LOW: inshore individuals with antifreeze levels ≤ 0.09. Significance values after Bonferroni adjustment for 3 simultaneous tests, *: p< 0.017; **: p<0.003). Significance levels were estimated by Montecarlo simulations (1000 runs per comparison).

COMPARISON	n_1	n_2	Rogers'	Allele sharing	Average squared
(1) INSHORE VS OFFSHORE	308	140	0.055 (p=0.060)	0.319** (p=0)	220.22 (p=0.414)
(2) IN-HIGH VS OFFSHORE	123	124	0.061 (p=0.340)	0.292** (p=0)	225.83 (p=0.404)
(3) IN-LOW VS OFFSHORE	58	124	0.077 (p=0.329)	0.292 (p=0.246)	202.26 (p=0.794)
(4) IN-HIGH VS IN-LOW	123	58	0.069 (p=0.077)	0.277 (p=0.387)	207.18 (p=0.673)
1992[+]					
(1) INSHORE VS OFFSHORE	68	40	0.106 (p=0.081)	0.333* (p=0.005)	213.90 (p=0.426)
1993[++]					
(1) INSHORE VS OFFSHORE	139	60	0.081 (p=0.104)	0.364** (p=0)	242.88 (p=0.329)
(2) IN-HIGH VS OFFSHORE	69	54	0.085 (p=0.473)	0.383** (p=0)	251.22 (p=0.417)
1994					
(1) INSHORE VS OFFSHORE	101	40	0.094 (p=0.241)	0.308 (p=0.061)	195.22 (p=0.572)
(2) IN-HIGH VS OFFSHORE	38	35	0.121 (p=0.184)	0.346 (p=0.097)	201.81 (p=0.408)
(3) IN-LOW VS OFFSHORE	47	35	0.112 (p=0.278)	0.306 (p=0.245)	193.26 (p=0.639)
(4) IN-HIGH VS IN-LOW	38	47	0.115 (p=0.155)	0.296 (p=0.272)	203.92 (p=0.313)
SPAWNING GROUP 1993 (HEL)					
(3) INSHORE SPAWNING GROUP (HEL) VS OFFSHORE (1993)	25	30	0.132 (p=0.348)	0.528** (p=0)	246.09 (p=0.434)
(4) INSHORE SPAWNING GROUP (HEL) VS IN-HIGH	25	25[+++]	0.118 (p=0.700)	0.425 (p=0.036)	233.34 (p=0.440)

Footnotes:
[+]: Sample sizes for individuals with known antifreeze levels, either high or low, were too low for meaningful comparisons in 1992.
[++]: Sample size for individuals with known low antifreeze levels were too low for meaningful comparisons in 1993.
[+++]: To equalize sample sizes the test was conducted between the 25 individuals from the spawning group (HEL) and a random set of 25 individuals from the IN-HIGH group (n=69). The test was repeated 10 times, each with a different random set of 25 IN-HIGH individuals. Results are averages over the 10 tests. As for the other cases, each test consisted of 1000 bootstrap runs.

Table 5. Population sample structure as determined by (I) Rst, and (II) Fst. Comparison: (a) among all 14 sample populations; between: (b) pooled offshore and all inshore cod; (c) pooled offshore and inshore cod with high (≥0.20 °CC) thermal hysteresis; (d) pooled offshore and inshore cod with low (i.e., ≤0.09 °CC) thermal hysteresis; and (e) between inshore cod with high and inshore cod with low thermal hysteresis (*: p≤0.01; **: p≤0.002 with Bonferroni adjustment assuming 5 simultaneous tests.)

(I) Rst

	14 POP	**INSHORE VS OFFSHORE**			**IN-HIGH VS IN-LOW**
	(a)	**(b)** (IN VS OFF)	**(c)** (IN-HIGH VS OFF)	**(d)** (IN-LOW VS OFF)	**(e)**
Gmo2	0.0800 (p=0.040)	0.0502 (p=0.326)	0.0056 (p=0.047)	0.0712 (p=0.212)	0.1704 (p=0.095)
Gmo4	-0.0047 (p=NA)	-0.0154 (p=NA)	0.0003 (p=0.284)	0.0535 (p=0.356)	0.0297 (p=0.210)
Gmo120	-0.0354 (p=NA)	0.0089 (p=0.364)	0.0131* (p=0.004)	0.0113 (p=0.421)	-0.0101 (p=NA)
Gmo132	0.0111 (p=0.253)	0.0267 (p=0.148)	-0.0004 (p=NA)	0.0085 (p=0.482)	0.0329 (p=0.162)
Gmo145	0.0235 (p=0.080)	0.0005 (p=0.497)	0.0102* (p=0.010)	0.0160 (p=0.441)	0.0115 (p=0.378)
Overall	-0.0002 (p=NA)	0.0037 (p=0.422)	0.0069* (p=0.006)	0.0319 (p=0.232)	0.0357 (p=0.096)
Nm	--	33.70	17.97	3.79	3.37

(II) Fst

	(a)	(b)	(c)	(d)	(e)
Gmo2	-0.0018 (p=NA)	-0.0008 (p=NA)	-0.0024 (p=NA)	0.0044 (p=0.112)	-0.0024 (p=NA)
Gmo4	0.0011 (p=0.228)	0.0004 (p=0.299)	-0.0007 (p=NA)	0.0015 (p=0.199)	0.0004 (p=0.403)
Gmo120	0.0016 (p=0.137)	0.0016 (p=0.050)	0.0027 (p=0.030)	0.0015 (p=0.195)	-0.0015 (p=NA)
Gmo132	-0.0025 (p=NA)	0.0033 (p=0.067)	0.0012 (p=0.256)	-0.0034 (p=NA)	-0.0026 (p=NA)
Gmo145	0.0037 (p=0.018)	<0.0001 (p=0.423)	0.0001 (p=0.419)	-0.0005 (p=NA)	-0.0004 (p=NA)
Overall	0.0006 (p=0.250)	0.0008 (p=0.073)	0.0002 (p=0.344)	0.0008 (p=0.217)	-0.0012 (p=NA)
Nm	416.42	312.25	1249.75	312.25	--

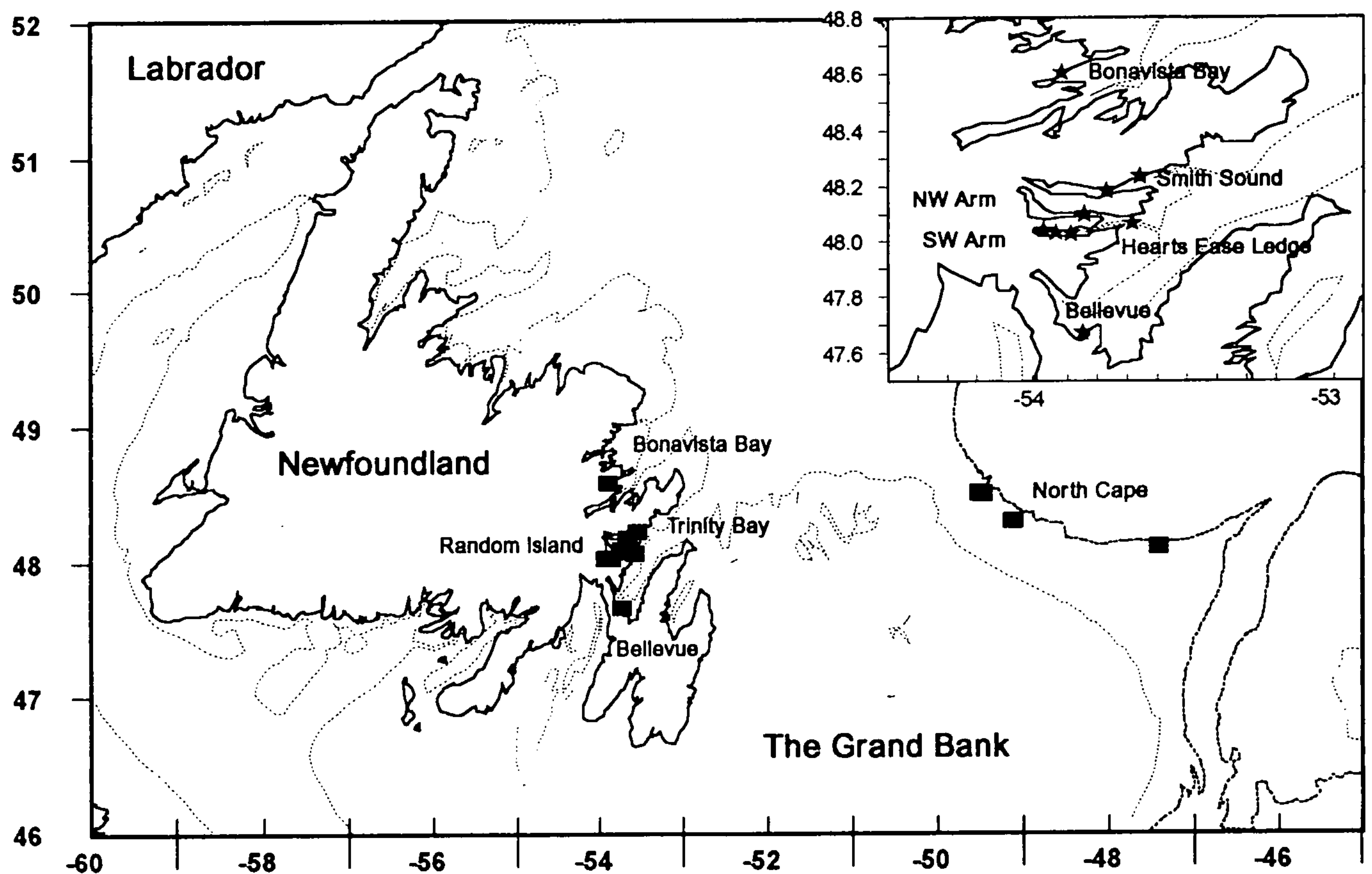


Figure 1. Bathymetric chart (coastline, 200 m, and 1000 m isobaths) of Newfoundland and the northern Grand Banks showing cod collection locations. Samples from offshore are from the North Cape region, samples from inshore are from the Random Island and Bellevue regions of Trinity Bay and Bonavista Bay (enlarged inset). Cod from both regions are considered part of the Northern cod complex (North Atlantic Fisheries Organization, i.e., NAFO, Divisions 2J, 3K, and 3L).

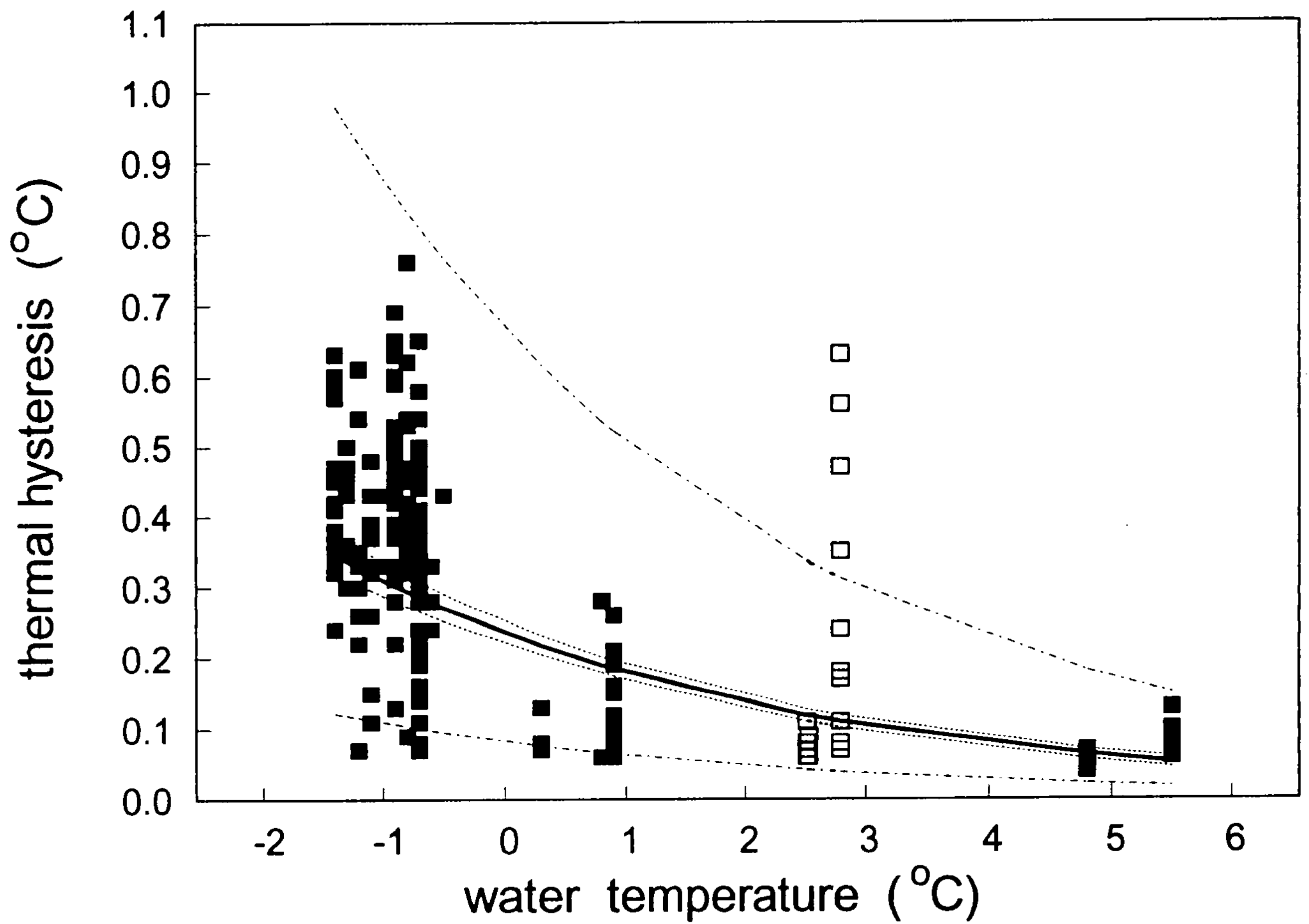
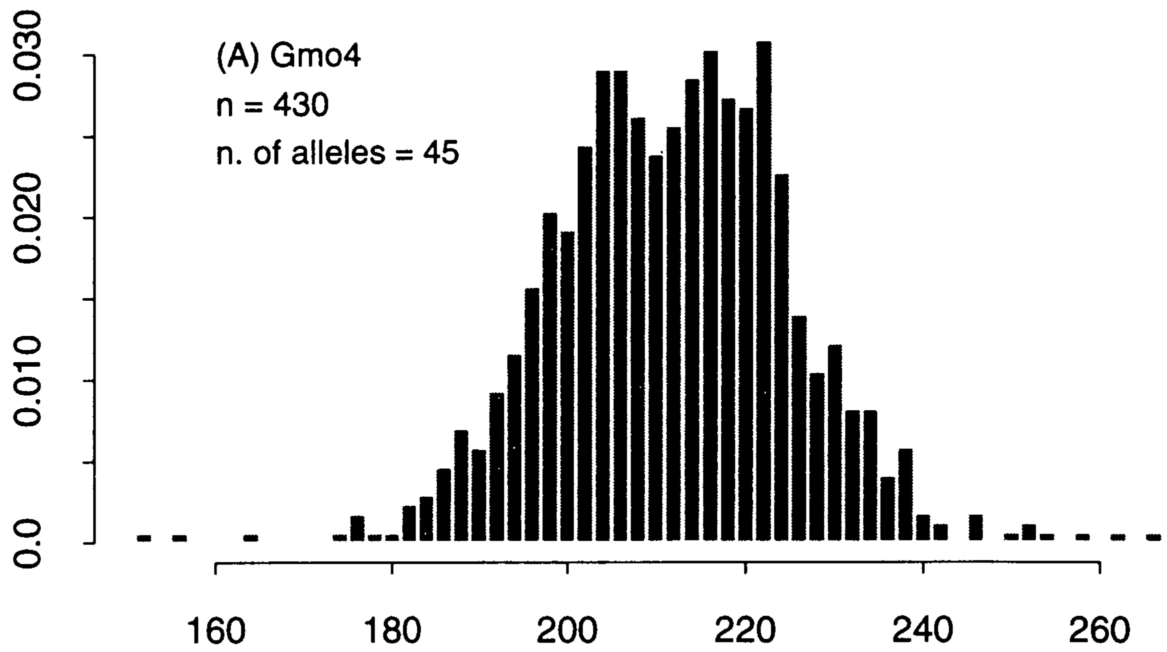


Figure 2. Relationship between cod thermal hysteresis and temperature of the water mass where the samples were collected. The solid heavy line is the fitted relationship (r^2=0.60), the fine dotted lines are the upper and lower 95% confidence limits for the population estimates and the fine dashed lines are the 95% confidence limits for individuals. Each data point is an individual and solid symbols are inshore collections and open symbols are offshore collections.

Figure 3. Allele frequency histograms for loci Gmo4 and Gmo120

FREQUENCY



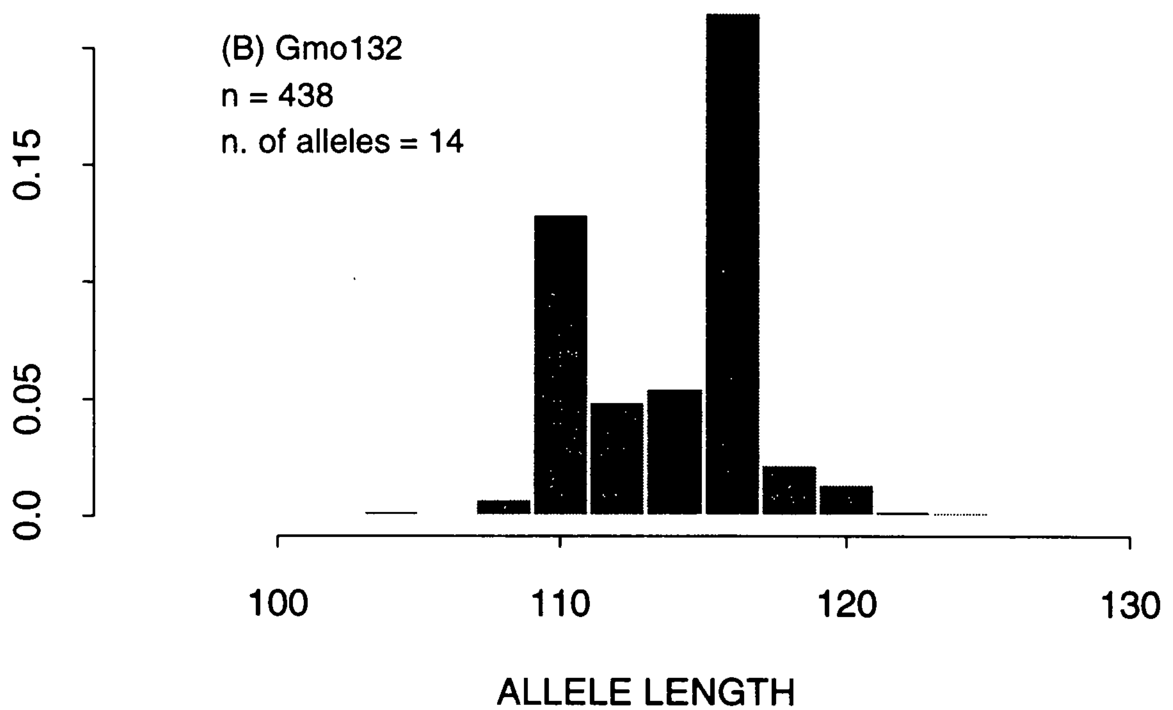


Figure 4. Genetic distance measures among all 14 sample populations: (A) Rogers' genetic distance; (B) Allele sharing distance; and (C) Average Squared distance. The allele sharing distance is based on the proportion, P, of alleles that are shared between the multiple locus genotypes of two populations, averaged over loci. The distance measure between two samples is therefore 1 - P.

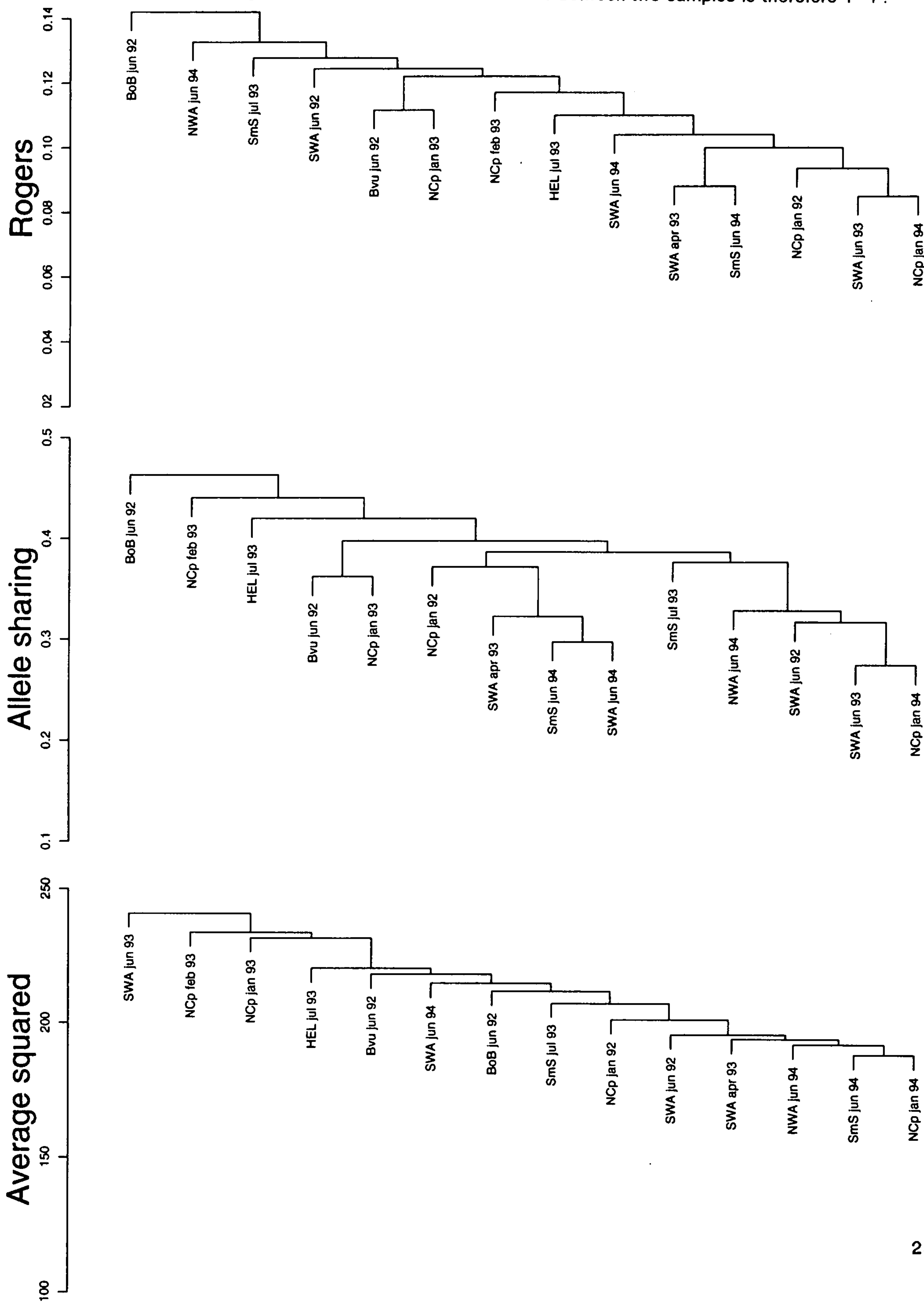


Figure 5. Density functions for inshore high (≥0.20 °C) thermal hysteresis cod, inshore low (≤0.09 °C) thermal hysteresis cod and offshore cod.