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Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada

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Polymorphisms at five microsatellite DNA loci provide evidence that Atlantic cod *Gadus morhua* inhabiting Gilbert Bay, Labrador are genetically distinguishable from offshore cod on the north-east Newfoundland shelf and from inshore cod in Trinity Bay, Newfoundland. Antifreeze activity in the blood suggests that Gilbert Bay cod overwinter within the Bay. Gilbert Bay cod are also smaller (weight and length) for their age and consequently less fecund for their age, than cod elsewhere within the northern cod complex. The productivity and recruitment potential of coastal cod off Labrador may thus be much lower than that of offshore northern cod or of inshore cod farther south, implying that a more conservative management strategy may be required for cod from coastal Labrador than traditionally practised for northern cod inhabiting less harsh environments. Relatively high F_{ST} and R_{ST} measures of population structure suggest that important barriers to gene flow exist among five components that include two inshore (Gilbert and Trinity Bay) and three offshore cod aggregations on the north-east Newfoundland Shelf and the Grand Bank. D_A and D_{SW} estimates of genetic distance that involve Gilbert Bay cod are approximately three- and 10-fold larger, respectively, than estimates not involving Gilbert Bay cod. The differences between inshore cod from Gilbert Bay and Trinity Bay raise the possibility that other genetically distinguishable coastal populations may exist, or may have existed prior to the northern cod fishery collapse. Harvesting strategies for northern cod should recognize the existence of genetic diversity between inshore and offshore components as well as among coastal components.

Key words: genetic structure; microsatellite; *Gadus morhua*; inshore cod; recruitment potential; Labrador.

INTRODUCTION

Intraspecific genetic variation in widely distributed marine fish species is threatened in several important ways (Philipp *et al*., 1995; Ryman *et al*., 1995; Thorpe *et al*., 1995) most notably by local population extinction resulting from excess exploitation and habitat loss, and by species displacement following reduction in population size. At the same time, gene flow among population components in marine fish species is generally high (Ward *et al*., 1994; Shaklee & Bentzen, 1998;

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Waples, 1998). Thus, when addressing conservation objectives for exploited fish stock complexes it is often difficult to determine whether the extent of dispersal and gene flow among components is too low for the stock complex to be managed as a single panmictic unit. Conversely, it is essentially impossible to determine whether gene flow among components is high enough to warrant management of a stock complex as a panmictic unit using genetic data alone (Waples, 1998), and information on the species' ecology, spawning and migration patterns, as well as on the regional oceanography is generally required (Ruzzante *et al*., 1998, 1999).

Despite the difficulties in estimation, some measure of genetic structure, however approximate, is essential for the conservation of genetic resources and for the proper analysis of population dynamics of marine species, especially for populations under intensive exploitation. Failure to recognize the existence of population structure in exploited species can lead to the depletion of less productive populations. Concerns about the potential losses of genetic diversity within the northern cod *Gadus morhua* L., and other cod stocks in the North West Atlantic raised by the recent collapse of these fisheries, have prompted a more accurate quantification of the relationships and mixing patterns among stock components. Such an initiative was taken recently using a mixed stock analysis approach with cod from the Gulf of St Lawrence and vicinity (Ruzzante *et al*., 2000).

Overfishing is the simplest of all hypotheses that have been put forward to explain the decline in abundance of Atlantic cod that resulted in the collapse of cod fisheries in the north-west Atlantic (Myers *et al*., 1996), and this hypothesis has not been refuted as the primary explanation for the collapse (Hutchings & Myers, 1994; Taggart *et al*., 1994; Hutchings, 1996; Myers *et al*., 1997*a*,*b*). Very high fishing mortalities were imposed on both the offshore and inshore components of the complex (Hutchings & Myers, 1994; Taggart *et al*., 1994; Myers *et al*., 1997*a*), which until recently were subject to the same regulations of fishing effort. Recent studies based on microsatellite DNA polymorphism done in concert with estimates of antifreeze protein activity (Ruzzante *et al*., 1996*b*, 1997, 1999; Taggart *et al*., 1998) have demonstrated the existence of temporally stable genetic differences between inshore overwintering cod from the area of Trinity Bay, Newfoundland, and offshore overwintering cod from the northern Grand Bank. These most recent findings are consistent with the hypothesis (Templeman, 1962; R. Wells, unpublished data) that distinct inshore and offshore components make up part of the northern cod complex and that these components either separate at certain critical (i.e., spawning) seasons or do not intermingle greatly. Because of their likely lower productivity, inshore cod populations were at relatively high risk of commercial extinction (Myers *et al*., 1997*a*).

The present paper examines the question of whether cod collected from Gilbert Bay, Labrador, a region that has historically sustained an inshore fishery (Powell, 1987) are genetically and phenotypically (life history and biochemical characteristics) distinguishable from northern cod collected offshore on the north-east Newfoundland Shelf and from cod collected elsewhere from inshore Newfoundland. The results are relevant to the recovery of the northern cod complex and more generally, to the conservation of genetic resources in exploited marine fish.

MATERIALS AND METHODS

SAMPLING

Gilbert Bay

Three samples of adult cod were collected in Gilbert Bay, Labrador (52°38′N, 56°01′W) [Fig. 1(a)] in October 1996 (n=12), May 1997 (n=37) and August 1997 (n=42), respectively. Cod were sampled from two separate locations within Gilbert Bay in each of the October 1996 and May 1997 collections; in August 1997 cod were collected from a single location within Gilbert Bay [Table I; Fig. 1(b)]. No age information is available on the cod collected in October 1996 as these fish were released subsequent to measurement and collection of blood samples (see details below).

The cod collected in May 1997 (n=37) were sexed, weighed (whole body mass, g) and measured (fork length, cm). Sagittal otoliths were removed and used for ageing (years). Fish were assigned to a sexual maturity index following Templeman *et al.* (1978). The wet gravimetric methods of Bagenal (1978) and Kjesbu (1989) were used to estimate the potential fecundity (number of vitellogenic oocytes in the ovary prior to spawning) of each female. Blood samples for genetic and antifreeze analyses (see details below) were obtained from each fish.

The life history and the genetic composition of the cod from Gilbert Bay were compared with four pools of northern cod samples obtained between 1992 and 1995 from two offshore areas on the north-east Newfoundland Shelf (NORTH, n=174 and SAND, n=96), from the North Cape region of the Grand Bank (SOUTH, n=249) and from the inshore region of Trinity Bay, Newfoundland (TRINITY, n=303) [Fig. 1(a)]. These samples were examined in previous studies and details of their collection are found in Ruzzante *et al.* (1996*b*, 1997, 1998) (Table I).

TISSUE COLLECTION AND DNA EXTRACTION

Blood samples were collected and processed for DNA as described by Ruzzante *et al.* (1998). Variation was analysed in five microsatellite DNA loci. PCR amplification and analysis of these five loci (Gmo2, Gmo4, Gmo120, Gmo132, and Gmo145) were conducted as described in Ruzzante *et al.* (1998). These five loci were used in comparisons involving Gilbert Bay cod with offshore cod from the north-east Newfoundland Shelf and the Grand Bank, as well as with inshore cod from Trinity Bay, Newfoundland (Ruzzante *et al.*, 1996*a*, 1996*b*, 1997, 1998).

ANTIFREEZE GLYCOPROTEIN ANALYSIS

Cod collected in May 1997 (n=37) were examined for antifreeze glycoprotein activity in the blood (Fletcher *et al.*, 1987). Upon capture, blood samples were taken from a caudal blood vessel using a 21- or 23-gauge needle and 3-cm^3 syringe. Each sample was placed immediately into a Vacutainer (Becton Dickinson) containing sodium heparin and held on ice until it could be centrifuged later in the day, when the plasma samples were removed from the blood cells, placed in 1·5-ml Eppendorf tubes and stored at $-20°$ C. Samples were later analysed for antifreeze activity by measuring thermal hysteresis (TH, the difference between the sample melting and freezing points). TH is directly proportional to the concentration of antifreeze present in the plasma (Kao *et al.*, 1986) and in northern Atlantic cod can be used as a physiological time tag to estimate the period that an individual fish has been resident in sub-zero degree water (Goddard *et al.*, 1994).

DATA ANALYSIS

Life history

Length and weight data from Gilbert Bay cod were obtained from 36 of the 37 fish captured in May 1997 (one fish was not aged and was removed from analysis). These data were compared with cod collected offshore during a research vessel survey off Labrador (n=177; Shelton *et al.*, 1996) and with cod collected inshore during April 1995 in the Smith Sound area of Trinity Bay [n=50; Fig. 1(a)]. Mean length-at-age from the research vessel survey were obtained from Table 31*a* in Shelton *et al.* (1996) and are not

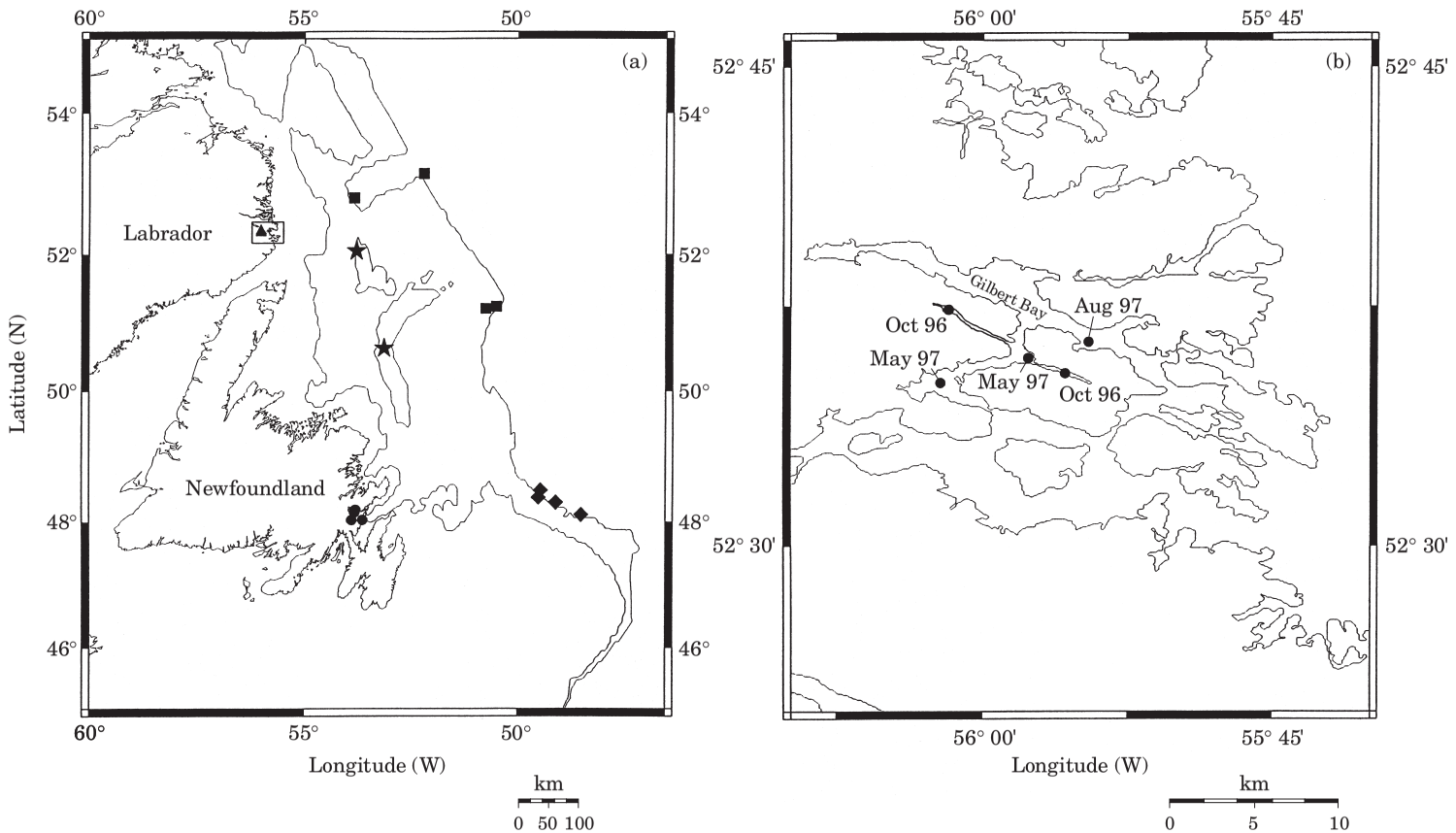

FIG. 1. (a) Bathymetric chart (coastline, 200-m and 400-m isobaths) of the north-west Atlantic showing locations where Atlantic cod were collected for genetic analysis in Gilbert Bay [▲; see (b) for detail]; northern cod, NORTH (■); northern cod, SOUTH (◆); St Anthony Basin and Notre Dame Channel SAND (★) and Trinity Bay (●). (b) Coastline chart of Gilbert Bay, Labrador, showing the nearly land-locked locations where Atlantic cod were collected for genetic analyses in October 1996 (n=12), in May 1997 (n=37), and in August 1997 (n=42).

TABLE I. Summary statistics for Atlantic cod *Gadus morhua* used in morphometrics and antifreeze analysis, and in microsatellite DNA analysis of population structure showing sample size (*n*) for number of individuals, range in latitude and longitude of capture; depth and water temperature range at the capture location; and length and age ranges in each sample

Group	Date of collection	*n*	Latitude	Longitude	Depth range (m)	Temperature range (° C)	Range in length (cm)	Range in age (years)
Gilbert Bay	October 1996	12	52·57–52·62	− 55·99–(− 56·03)	7–20	6·3–7	44·5–70	NA
Gilbert Bay	May 1997	37	52·54–52·59	− 55·93–(− 56·02)	0–3	0–4	38–60	4–15
Gilbert Bay	August 1997	42	52·60	− 55·92	0–28	1·1–8·9	43–73	NA
NORTH (*)	1992–1994	174	51·22–53·15	− 53·83–(− 50·51)	296–500	2·4–3·8	21–50	1–5
SAND (*)	June 1994	96	50·66–52·07	− 53·78–(− 53·13)	401–448	2·3–2·6	23–47	NA
SOUTH (*)	1992–1994	249	46·09–48·49	− 49·51–(− 47·59)	380–462	1·6–3·5	21–75	1–8
TRINITY (*)	1992–1995	303	48·03–48·18	− 53·89–(− 53·63)	15–275	− 1·4–(− 0·9)	24–85	6–9 and NA

NA, Not applicable.

Details on the composition of the samples from NORTH, SAND, SOUTH and TRINITY have been published elsewhere (Ruzzante *et al.*, 1996*b*, 1997, 1998). These samples, which have been analysed in a different context, are marked with *.

weighted by sample size since numbers caught at age were not provided by Shelton *et al.* (1996): they included the years 1978–1995. Statistical analyses of life history data were conducted with the SAS routine PROC GLM (SAS Inc., 1996). Regressions were performed on log transformed data and slopes were compared using an ANCOVA format within PROC GLM.

Genetics

Homogeneity of allele frequency distributions was tested among samples as was departure from Hardy–Weinberg equilibrium (HWE), and genotypic disequilibrium between two loci using χ^2 pseudoprobability contingency tests following Weir (1996). All genetic tests were conducted using Splus© (MathSoft Inc., 1996). Tests of homogeneity were made by randomization of alleles across individuals and populations (1000 bootstrap samples; Manly, 1991). Tests of HWE were made with both goodness-of-fit and log-likelihood ratio tests and were conducted by randomization of alleles within the Gilbert Bay collection. Tests of genotypic disequilibrium were made by permutation of alleles across individuals for the Gilbert Bay collection. Population structure was estimated with F_{ST} (Wright, 1951) and R_{ST} (Slatkin, 1995). Further details on procedures for obtaining these estimates are in Ruzzante *et al.* (1998, 2000). Pairwise genetic distances among populations based on the Stepwise Mutational Model (SMM) were estimated using D_{SW} (Shriver *et al.*, 1995). We also estimated D_A (Nei *et al.*, 1983), a non-SMM estimate of genetic distance with low variance relative to other non-SMM measures (Takezaki & Nei, 1996; Ruzzante, 1998). Significance for both distance measures was estimated by bootstrapping genotypes (1000 resampling trials with replacement) across individuals and populations for each locus separately. In all cases significance levels were adjusted for multiple comparisons using the sequential Bonferroni approach (Rice, 1989). This procedure, however, does not correct for the lack of independence among comparisons.

RESULTS

LIFE HISTORY

Gilbert Bay cod were significantly smaller for their age than offshore cod from the north-east Newfoundland shelf off Labrador, than cod from Trinity Bay (F=31·99, n=213; P<0·001 and F=70·08, n=86; P<0·001, respectively; Fig. 2). There was no difference between Trinity Bay cod and offshore cod in length-at-age (P=0·5464, F=0·36, n=227, Fig. 2). The Gilbert Bay sample reported here is a subsample of a total of 159 cod caught in Gilbert Bay during 1996 and 1997 (Smedbol, 1999). The length (L) at age (A) relationship of this subsample ($L=29{\cdot}5A^{0{\cdot}259}$, n=36) is similar to the relationship reported by Smedbol (1999) for the total sample ($L=33{\cdot}2A^{0{\cdot}183}$, n=159). These two regression lines were not different (P=0·3847, F=0·76, n=196), therefore the subsample of 37 cod for which there is genetic information is unbiased. Thus, Gilbert Bay cod appear to grow more slowly and consequently have a lower fecundity-at-age than offshore northern cod and inshore cod in Trinity Bay (Fig. 3).

ANTIFREEZE ANALYSIS

All 37 cod sampled in Gilbert Bay on 26 May 1997 had antifreeze glycoproteins in their plasma, and almost 80% had TH levels >0·3° C (Fig. 4). The high average TH value (i.e., 0·375, S.E. 0·015) suggests that this sample of

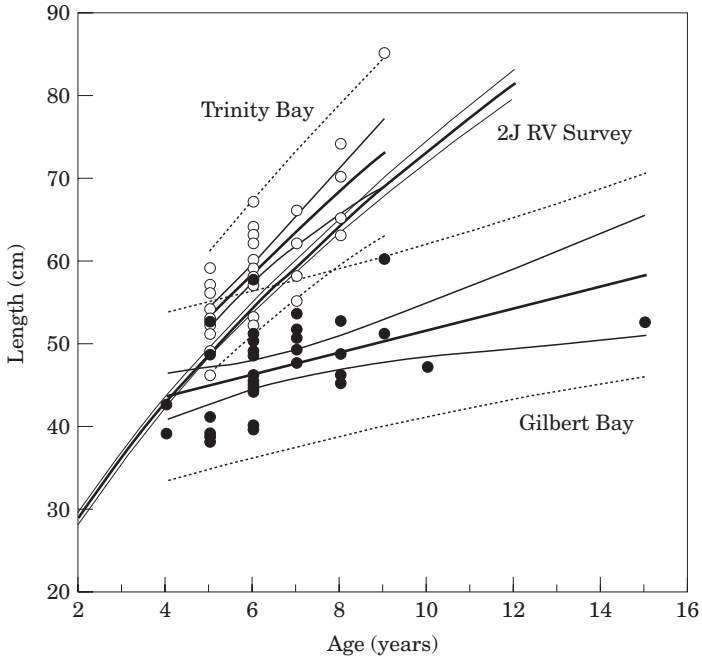

FIG. 2. Length-at-age relation for Gilbert Bay cod (●, $n=36$) collected in May 1997 relative to that for cod from Trinity Bay (○, $n=50$) collected in April 1995 and cod collected during research vessel (RV) surveys off Labrador (NAFO Division 2J) between 1978 and 1995 (based on annual average length-at-age in Shelton *et al.*, 1996; Table 31). For each population the regression (——), and the upper and lower 95% CL for the regression (——) and for the observations (· · ·) are provided.

cod had been exposed to sub-zero temperature water (inshore) for a minimum of 60 days (see Goddard *et al.*, 1994 for method of estimation) and thus can be considered to have overwintered inshore in the Gilbert Bay region.

POPULATION GENETICS: VARIATION WITHIN GILBERT BAY

Single locus statistics, genotypic disequilibrium, and Hardy–Weinberg equilibrium

The number of alleles per locus among cod from Gilbert Bay ($n=91$) ranged between seven (Gmo132) and 25 (Gmo4; Table II), and observed and expected heterozygosities ranged from 0·739 and 0·738 to 0·928 and 0·901, respectively (Table II). There was no evidence of departure from HWE with either the goodness of fit or the log-likelihood ratio tests for any of the five loci ($P \geq 0{\cdot}042$, $\alpha=0{\cdot}010$ after correction for five simultaneous tests). Two independent pairs of loci showed marginal evidence of genotypic disequilibrium among cod from Gilbert Bay ($n=91$; Gmo2–Gmo4: $P=0{\cdot}037$, and Gmo132–Gmo145: $P=0{\cdot}038$, otherwise $P \geq 0{\cdot}133$) but no test of genotypic disequilibrium was significant after a sequential Bonferroni correction for 10 simultaneous tests ($\alpha=0{\cdot}005$).

Multilocus analysis of population structure

Analyses of F_{ST} and R_{ST} estimates among the three temporal collections within Gilbert Bay revealed no evidence of population genetic structure with

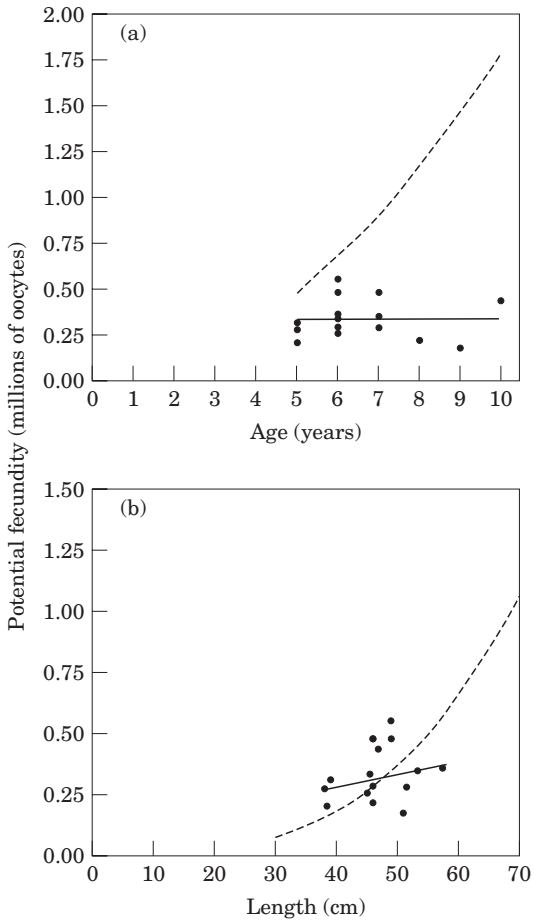

FIG. 3. Potential fecundity as a function of: (a) age (years); and (b) length (cm) for Gilbert Bay cod collected in May 1997 (n=15) and in comparison to the relations for Trinity Bay cod reported by Pinhorn (1984) shown by the dashed curve.

either measure (Table III) when estimated over all loci. When considered individually, one locus (Gmo145) showed marginal evidence of structure with F_{ST} and R_{ST} (Table III).

Genetic distance measures

When measured with D_A, the small (n=12) October 1996 collection of cod from Gilbert Bay appeared distinguishable from cod collected in August 1997 (n=42), and marginally distinguishable from cod collected in May 1997 [n=37; although this last difference disappeared after a Bonferroni correction for three simultaneous tests (α=0·017); Table IV]. There was no difference between the May (n=37) and August 1997 (n=42) collections when measured with D_A (Table IV). When measured with D_{SW}, there was no difference between temporal collections within Gilbert Bay (Table IV).

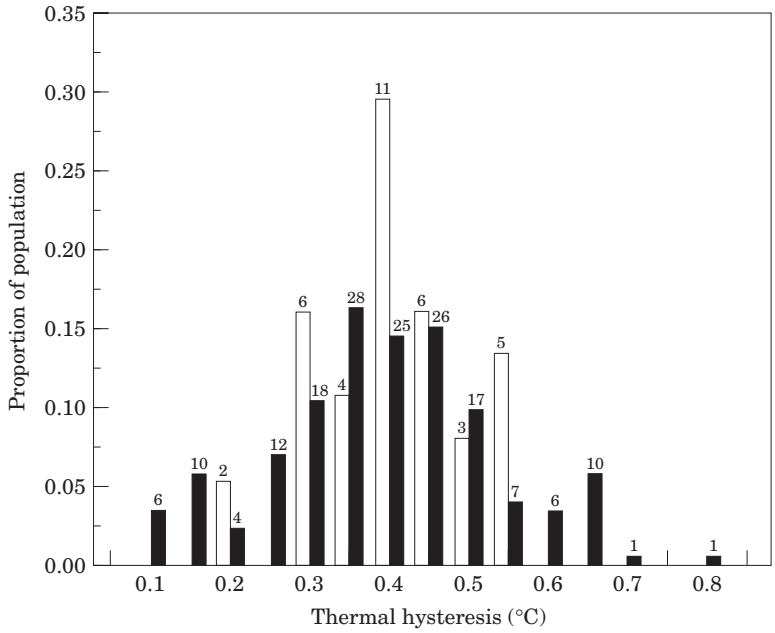

FIG. 4. Frequency histogram of thermal hysteresis (n for each class provided) from individual cod collected in Gilbert Bay in May 1997 (□, $n=37$) and in Trinity Bay (Southwest Arm; ■, $n=171$, data from Ruzzante *et al.*, 1996*b*, 1997) in June 1992 and April and June 1993. Note that the May 1997 cod from Gilbert Bay were all caught in above-zero temperature water (see Sampling and Table I).

TABLE II. Single locus statistics for Gilbert Bay cod ($n=91$) and overall samples as listed in Table I

Locus	Location	n	N	Allele range	H_{obs}	H_{exp}	D
Gmo2	Gilbert Bay	89	8	106–130	0·787	0·763	0·031
	Overall	851	27	100–208	0·770	0·804	− 0·043
Gmo4	Gilbert Bay	83	25	183–237	0·928	0·901	0·029
	Overall	822	57	113–275	0·970	0·958	0·012
Gmo120	Gilbert Bay	88	19	172–212	0·875	0·857	0·021
	Overall	804	47	138–238	0·959	0·950	0·010
Gmo132	Gilbert Bay	88	7	105–121	0·739	0·738	0·001
	Overall	838	15	103–135	0·727	0·731	− 0·005
Gmo145	Gilbert Bay	87	19	159–197	0·908	0·884	0·028
	Overall	834	51	137–221	0·942	0·943	− 0·000

n, Number of individuals; N, number of alleles, allele range given in basepairs; H_{obs} and H_{exp}, observed and expected heterozygosities respectively; D, deficiency of heterozygotes measured as $(H_{obs} - H_{exp})/H_{exp}$.

POPULATION GENETICS: GILBERT BAY COD IN COMPARISON TO OFFSHORE AND INSHORE NORTHERN COD

Single locus statistics

The variation at single loci among cod from Gilbert Bay was compared with that found over all samples including those from offshore locations on the

TABLE III. Estimates of population structure among three temporal samples of adult cod from within Gilbert Bay

Estimate	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Overall
F_{ST}	−0·0042	−0·0033	0·0006	−0·0006	0·0251**	0·0039
R_{ST}	0·0011	−0·0246	0·0013	−0·0261	0·1013**	0·0127

**$P<0·010$.

TABLE IV. Estimates of pairwise genetic distance between temporal samples from Gilbert Bay. D_A above diagonal and D_{SW} below diagonal

Sample	October 1996	May 1997	August 1997
October 1996 ($n=12$)	—	0·175*	0·114***
May 1997 ($n=37$)	0·035	—	0·136
August 1997 ($n=42$)	0·060	−0·080	—

*$P<0·050$; ***$P<0·001$.

north-east Newfoundland Shelf and the Grand Bank, and from inshore locations in Trinity Bay, Newfoundland (Table II). For all five loci, the range in allele size among cod from Gilbert Bay was well within the range of variation over all samples (Table II). Observed and expected heterozygosities over all samples ranged from 0·727 to 0·970, and from 0·731 to 0·958, respectively, and there was no apparent tendency for the Gilbert Bay sample to exhibit higher or lower heterozygosity than overall (Table II). Expected and observed heterozygosities remained largely unchanged whether calculated exclusively among Gilbert Bay cod or overall (Table II).

Population structure

Pseudoprobability χ^2 tests of homogeneity revealed that for each of the five loci examined, allele frequency distributions differed ($P<0·001$) among the five samples of cod from Gilbert Bay ($n=91$), the north-east Newfoundland Shelf area of Hamilton and Belle Isle Banks (NORTH; $n=174$); the St Anthony Basin and Notre Dame Channel area (SAND; $n=96$); the Grand Bank area (SOUTH; $n=249$) and the Trinity Bay area (TRINITY; $n=303$).

Analyses of F_{ST} and R_{ST} estimates among the five components of northern cod revealed evidence of population genetic structure with both measures when considered over all loci (Table V). Single locus estimates indicated that, when measured with F_{ST}, all five loci reflected the structure with the possible exception of Gmo132; whereas when measured with R_{ST} three loci, Gmo4, Gmo120, and Gmo145 reflected the structure (Table V).

TABLE V. Estimates of population structure among Atlantic cod *Gadus morhua* collections from five locations within the geographic range of Northern cod: Gilbert Bay (n=91), north-east Newfoundland Shelf (NORTH, n=174), St Anthony Basin and Notre Dame Channel (SAND, n=96), Grand Bank (SOUTH, n=249), and Trinity Bay (TRINITY, n=303)

Estimate	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Overall
F_{ST}	0·017***	0·011***	0·011***	0·005**	0·008***	0·010***
R_{ST}	− 0·001 (NA)	0·033***	0·010**	− 0·002 (NA)	0·0389***	0·016***

**P<0·010, ***P<0·001.
NA, Not applicable.
Estimates are based on five loci: Gmo2, Gmo4, Gmo120, Gmo132, Gmo145.

TABLE VI. Estimates of pairwise genetic distance between inshore (i.e., Gilbert and Trinity Bay) and offshore (i.e., NORTH, SAND, and SOUTH) components of northern cod *Gadus morhua* complex. D_A above diagonal and D_{SW} below diagnonal

Sample location	n	Gilbert Bay	NORTH	SAND	SOUTH	TRINITY
Gilbert Bay	91	—	0·173***	0·204***	0·181***	0·176***
NORTH	174	0·309***	—	0·052*	0·037**	0·036***
SAND	96	0·590***	0·054***	—	0·044	0·047**
SOUTH	249	0·462***	0·048***	0·037**	—	0·032***
TRINITY	303	0·485***	0·032**	− 0·002	0·016**	—

a=0·05/10=0·005 with initial K of sequential Bonferroni correction (Rice, 1989) K=10.
*P<0·050; **P<0·010; ***P<0·001.
Estimates are based on variability at five loci: Gmo2, Gmo4, Gmo120, Gmo132, Gmo145.

Genetic distance measures

Genetic distance analysis was conducted with the collections from Gilbert Bay considered separately in two groups [October 1996 and May 1997 (n=49), and August 1997 (n=42)] and pooled into a single group (n=91). When considered separately, both groups of samples from Gilbert Bay differed significantly (P<0·001) from all other collections of offshore and inshore northern cod with both measures of genetic distance and they did not differ from each other with either measure (D_A=0·095, P=0·643; D_{SW}=0·018, P=0·241). The lack of difference between the two groups of samples from Gilbert Bay reflects (short-term) temporal stability in the genetic composition of cod in this region with respect to that of the remaining groups of offshore and inshore northern cod. When pooled, cod from Gilbert Bay (n=91) remained significantly different from all other samples with both measures of genetic distance (Table VI). Distance estimates involving the Gilbert Bay cod were in general three- to four-fold larger than those between any pair of northern cod samples when measured with D_A, and approximately an order of magnitude larger when measured with D_{SW} (Table VI). Other pairwise comparisons were also significant with one or both measures of genetic distance: for example, the NORTH

sample (n=174) differed from TRINITY cod (n=303) when measured with D_A and from SAND (n=96), SOUTH (n=249) and TRINITY when measured with D_{SW} (Table VI). SAND and SOUTH differed from TRINITY with both measures (Table VI). The two measures of genetic distance, D_A and D_{SW}, were highly correlated (r=0·974, n=10, P<0·001).

DISCUSSION

Atlantic cod inhabiting Gilbert Bay, Labrador are genetically distinguishable from other inshore and offshore northern cod components. Further, Gilbert Bay cod grow more slowly and consequently exhibit a lower fecundity-at-age relationship than northern cod elsewhere; thus their production and recruitment capacity is much lower. These results indicate that Gilbert Bay cod, as well as other potentially unique components of northern cod, are deserving of conservation measures or at least of managing regimes that take account of their uniqueness. If these genetically distinguishable components are not conserved vigorously, both in terms of population numbers and age structure within the population, they may be extirpated.

Temperature is responsible for the greatest proportion of variation in growth for Atlantic cod throughout their range (Brander, 1994). Thus, the relatively small size-at-age for Gilbert Bay cod suggests that they experience lower temperatures than any other northern cod (offshore and inshore). The high levels of antifreeze activity measured as thermal hysteresis in the plasma lead to the conclusion that the Gilbert Bay cod overwinter in sub-zero inshore waters (temperature below the seasonal thermocline is sub-zero year-round; J. S. Wroblewski, unpublished data) and not in >0° C offshore waters as the majority of northern cod do. The Gilbert Bay cod assessed for antifreeze activity were collected at the end of May 1997 from the surface layer that had a shallow (4 m) thermocline above which temperatures ranged from 2·5 to 5·8° C and below which temperatures were consistently <0° C. The time of year, local temperature and understanding of the pattern of antifreeze production in cod (Fletcher *et al.*, 1987) lead to the conclusion that the antifreeze levels in the cod were decreasing from TH values of *c.* 0·5° C (Fig. 4). Such values are attained after >75 days of <0° C exposure and remain high as long as the fish stay in sub-zero water (Goddard *et al.*, 1994). Antifreeze is lost from the blood plasma at a rate proportional to the temperature of the fish—the biological half-life of antifreeze in cod being 15·6 ± 5 days at 5° C, 37·3 ± 2·9 days at 1° C and >100 days at <0° C (Fletcher *et al.*, 1987). Incursions of cod into the warmer surface layer in Gilbert Bay would initiate antifreeze clearance and provide the range of thermal hysteresis estimates observed (Fig. 4). These observations and the interpretation are consistent with those reported for overwintering cod in Trinity Bay cod (Ruzzante *et al.*, 1996*b*, 1999).

Sonic tagging studies conducted recently in Gilbert Bay (J. S. Wroblewski, unpublished data) show that cod not only overwinter in Gilbert Bay, they remain active in sub-zero temperatures throughout the winter. Observations of their reproductive stages indicate these cod spawn in late spring and early summer, a situation similar to that found in cod that overwinter in Trinity Bay (Smedbol & Wroblewski, 1997; Smedbol *et al.*, 1998) and in cod from Ogac Lake, Baffin

Island (Patriquin, 1967). Spawning by cod within Gilbert Bay, therefore, takes place much later than spawning by cod in the offshore region (January–March; Myers *et al.*, 1993) suggesting temporal barriers to gene flow between inshore cod (both from Gilbert Bay and Trinity Bay) and offshore northern cod. Other lines of evidence for year-round presence of cod within Gilbert Bay include the existence of a historical (at least since 1973) longline, trap, and gillnet fishery in the inner reaches of the bay in the early spring, before the offshore–inshore migrating cod arrive inshore (Powell, 1987). Such a two-stage fishery pattern is consistent with that reported for other embayments in Newfoundland (G. R. Lilly, unpublished data).

Recently, Ruzzante *et al.* (1998) used genetic evidence interpreted within an oceanographic framework, as well as evidence of inshore overwintering (Goddard *et al.*, 1992, 1994; Wroblewski *et al.*, 1994) and of spawning in coastal regions of Newfoundland (Wroblewski *et al.*, 1996; Smedbol & Wroblewski, 1997; Smedbol *et al.*, 1998) to argue that the most appropriate model of stock structure for northern cod was one of several components. One of these components was an along-shelf migratory component with inshore or nearshore overwintering and spawning fidelity along coastal Newfoundland that could show some degree of genetic heterogeneity and thus, reproductive isolation, among inshore regions (Ruzzante *et al.*, 1998). Similar inshore heterogeneity was reported in Ruzzante *et al.* (1996*b*, 1997). In fact, there is historical precedence for considering ‘ north–south and inshore–offshore sub-stocks ’ (Templeman, 1962) related to the idea that ‘ there are probably substocks within this complex ’ (R. Wells, unpublished data). Four decades ago Templeman & Fleming (1953) (reproduced as Fig. 11 in Halliday & Pinhorn, 1990) considered the coastal regions of eastern Newfoundland and Labrador as distinct sub-divisions relative to the offshore areas of the north-east Newfoundland Shelf.

The present study has demonstrated that such genetic, life history, and behavioural differences extend northward from coastal Newfoundland to coastal Labrador and in at least two cases the population subdivision (components) observed are identifiable at bay scales. The relatively high and temporally stable (short-term) levels of population structure estimated under both, stepwise mutation model (SMM) assumptions and under non-SMM assumptions, as well as the consistency across individual loci when using F_{ST} (Table III) indicate that there are important barriers to gene flow among the five population components that include two inshore (i.e., Gilbert Bay and Trinity Bay) and three offshore cod aggregations from the north-east Newfoundland Shelf and the Grand Bank region (NORTH, SAND, SOUTH; Table III; see also Table 2 in Ruzzante *et al.*, 1998). Genetic distances illustrate that Gilbert Bay cod are more reproductively isolated than are the other identified components and raises the question of at which geographic scales are coastal cod populations likely to be genetically distinguishable?

Using a combination of genetic evidence and results from tagging experiments conducted over three decades starting in the 1960s and compiled by Taggart (1997), Taggart *et al.* (1998) hypothesized that significant structure in localized coastal cod populations in the North West Atlantic is likely to be revealed at scales of 60–100 nautical miles if studies are focused on spawning aggregations and use a combination of genetic and behavioural metrics (e.g. nuclear DNA

variation and conventional tagging; reviewed in Ruzzante *et al.*, 1999). Assuming other inshore bay cod populations along the north-east Newfoundland Shelf have not already been overfished, it is probable that strong conservation measures, or at the very least a management scheme that takes into consideration the distinctiveness of inshore cod populations with respect to offshore northern cod and arguably to each other, are necessary to preserve the genetic variation among coastal cod populations along the north-east Newfoundland shelf. Studies like the one reported here and others cited above provide some of the first steps toward addressing issues related to the conservation of diversity in exploited, high gene flow, marine fish species.

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