The effects of isolation and colonization history on the genetic structure of marine-relict populations of Atlantic cod (*Gadus morhua*) in the Canadian Arctic

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Abstract: The genetic consequences of extended periods at low population size are fundamental to the conservation of depleted species such as the Atlantic cod (*Gadus morhua*). We compared microsatellite genetic variability among cod populations in Canadian Arctic lakes with that of Gilbert Bay resident and inshore cod from eastern Canada. The Arctic populations had the lowest genetic diversity and were the most strongly genetically structured and distinct. By contrast, eastern Canadian samples expressed high allelic diversity and were not significantly genetically structured or distinct relative to each other, whereas Gilbert Bay resident cod were intermediate to the Arctic and eastern Canadian groups. Our results are consistent with the hypothesis that the Arctic populations were colonized between 8000 and 5000 years ago and have experienced little or no gene flow since that time. Despite isolation at the extreme of the species' range, the Arctic populations have retained relatively high heterozygosities and high genetic effective population sizes relative to census sizes (N_e–N_c ratios). Potential explanations for this include the absence of fishing pressure, allowing for the persistence of large, highly fecund individuals, as well as biotic (e.g., absence of planktivores) and abiotic (e.g., low environmental stochasticity) factors in the Arctic lakes that minimize individual variance in reproductive success.

Résumé : La connaissance des conséquences génétiques de longues périodes de faible densité de population est essentielle pour la conservation des espèces surexploitées, telles que la morue franche (*Gadus morhua*). Nous avons comparé la variabilité génétique des microsatellites de populations de lacs de l'arctique canadien avec celles de morues habitant la baie de Gilbert et les côtes de l'Est canadien. Les populations arctiques possèdent la diversité génétique la plus faible et elles sont celles qui sont les plus structurées et distinctes du point de vue génétique. En revanche, les échantillons de l'Est canadien montrent une forte diversité allélique, ne sont pas significativement structurées génétiquement et ne se distinguent pas l'une de l'autre; les morues vivant dans la baie de Gilbert sont intermédiaires entre les groupes de l'arctique et de l'Est canadien. Nos résultats sont compatibles avec l'hypothèse selon laquelle les populations arctiques ont été établies il y entre 8000 et 5000 ans et qu'elles n'ont connu que peu ou pas de flux génétique depuis cette époque. Malgré leur isolement à l'extrême marge de la répartition de l'espèce, les populations arctiques ont conservé une forte hétérozygotie et des tailles de population génétique effective élevées par rapport aux tailles d'inventaire (rapports N_e–N_c). Les explications possibles de cette situation comprennent l'absence de pression de pêche qui permet la persistance de gros individus à forte fécondité, mais aussi des facteurs biotiques (par ex., l'absence de planctonophages) et abiotiques (par ex., la stochasticité environnementale faible) dans les lacs arctiques, ce qui minimise la variance individuelle du succès reproductif.

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Introduction

The temporal stability and spatial scale of population structure is fundamental to our understanding of population dynamics and species' biology. In freshwater environments, dispersal barriers such as impassable river features or watershed separations can limit gene flow, thus generating high genetic variability and genetic distance among population units. By contrast, there are fewer barriers to dispersal in the marine environment, where it is generally believed that migration by various life-history stages of most marine species is too frequent and too extensive for significant genetic divergence to arise (Ward et al. 1994). Furthermore, factors such as natural selection and historical contact can upset the equilibrium between drift and gene flow, making allelic frequencies difficult to interpret for ma-

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rine fishes (Hilbish 1996). As a result, the genetic effects of isolation and small population size are largely unknown for most marine taxa. This issue is becoming increasingly important as global fisheries continue to deplete many species and populations are driven to unnaturally low numbers for extended periods of time, while remaining aggregations in geographically disjunct locales may experience limited gene flow. A well-known example of this is the Atlantic cod (*Gadus morhua*) in the Northwest Atlantic, where stocks have failed to recover from historically unprecedented declines despite a significant reduction in fishing pressure (Hutchings and Reynolds 2004).

## Population structure of Atlantic cod

Evidence from vertebral data (Templeman 1981), geographic surveys (Hutchings et al. 1993), and tagging studies (Taggart et al. 1995) suggest that Atlantic cod spawning areas in the Northwest Atlantic are spatially discrete and temporally stable. The degree to which these aggregations represent reproductively isolated units has been intensely scrutinized. Studies using allozyme (e.g., Cross and Payne 1978) and mitochondrial DNA markers (e.g., Carr et al. 1995; Árnason et al. 2000) were unable to reject the null hypothesis of no genetic differentiation among putative populations of Northwest Atlantic cod. In contrast, the analysis of variation at highly polymorphic neutral microsatellite markers has revealed weak yet detectable spatial and temporally stable genetic differences between cod overwintering in inshore and offshore Newfoundland waters and between spawning complexes from various offshore regions (e.g., Bentzen et al. 1996; Ruzzante et al. 1996; Beacham et al. 2002). Spatial patterns of genetic differentiation among Northwest Atlantic cod populations are consistent with an isolation-by-distance model, whereby cod from more distant locations tend to be more genetically distinct (Pogson et al. 2001).

Ruzzante et al. (2000) compared microsatellite DNA variability in cod from Gilbert Bay, Labrador, with samples from Trinity Bay, Newfoundland, and the Northeast Newfoundland Shelf and found that samples from Gilbert Bay yielded distance measures 3- to 10-fold greater than analyses excluding Gilbert Bay. They concluded that genetically distinguishable coastal cod populations may exist or may have existed before the collapse of the northern cod fishery. Although Beacham et al. (2002) also found Gilbert Bay to be the most genetically distinct in their analysis of more than 5000 Northwest Atlantic cod, they found that annual variability within bays in northeastern Newfoundland was as great as variation among sites within any year, suggesting no evidence of bay-scale structure.

Marine-relict cod in Canadian Arctic lakes

Three marine-relict populations of Atlantic cod persist in saline coastal Arctic lakes on Baffin Island, Nunavut, Canada, at the northern extreme of the species' range in Canadian marine waters (Hardie and Hutchings 2003). Although Inuit Qaujimajatuqanginnut (traditional knowledge) of large cod in these lakes predates scientific reports, the cod population in Ogac Lake (OG), on the south shore of Frobisher Bay, has been known to science for only half a century (McLaren 1967; Patriquin 1967), and we have recently confirmed that two more populations, Qasigialiminiq (QL) and Tariujarusiq

Fig. 1. Sampling locations of 675 Atlantic cod (*Gadus morhua*) collected from Ogac (OG, *n* = 68), Qasigialiminiq (QL, *n* = 105), and Tariujarusiq (TL, *n* = 98) lakes on Baffin Island (Nunavut), Gilbert Bay (Labrador) (GB, *n* = 100), and Northwest Atlantic Fisheries Organization (NAFO) divisions 3PS (*n* = 68), 4T (*n* = 93), 4W (*n* = 57), and 4X (*n* = 91).
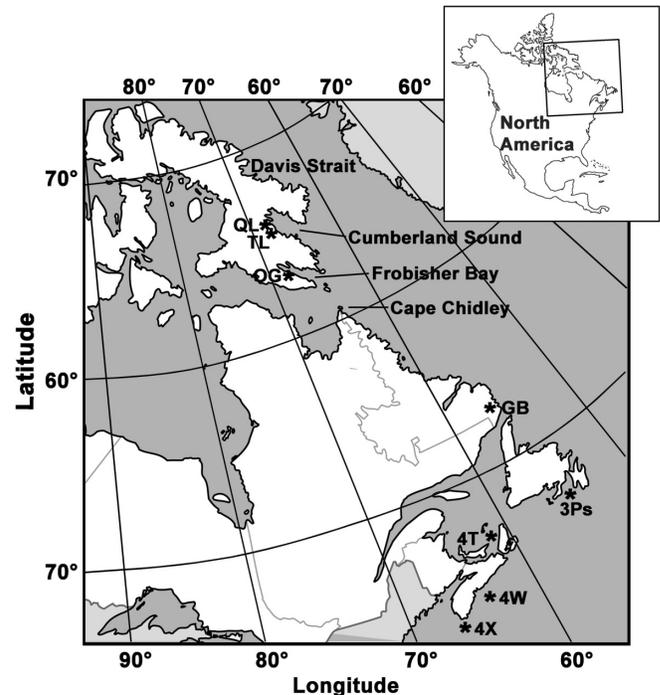
(TL), exist several hundred kilometres to the north, in the southwestern region of Cumberland Sound (Hardie and Hutchings 2003). The mitochondrial cytochrome *b* haplotypes of the cod in all three Arctic lakes are either the most common West Atlantic haplotype (haplotype A; sensu Carr et al. 1995) or single, third-position neutral mutations from this haplotype (haplotypes E and G; sensu Carr et al. 1995; D.C. Hardie and J.A. Hutchings, unpublished data). This supports a shared ancestry between the Arctic populations and progenitors of contemporary Northwest Atlantic cod and suggests that the use of a more rapidly evolving genetic marker is more likely to reveal the evolutionary history of these populations. Brooker (1994) used microsatellites to reveal low allelic richness and heterozygosity among cod from OG and found that they were significantly genetically distant from the two maritime Canadian cod stocks that were analyzed. Here, we extend the analysis using microsatellites to include two more Arctic populations, a bay-resident population, and several other eastern Canadian populations to consider the effects of isolation and colonization history on the genetic structure of the Arctic populations.

Currently, the northern limit of marine Atlantic cod populations occurs near Cape Chidley, at the northern tip of Labrador, and cod are absent from marine waters adjacent to the Arctic lakes (Fig. 1), which raises the questions of how and when these populations were colonized. The colonization and persistence of the Baffin Island populations depend on a suitable combination of (*i*) conditions that fostered a northward range expansion of the cod's range into Frobisher Bay and Cumberland Sound and (*ii*) appropriate changes in rela-

1832

Can. J. Fish. Aquat. Sci. Vol. 63, 2006

tive sea level to create these coastal lakes during the same postglacial period. Because changes in the latitudinal range of Atlantic cod in response to changes in ocean temperatures are known to occur over relatively short time scales (e.g., Jensen 1948), it is plausible that Atlantic cod expanded their range northward during a period of ameliorated Arctic Ocean conditions associated with the collapse of the Laurentide ice sheet between 10 000 and 7000 years before present (ybp) (Dyke 1979; Aitken and Gilbert 1989; Stewart et al. 1993). Several lines of evidence suggest that this region of the Canadian Arctic has experienced a drop of about 70 m in relative sea levels as the result of glacioisostatic rebound over the past 9000 years and that the region is currently at or near pre-Holocene levels (references in Dyke 1979; Doner 2001). What is clear is that all three lakes derive from marine origins and were formed by glacioisostatic rebound over the last 9000 to 5000 years.

The present study

We employ genetic data from seven hypervariable microsatellite loci to elucidate the genetic characteristics of Atlantic cod populations in Arctic lakes at the northern extreme of the species' range compared with coastal and oceanic relatives. We test a hypothesis about the colonization history of these unique populations and use them as a microcosm of marine stocks to examine the genetic consequences of extended periods of isolation and low population size.

Elucidating the evolutionary history and genetic characteristics of these populations is interesting in its own right, as they have persisted for a long time in a highly unusual habitat at the extreme of the species' range. Furthermore, these isolated populations provide a unique opportunity to make inferences about the projected effects of extended periods of isolation at low population size on depleted cod stocks and other high-gene-flow marine species currently of conservation concern. The risk of loss of genetic diversity in stocks held at depressed population sizes for extended periods is heightened by the fact that genetic effective population size (N_e), a metric of the number of mature individuals that successfully contribute to subsequent generations, is invariably much lower than the census population size (N_c).

Our working hypothesis for the colonization history of the Arctic lacustrine populations, based on the geological record and paleobiological information, is that Atlantic cod colonized the lakes no more than 8000 ybp during a northward species' range expansion into Frobisher Bay and Cumberland Sound in response to a postglacial warming period. Aside from a probable period of low gene flow when cod were still present in the area and before the lakes had rebounded to sufficiently high levels to prevent migration, the populations would have been isolated from each other and from marine stocks since that time.

Materials and methods

Arctic study lakes

Ogac, Qasigialiminiq, and Tariujarusiq lakes are coastal meromictic lakes displaying strong vertical stability of thermal and salinity strata, which consist of a warmer, freshwater layer above a deeper saline layer, which itself overlies an anoxic hypersaline bottom layer. Stratification is maintained perennially by the influx of the highest spring tides, which reach 11.9 m in the vicinity of OG (Resor Island) and 6.9 m in the vicinity of QL and TL (Imigen Island), and breach the lake in monthly series during the open-water season (D.C. Hardie and J.A. Hutchings, unpublished data). The freshwater surface layer, which is deepest at the time of cod spawning (Patriquin 1967), may retain cod eggs in the lake, as they are negatively buoyant at low salinities. The lakes contain a simple community of marine organisms, which either persist as truly landlocked forms or are carried into the lake during tidal inflows. Atlantic cod are the only fish species present, and conspecifics make up a large part of the diets of adult cod, particularly of larger specimens (Patriquin 1967; D.C. Hardie and J.A. Hutchings, unpublished data). A resident cod population in Gilbert Bay (GB), Labrador, shares some features with the populations of these Arctic lakes, including strong thermal and salinity stratification and similar freshwater and tidal influences (Morris et al. 2002).

Sample collection

A total of 675 Atlantic cod were collected from eight sites (Fig. 1). Eastern Canadian samples were obtained from Fisheries and Oceans Canada trawl surveys of North Atlantic Fishery Organization (NAFO) divisions 3Ps, 4T, 4W, and 4X in 2002. Gilbert Bay samples were collected by hook and line in 2004 (C.J. Morris, Science, Oceans and Environment Branch, Department of Fisheries and Oceans, St. John's, Newfoundland and Labrador). Samples from Arctic lakes were collected using minnow traps, gill nets, and hook and line in 2003 and 2004.

Microsatellite DNA

Genomic DNA was extracted from tissue stored in 95% ethanol, using the DNEasy Tissue Kit following manufacturer's instructions (Cat. No. 69504; QIAGEN Inc., Mississauga, Ontario, Canada). Six individuals were duplicated in each 96-well extraction plate to ensure consistent scoring of microsatellite alleles. The following seven microsatellite loci were amplified by polymerase chain reaction (PCR) using a PTC-200 thermal cycler (MJ Research PTC-0200; Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) and following conditions specified by the following authors: *Gmo3, Gmo8, Gmo19, Gmo34, Gmo35* (Miller et al. 2000), *Tch5* (O'Reilly et al. 2000), and *Mae9* (O'Reilly et al. 2002). Reactions were carried out in 10 µL volumes containing 50 mmol·L^{-1} KCl, 20 mmol·L^{-1} Tris (pH 8.4), 0.2 mmol·L^{-1} of each dNTP, 0.5 mmol·L^{-1} of each Hex-labelled primer, 0.8 µL *Taq* DNA polymerase, and 20–100 ng of template DNA. Following PCR, 3 µL of product were added to 8 µL of deionized formamide (5% *w/v* bromophenol blue), denatured for 10 min at 95 °C, and size-fractioned on a 0.4 mm, 6 mol·L^{-1}, 6% denaturing polyacrylamide gel for 1–2 h (depending on the size of the expected product) alongside a 4 bp allelic ladder constructed out of genomic DNA (run next to a commercial 60–400 bp fluorescent (CRX) ladder (Cat. No DG6221; Promega, Madison, Wisconsin)). Ladder and microsatellite alleles were visualized using an FMBIO II® florescent imaging system (Hitachi Software Engineering America Ltd., San Francisco, California). The six duplicated samples from each extraction plate were scored blind and checked against each other for consistency. All gels were

scored by two individuals and either run again (28 individuals at various loci) or excluded from analysis (three individuals were excluded at all loci) if there were any inconsistencies.

Statistical analysis

Microsatellite diversity within each sample was tested for observed (H_O) and expected (H_E) heterozygosities, Hardy–Weinberg equilibrium (HWE) for each locus in each population (probability test), and evidence of linkage disequilibrium, using GENEPOP 3.4 (Raymond and Rousset 1995). Mean allelic richness (A) was corrected for a minimum sample size of 57 (A_c), using the rarefaction method of Goudet (2001), and compared using analysis of variance (ANOVA; Analyse-It Software Ltd., Leeds, UK). Allele distributions were compared, and global and pairwise variance in microsatellite allele identity (F_{st}; Wright 1951) and size (R_{st}; Slatkin 1995) were estimated using GENEPOP 3.4. Significance tests for F_{st} were performed by bootstrapping across loci 1000 times per individual comparison (Goudet 2001).

We compared variance in R_{st} and F_{st} to test the relative importance of mutation (R_{st}) and drift (F_{st}) for population differentiation, using SPAGeDi 1.1 (Hardy and Vekemens 2002) following Hardy et al. (2003). This test does not rely on assumptions of constant population size or mutation–drift equilibrium and is expected to remain exact even when these assumptions are violated. Although the test is sensitive to nonneutrality of loci if different alleles size ranges are selected for in different populations, it is robust if there is selection for the same range of allele sizes across the species' range (Hardy et al. 2003). This approach has been used in other studies of north temperate fishes to infer dynamics of colonization history (e.g., Fraser and Bernatchez 2005). We tested the null hypothesis $R_{st} = F_{st}$ (i.e., differentiation is driven mainly by drift, thus F_{st} is more appropriate) and the alternative hypothesis $R_{st} > F_{st}$ (i.e., differentiation is driven mainly by stepwise mutational model (SMM) like mutations, thus R_{st} is more appropriate), using a permutation test of allele sizes (5000 random permutations) to generate a simulated distribution of R_{st} values. Nei's (1978) unbiased genetic distances (D) among sampling locations were calculated and tested for significance using a permutation test with 1000 iterations of the data (Genetix Version 4.05 for Windows; Belkhir et al. 1996). The sequential Bonferroni correction was applied to correct for x simultaneous tests where appropriate ($\alpha = 0.05/x$) (Rice 1989).

Testing models of colonization history and genetic differentiation

We used EasyPop (Balloux 2001) to simulate the colonization history of the Arctic populations in order to test which combinations of genetic effective population size (N_e) and migration rate (m) could explain observed among-population variation in F_{st}, observed heterozygosity (H_O), and allelic richness (A). Unfortunately, N_e must be held constant throughout simulations performed using EasyPop, so it is not possible to simulate population growth after a founder event by a smaller number of individuals. Several other user-selected parameters are required, for which we selected, as a priority, the most realistic values, and secondarily, values towards the "conservative" end of the range of realistic options (i.e., those most likely to allow the populations to reach values of

the genetic parameters described above). We used generation times (G) of 7 years, which is the mean age of mature cod from 50 samples from each of the three Arctic lakes, and 12 years, which is the generation time of the northernmost cod stocks for which data are available to obtain these estimates (Hutchings 1999). We used a colonization time of 8000 ybp (667 and 1143 generations for $G = 7$ and $G = 12$, respectively) based on the most conservative (oldest) period when Atlantic cod are likely to have colonized the lakes. We applied the island model of migration and allowed free recombination, as these were the most realistic descriptors of the colonization of these lakes. We used a microsatellite mutation rate of 0.0005, which is at the conservative end of the standard microsatellite mutation rate range suggested by Jarne and Lagoda (1996). We adopted the mean allelic richness across loci (17 possible allelic states) from eastern Canadian population groups from the current study and allowed maximal initial population variation as we found no evidence of a recent population bottleneck (see below). We selected a mixed mutation model, which tends to fit microsatellite data sets better than other options (Di Rienzo et al. 1994), at a proportion of 90% SMM – 10% KAM (K-allele model), also recommended as providing the best fit for microsatellite data (Cornuet and Luikart 1996).

We used BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) to test for evidence of a recent reduction in population size in the three Arctic populations. This program compares observed with expected gene diversity, which is computed from the observed number of alleles given the sample size under the assumption of mutation–drift equilibrium. Populations having recently experienced a reduction in effective population size exhibit a correlative reduction in allele number and gene diversity. Because the former is reduced faster than the latter, observed gene diversity in recently bottlenecked populations tends to be higher than expected equilibrium gene diversity (Luikart et al. 1999). This is tested using the qualitative sign and Wilcoxon tests of whether populations show a significant number of loci with heterozygosity excess and the quantitative mode-shift test of allele frequency distributions. The latter tests whether the allele distribution is approximately L-shaped (as expected under mutation–drift equilibrium) or not (recently bottlenecked populations are mode-shifted). We used 5000 iterations of the two-phase model (TPM), consisting mostly (90%) of one-step changes but allowing 10% multistep changes, as recommended for microsatellite data (Cornuet and Luikart 1996), with 30% variance for the TPM.

Results

Microsatellite DNA

The number of alleles per locus varied from 1 to 12 in the three Arctic populations, from 2 to 24 in Gilbert Bay, and from 2 to 45 in the eastern Canadian populations (Table 1). There was a significant difference in mean corrected allelic richness among the Arctic populations (4.7–6.4), Gilbert Bay (10.1), and the eastern Canadian populations (14.9–16.4) (ANOVA, $P = 0.04$). Pairwise linkage disequilibrium analysis (Fisher's method) verified that all loci were independent across all populations ($\alpha = 0.05/x$). The only populations that were not in Hardy–Weinberg equilibrium (HWE) were OG

1834

Can. J. Fish. Aquat. Sci. Vol. 63, 2006

Table 1. Per-locus total allelic richness, allelic richness corrected to a sample size of 57 individuals (in parentheses), mean total allelic richness ($\hat{A}$), and mean corrected allelic richness ($\hat{A}_c$) for Atlantic cod (*Gadus morhua*) populations from Arctic lakes on Baffin Island, Nunavut (OG, QL, TL), Gilbert Bay, Labrador (GB), and Northwest Atlantic Fisheries Organization (NAFO) divisions 3PS, 4T, 4W, and 4X.

	N	Gmo3	Gmo8	Gmo19	Gmo34	Gmo35	Mae9	Tch5	$\hat{A}$ ($\hat{A}_c$)
OG	68	3 (3)	10 (9.6)	6 (6)	2 (2)	5 (5)	7 (6.8)	9 (8.9)	5.9 (5.7)
QL	105	3 (3)	9 (8.1)	7 (6.8)	3 (2.9)	6 (6)	12 (11.9)	9 (8.8)	6.8 (6.4)
TL	93	3 (2.9)	6 (5.6)	6 (5.9)	1 (1)	6 (5.9)	7 (7)	6 (5.6)	4.8 (4.7)
GB	100	2 (2)	14 (12.8)	16 (14.2)	5 (5)	6 (5.9)	24 (20)	11 (10)	10.0 (9.1)
3PS	68	5 (4.7)	21 (20.4)	25 (23.6)	5 (4.8)	7 (7)	43 (39.7)	17 (16.7)	16.7(14.9)
4T	93	5 (3.8)	25 (20.8)	27 (23.7)	5 (5.0)	7 (6.6)	45 (37.2)	21 (19.4)	16.7(14.9)
4W	57	3	18	23	6	7	42	19	16.9
4X	91	5 (4.5)	20 (17.8)	25 (22.5)	5 (4.6)	7 (6.6)	45 (38.3)	20 (18.5)	16.1(14.6)
$\hat{A}$		8	37	38	6	7	67	24	

Table 2. F_{IS} values (inbreeding coefficient; Weir and Cockeram 1984), p values for the probability test for Hardy–Weinberg equilibrium across each locus and population, and expected (H_E) and observed (H_O) heterozygosity for seven microsatellite loci from eight populations of Atlantic cod (*Gadus morhua*).

	Gmo3	Gmo8	Gmo19	Gmo34	Gmo35	Mae9	Tch5	p value	H_E	H_O
OG	–0.017	–0.001	0.019	0.151	0.113*	–0.086	–0.101	0.04	0.60	0.60
QL	–0.168	–0.040	0.007	–0.129	–0.036	0.003	0.052	0.62	0.70	0.72
TL	–0.042	–0.081	0.156*	fixed	–0.099	–0.090	0.474*	<0.01	0.60	0.56
GB	–0.016	0.028	0.048	–0.017	–0.065	0.029	0.080	0.81	0.70	0.69
3PS	0.038	0.003	–0.041	–0.021	–0.009	0.047	0.089	0.59	0.76	0.75
4T	–0.088	–0.028	–0.042	–0.007	0.038	0.034	–0.020	0.36	0.77	0.77
4W	0.101	–0.008	0.041	–0.019	0.022	0.008	–0.022	0.66	0.78	0.78
4X	–0.037	–0.045	0.043	–0.049	–0.056	0.080*	0.011	0.59	0.77	0.77
p value	0.899	0.239	0.129	0.562	0.282	0.387	<0.01			
H_E	0.33	0.82	0.85	0.54	0.73	0.86	0.85			
H_O	0.34	0.84	0.82	0.55	0.73	0.85	0.80			

Note: Significant F_{IS} values ($\alpha = 0.05$, adjusted for multiple comparisons) are indicated by an asterisk.

and TL. These two populations showed the only per-locus deviations from HWE, which occurred at *Gmo35* in OG and at *Tch5* and *Gmo34* (fixed for one allele) in TL (Table 2; Fig. 2).

Many alleles present in the eastern Canadian populations were absent from the Arctic populations and, to a lesser degree, from the Gilbert Bay population (Fig. 2). There was no significant difference ($\alpha = 0.05/x$) in the frequency distribution of alleles between any of the eastern Canadian populations at any loci. In contrast, the frequency distribution of alleles was unique at every locus within each Arctic population, with the exception of one comparison between TL and GB at the *Gmo3* locus. The frequency distribution of alleles for cod from Gilbert Bay was unique at all loci except *Gmo3* and *Gmo35*, for which the distributions did not differ significantly from the eastern Canadian populations. There were three unique alleles present at *Gmo8* in the OG population.

There was high variance in allelic identity among the Arctic populations ($F_{st} = 0.20$–0.23). Variance was also high between any of the Arctic populations and Gilbert Bay ($F_{st} = 0.16$–0.21) and between any of the Arctic populations and any of the eastern Canadian populations ($F_{st} = 0.08$–0.14). Variance was lower between Gilbert Bay and any of the eastern Canadian populations ($F_{st} = 0.05$–0.06), and there was

no significant variance in allelic identity among any of the eastern Canadian populations ($F_{st} < 0.003$) (Table 3).

We were unable to reject the null hypothesis (H_0: $R_{st} = F_{st}$) used to assess the relative importance of mutation (R_{st}) and drift (F_{st}) for population differentiation (global $p > 0.3$, per-locus $p > 0.3$ at all loci except *Gmo19* where $p = 0.06$). This suggested that allele-identity-based measures of genetic structure and distance were more appropriate and led to the use of F_{st} and Nei's (1978) unbiased genetic distance (D).

The Arctic populations were significantly genetically distant from each other ($D = 0.484$–0.596) and from Gilbert Bay samples ($D = 0.527$–0.611) and less distant from the eastern Canadian samples ($D = 0.242$–0.395) at the 5% significance level after correction for multiple comparisons (Table 3). The samples from Gilbert Bay were also significantly genetically distant from eastern Canadian samples, although only moderately so ($D = 0.156$–0.177) relative to comparisons involving Arctic populations. There was no significant evidence of genetic distance among groups of eastern Canadian samples.

Effective population size (N_e) of Arctic populations

Simulating the isolation of the three Arctic populations showed that even under the most conservative scenario of

Fig. 2. Frequency distributions of allele sizes at seven microsatellite loci for Atlantic cod (*Gadus morhua*) populations from Arctic lakes on Baffin Island, Nunavut (OG, QL, TL), Gilbert Bay, Labrador (GB), and Northwest Atlantic Fisheries Organization (NAFO) divisions 3PS, 4T, 4W, and 4X.
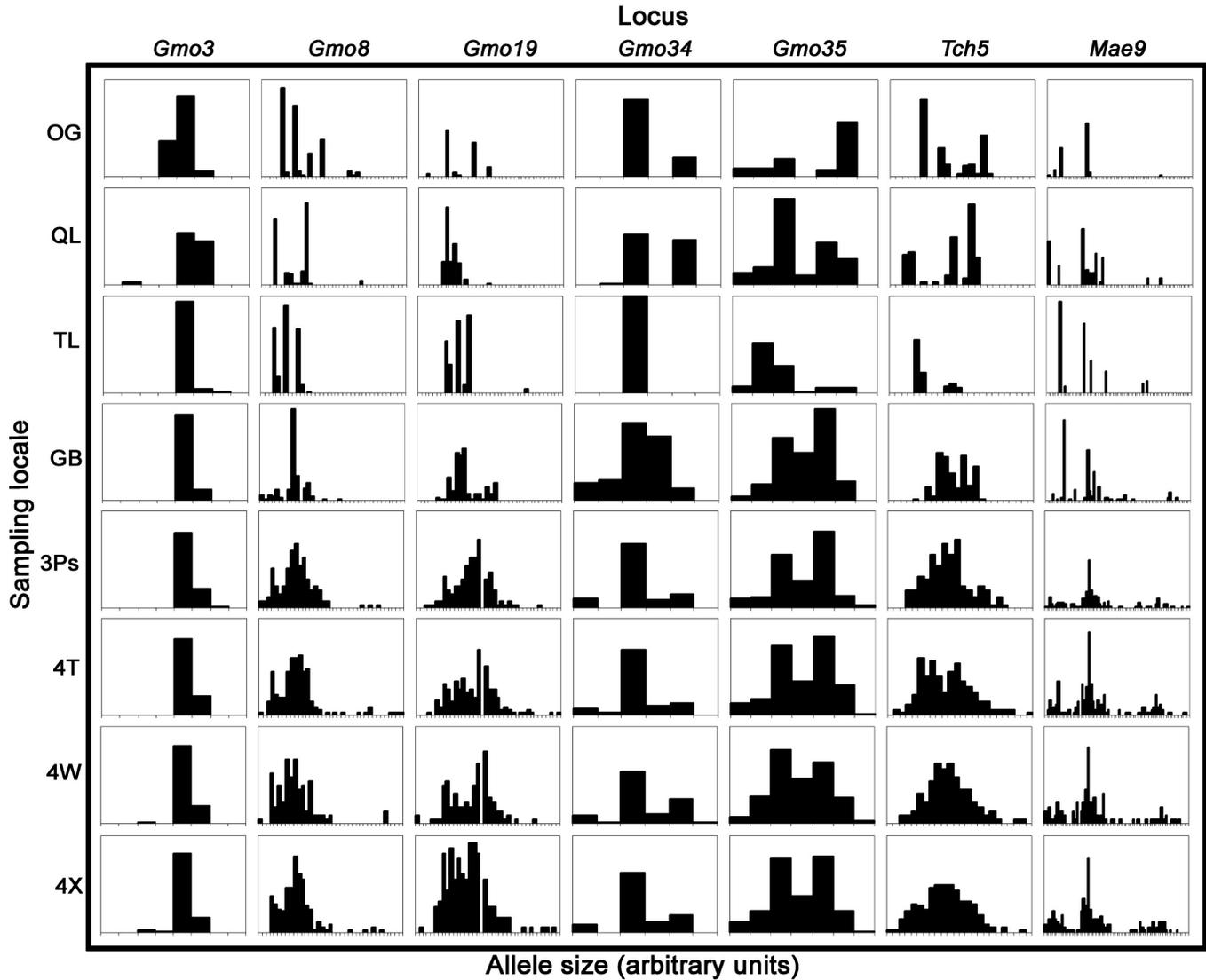
initial conditions, genetic effective population size must have been in the range of 300–1000, with no or extremely little migration over the last 8000 years, in order to account for the high levels of observed variance in allelic identity (Table 4). For example, simulations based on the most conservative model parameters (high number of generations (1143), low founding allelic richness, low mutation rate) suggest that a sustained N_e of 1000 with no migration for 1143 generations (8000 years) would have been necessary to generate the observed global F_{st} values of 0.22. All else being equal, simulating even very low amounts of migration (0.001) requires an N_e of 300 in order to simulate $F_{st} = 0.22$ for either generation time.

For illustrative purposes, the equation

(1) $H_E = 4N_e\mu/(4N_e\mu + 1)$

shows that low heterozygosity infers a small effective population size. Based on eq. 1, the actual ($\hat{H}_E = 0.63$; Table 2)

and modelled ($\hat{H} = 0.66$; Table 4) heterozygosities yield N_e values of 850 and 975, respectively, which are similar to the most parsimonious estimate of N_e from our model (1000) for the shorter generation time.

There was no evidence of a recent reduction in population size based on either quantitative (sign test, $p > 0.35$; Wilcoxon test, $p > 0.37$) or qualitative (mode-shift test, normal L-shaped allele frequency distributions) analyses in any of the Arctic populations.

Discussion

Genetic consequences of extended periods of small population size

Perhaps the most unambiguous component of our analysis is that the Arctic populations of Atlantic cod are the most strongly genetically structured, the most genetically distinct, have the lowest allelic richness, and possess the most unique

Table 3. Multilocus variance in allelic identity as F_{st} (bottom half of matrix; Wright 1951) and Nei's (1978) unbiased genetic distance (top half of matrix) with significant values (α = 0.05) adjusted for multiple comparisons indicated by asterisk.

	OG	QL	TL	GB	3PS	4T	4W	4X
OG		0.596*	0.484*	0.611*	0.395*	0.355*	0.379*	0.362*
QL	0.1952*		0.565*	0.572*	0.322*	0.312*	0.269*	0.280*
TL	0.2381*	0.2234*		0.527*	0.274*	0.242*	0.275*	0.251*
GB	0.1966*	0.1563*	0.2141*		0.177*	0.173*	0.161*	0.156*
3PS	0.1407*	0.0943*	0.1432*	0.0576*		−0.003	0.008	−0.003
4T	0.1291*	0.0913*	0.1295*	0.0558*	−0.0008		0.008	0.001
4W	0.1359*	0.0806*	0.1456*	0.0526*	0.0025	0.0026		−0.002
4X	0.1299*	0.0834*	0.1324*	0.0510*	−0.0008	0.0004	−0.0005	

Table 4. Initial input variables for population divergence simulations using the program EasyPop (Balloux 2001) and resulting final allelic richness, heterozygosity, and global F_{st} predictions.

Genetic effective population size (N_e)	Migration (m)	Generations since isolation	Final allelic richness (observed 6)	Final heterozygosity (observed 0.63)	Final F_{st} (observed 0.22)
500	0	667	11	0.55	0.31
300	**0.001**	**667**	**8**	**0.60**	**0.22**
750	**0**	**667**	**14**	**0.67**	**0.21**
1000	0	667	14	0.71	0.16
750	0	1143	13	0.60	0.27
300	**0.001**	**1143**	**8**	**0.52**	**0.21**
750	0.001	1143	13	0.75	0.10
1000	**0**	**1143**	**14**	**0.66**	**0.22**
1250	0	1143	15	0.71	0.18

Note: Combinations of input parameters yielding the closest predictions of allelic richness, heterozygosity, and F_{st} to observed population values given in bold.

allele distributions. The eastern Canadian populations fall at the opposite extreme, and cod from Gilbert Bay are intermediate for all of the above genetic characteristics, as expected based on previous research (Ruzzante et al. 2000).

We expected an increase in allelic richness from the small, isolated populations in the Arctic lakes to the larger resident population in Gilbert Bay to the large, admixed eastern Canadian populations. Allelic richness is greater at any one time and new alleles persist for longer periods in larger populations because average time between mutation and fixation of new alleles is proportional to N_e (Beebee and Rowe 2004). The probability of the loss of rare alleles as the result of chance events is greater in smaller populations, which increases their vulnerability to factors such as inbreeding depression. The presence of three unique alleles in OG is intriguing and reinforces that this population has been isolated for a sufficient period for new mutations to become established. The trend of increasing allelic richness from Arctic (4.7–6.4) to Gilbert Bay (10.1) to eastern Canadian (14.9–16.4) populations is consistent with the hypothesis of increasing gene flow and effective population size across the three groups. This supports the hypothesis that the Arctic populations are comparatively small and have been isolated for a long time and is consistent with the low microsatellite allelic richness previously reported among cod from Ogac Lake (Brooker 1994). The moderate allelic richness of the Gilbert Bay population supports the hypothesis that oceanographic features act to retain early life history stages within the bay and that adult cod in this population are philopatric (Morris et al. 2002), although population size and gene flow are higher than in the Arctic lakes. The high allelic richness in the eastern

Canadian populations is consistent with the high dispersal and high effective population size model that is assumed for many marine species.

The result that OG and TL are out of Hardy–Weinberg equilibrium (HWE) is most likely due to single loci being out of HWE in each population (*Gmo35* in OG, *Tch5* in TL). The low values of $\hat{A}_c$ and H_E for TL and the fact this population was not in HWE are most likely due to the fixation of *Gmo34* for one allele in this population. TL also had the lowest population size of the three Arctic lakes (D.C. Hardie, unpublished data), thus having a higher probability of inbreeding, which may have contributed to a loss of heterozygosity. Overall, the results suggest that even if the Arctic populations experienced an early colonization bottleneck, population growth with approximately random mating has restored Hardy–Weinberg proportions at most loci. The lack of evidence for recent reductions in population sizes in the Arctic lakes further supports this, suggesting that population dynamics have been historically stable.

Genetic differentiation among populations

Genetic differentiation based on both allele identity (F_{st}) and size (R_{st}) was approximately two orders of magnitude higher for the Arctic populations than has previously been reported for the species (Ruzzante et al. 1999; Beacham et al. 2002). Although the values reported here are extremely high for cod, and for any marine species, they are not particularly unusual for freshwater fishes, for which measures of genetic structure of this magnitude and greater are common. The lack of evidence of SMM-like processes contributing to population differentiation suggests that the random loss of

alleles by genetic drift has been more important than mutation over the circa 1000 generations since the colonization of the Arctic lakes and accounts for most of the strong genetic structure among these populations and between each of them and the Gilbert Bay and eastern Canadian populations. Furthermore, the observation that the Arctic populations all show depressed allelic richness and unique allele frequency distributions provides further evidence of drift-related processes rather than mutation. These results are consistent with low effective population size in the Arctic populations, a situation in which drift-related processes would be expected to exceed mutation, especially over a relatively short evolutionary period. However, founder effects cannot be discounted as an explanation for lower allelic richness among the Arctic populations, although our results suggest that these events, if they did happen, have not been recent. However, it is plausible that a bottleneck occurred during colonization, such that some alleles from the founding population never made it into the Arctic lakes, and that evidence of this bottleneck was erased with the reaching of a new equilibrium between drift and mutation at the new N_e (approximately 0.2–4.0 founder-N_e generations; Luikart and Cornuet 1998). The range of genetic distances reported for any comparisons involving the Arctic populations are consistent with a previous comparison between OG and maritime cod samples ($D = 0.475$; Brooker 1994).

Colonization history: evidence for a high N_e–N_c ratio for a marine fish

Our results are consistent with the hypothesis that the Arctic populations were colonized by Northwest Atlantic cod, which could have expanded their range northward during a period of ameliorated Arctic Ocean conditions between 8000 and 5000 ybp. Since then, colder temperatures and increased ice cover likely acted to push Atlantic cod out of Canadian Arctic waters, except in the warmer saline lakes formed by glacioisostatic rebound during this same period where these unique populations have persisted in isolation from each other and from marine stocks. Small population size and the absence of migration set up an ideal situation for genetic drift to strongly alter genetic diversity, even on relatively short evolutionary time scales. This random loss of alleles, to the point of fixation in one instance, may have contributed to the genetic distinctiveness of the Arctic populations by several different metrics examined in this study, although founder effects could have contributed to their low allelic richness.

A further test of our colonization hypothesis was to model combinations of population size and migration rate to reveal whether the observed F_{st}, heterozygosity, and allelic richness values from the present study are consistent with our hypothesised divergence time of 5000 to 8000 ybp. Simulations based on colonization 8000 ybp suggest that a sustained N_e in the range of 300–1000 (depending on migration and generation time) would be necessary to attain observed values of heterozygosity and F_{st}, and calculations based on observed and modelled heterozygosities suggest a value of N_e closer to the upper end of this range, 850–975. Although the assumption that N_e remained constant throughout colonization may be somewhat unrealistic, our results suggest that the sizes of the Arctic populations have been stable for a long period. This result, coupled with the relatively high heterozygosities in the Arctic populations, is interesting from a conservation perspective, as it suggests that they may be genetically predisposed to be able to tolerate extended periods of low abundance and high inbreeding without suffering severe demographic or evolutionary consequences. Considering Patriquin's (1967) estimate that there were on the order of 10 000 cod greater than 25 cm long in the Ogac Lake population, the N_e–N_c ratio would have to be between 3×10^{-2} and 10^{-1}, which is orders of magnitude greater than the values suggested for marine fishes (Hutchings and Reynolds 2004), including Atlantic cod (Hutchinson et al. 2003), which range from 10^{-2} to 10^{-5}. Given the depauperate fauna in the lakes, 10 000 cod per lake appears a generous estimate, and it is unlikely that census size greatly exceeds this amount in any of the Arctic lakes (D.C. Hardie and J.A. Hutchings, unpublished data). As such, it appears that the N_e–N_c ratio may be higher, even much higher, in the Arctic populations than previously estimated for cod and other marine fishes.

Given that a higher N_c is unlikely, additional factors that could increase the N_e–N_c ratio are higher average individual reproductive success and (or) lower individual variability in reproductive success (Lande and Barrowclough 1987). There are several features of the Arctic populations, relative to oceanic stocks, that could act to decrease individual variability in reproductive success. First, the perennial stratification of the lakes provides a retention mechanism for cod eggs and larvae, which are negatively buoyant at low salinity and as a result are held in the deeper, stable, saline layer. The restricted size and highly stable nature of the saline layers may mean that recruits are not lost from the population to the same degree that oceanographic features such as storms, currents, and other stochastic events remove recruits from marine populations. Furthermore, these factors may help to ensure that spawning is unlikely to be prevented, interrupted, or otherwise affected unduly by environmental forces. At first consideration, the absence of other fish species in Arctic lakes would appear to be deleterious to fitness, forcing cod to resort to cannibalism or to forage on unusual prey such as sea urchins (*Strongylocentrotus droebachiensis*) and bivalves. However, compared with oceanic environments where larval predators such as herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) are abundant, the absence of any planktivorous fish species in the Arctic lakes may act to increase the number of recruits per spawner and lower individual variability in reproductive success. Larval predation is one of the greatest sources of mortality in marine fishes, and the contemporary increase in planktivorous fish stocks has been invoked as a possible mechanism contributing to the failure of depleted cod stocks to recover (Swain and Sinclair 2000; Walters and Kitchell 2001). In essence, these factors may act to dampen the "sweepstakes" effect thought to account for high variability in reproductive success and low N_e–N_c ratios in the stochastic marine environment (Hedgecock 1994). It may be possible to test these speculations by assessing genetic variability within larval cohorts, using a paternity assignment program such as Parentage (Emery et. al 2001).

Despite features that may contribute to the maintenance of a high N_e–N_c ratio, the persistence of Arctic populations at the extreme of the species' range, at low temperatures, in

1838

Can. J. Fish. Aquat. Sci. Vol. 63, 2006

depressed and variable salinity, under extended periods of ice cover, and among a sparse and depauperate fauna is remarkable. Although we present evidence of strong genetic structure and distinctiveness in these populations, they are in HWE and maintain reasonably high levels of heterozygosity, despite the relatively strong effect of drift or founder effects on allelic richness and allelic distributions. In addition to decreased individual variability in reproductive success via the mechanisms outlined above, two other related factors (the absence of fishing and large body size) may help to explain how these unlikely populations have persisted despite the expected genetic consequences of extended periods at low population size. Although marine cod approached 100 kg in size in the past, such individual sizes are unheard of today. Indeed, Atlantic cod of the size class observed in the Arctic lakes, up to 157 cm, are extremely rare in contemporary marine populations. Although the absence of large size classes in harvested fisheries is not uncommon, a much more disquieting phenomenon may be at play whereby fishing acts as an intense form of size-selective mortality, leading to evolutionary changes in the size structure of harvested species (Law 2000; Olsen et al. 2004). This too has been proposed as a factor contributing to the nonrecovery of collapsed marine fishes, especially for highly fecund "lottery" spawners, which rely on the strong relationship between body size and fecundity to ensure recruitment in the face of intense juvenile mortality (Hutchings 2005). It is possible that the absence of fishing in the remote Arctic populations has resulted in a large average size and high fecundity, which may combine with low variability in reproductive success in these sheltered lakes to ensure sufficient recruitment in these ostensibly difficult settings.

Acknowledgements

References

Aitken, A.E., and Gilbert, R. 1989. Holocene nearshore environments and sea-level history in Pangnirtung Fiord, Baffin Island, N.W.T., Canada. Arct. Alp. Res. **1**: 34–44.

Árnason, E., Petersen, P.H., Kristinsson, K., Sigurgislason, H., and Palsson, S. 2000. Mitochondrial cytochrome *b* DNA sequence variation of Atlantic cod from Iceland and Greenland. J. Fish Biol. **56**: 409–430.

Balloux, F. 2001. EASYPOP (version 1.7). A computer program for the simulation of population genetics. J. Hered. **92**: 301–302.

Beacham, T.D., Brattey, J., Miller, K.M., Le, K.D., and Withler, R.E. 2002. Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. ICES J. Mar. Sci. **59**: 650–665.

Beebee, T., and Rowe, G. 2004. An introduction to molecular ecology. Oxford University Press, New York.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. 1996. GENETIX 4.05, logiciel sous Windows™ pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus mohua*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**: 2706–2721.

Brooker, A.L. 1994. Polymorphic microsatellites: tools for measuring genetic diversity in subpopulations of Atlantic cod (*Gadus morhua*). M.Sc. thesis, Department of Biology, Dalhousie University, Halifax, N.S.

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

Cornuet, J.M., and Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, **144**: 2001–2014.

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

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

Doner, L.A. 2001. Late Holocene paleolimnology and paleoclimatology from sub-arctic lakes in Nunavut, Canada, and Iceland. PhD. thesis, Institute of Arctic and Alpine Research, University of Colorado, Boulder, Co.

Dyke, A.S. 1979. Glacial and sea-level history of southwestern Cumberland Peninsula, Baffin Island, N.W.T., Canada. Arct. Alp. Res. **11**: 179–202

Emery, A.M., Wilson, I.J., Craig, S., Boyle, R.L., and Noble, L.R. 2001. Assignment of paternity groups without access to parental genotypes: multiple mating and development plasticity in squid. Mol. Ecol. **10**: 1265–1278.

Fraser, D.J., and Bernatchez, L. 2005. Allopatric origins of sympatric brook charr populations: colonization history and admixture. Mol. Ecol. **14**: 1497–1509.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices. Version 2.9.3. Université de Lausanne, Institut d'Écologie, Lausanne, France.

Hardie, D.C., and Hutchings, J.A. 2003. Landlocked Atlantic cod (*Gadus morhua*) in the Canadian Arctic. *In* Proceedings of the 7th ACUNS (Inter)National Student Conference on Northern

Studies, 23–26 October 2003, Edmonton, Alta. CCI Press, Edmonton, Alberta. pp. 194–196.

Hardy, O.J., and Vekemens, X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. **2**: 618–620.

Hardy, O.J., Charbonnel, N., Frèville, H., and Heuertz, M. 2003. Microsatellite allele sizes: a simple test to asses their significance on genetic differentiation. Mol. Ecol. **163**: 1467–1482.

Hedgecock, D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? *In* Genetics and evolution of aquatic organisms. *Edited by* A.R. Beaumont. Chapman and Hall, London. pp. 122–134.

Hilbish, T.J. 1996. Population genetics of marine species: the interaction of natural selection and historically differentiated populations. J. Exp. Mar. Biol. Ecol. **200**: 67–83.

Hutchings, J.A. 1999. Influence of growth and survival costs of reproduction on Atlantic cod, *Gadus morhua*, populations growth rate. Can. J. Fish. Aquat. Sci. **56**: 1612–1623.

Hutchings, J.A. 2005. Life history consequences of overexploitation to population recovery in Northwest Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **64**: 824–832.

Hutchings, J.A., and Reynolds, J.D. 2004. Marine fish population collapses: consequences for recovery and extinction risk. Bioscience, **54**: 297–309.

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

Hutchinson, W.F., van Oosterhout, C., Rogers, S.I., and Carvalho, G.R. 2003. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). Proc. R. Soc. Lond. B, **270**: 2125–2132.

Jarne, P., and Lagoda, J.L. 1996. Microsatellites, from molecules to populations and back. Trends Ecol. Evol. **11**: 424–429.

Jensen, A.S. 1948. Contributions to the ichthyofauna of Greenland. Spolia Zool. Mus. Haun. IX: 8–24.

Lande, R., and Barrowclough, G.F. 1987. Effective population size, genetic variation, and their use in population management. *In* Viable populations for conservation. *Edited by* M.E. Soule. Cambridge University Press, Cambridge. pp. 87–123.

Law, R. 2000. Fishing, selection, and phenotypic evolution. ICES J. Mar. Sci. **57**: 659–668.

Luikart, G., and Cornuet, J.M. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Cons. Biol. **12**: 228–237.

Luikart, G., Cornuet, J.M., and Allendorf, F.W. 1999. Temporal changes in allele frequencies provide estimates of population bottleneck size. Conserv. Biol. **13**: 523–530.

McLaren, I.A. 1967. Physical and chemical characteristics of Ogac Lake, a landlocked fiord on Baffin Island. J. Fish. Res. Board Can. **24**: 981–1015.

Miller, K.M., Le, K.D., and Beacham, T.D. 2000. Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). Mol. Ecol. **9**: 238–239.

Morris, C.J., Simms, J.M., and Anderson, T.C. 2002. Biophysical overview of Gilbert Bay: a proposed Marine Protected Area in Labrador. Can. Ms. Rep. Fish. Aquat. Sci. No. 2595. Department of Fisheries and Oceans Canada, St. John's, Newfoundland.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

Olsen, E.M., Heino, M., Lilly, G.R., Morgan, M.J., Brattey, J., Ernande, B., and Dieckmann, U. 2004. Maturation trends indica-

tive of rapid evolution preceded the collapse of northern cod. Nature (London), **428**: 932–935.

O'Reilly, P.T., Canino, M.F., Bailey, K.M., and Bentzen, P. 2000. Isolation of twenty low stutter di- and tetranucleotide microsatellites for population analyses of walleye pollock and other gadoids. J. Fish Biol. **56**: 1074–1086.

O'Reilly, P.T., McPherson, A.A., Kenchington, E., Taggart, C., Jones, M.W., and Bentzen, P. 2002. Isolation and characterization of tetranucleotide microsatellites from Atlantic haddock (*Melanogrammus aeglefinus*). Mar. Biotechnol. **4**: 418–422.

Patriquin, D.G. 1967. Biology of *Gadus morhua* in Ogac Lake, a landlocked fiord on Baffin Island. J. Fish. Res. Board Can. **24**: 2573–2594.

Pogson, G.H., Christopher, T., Mesa, K.A., and Boutilier, R.G. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. Evolution, **55**: 131–146.

Raymond, M., and Rousset, F. 1995. GENEPOP (3.3): populations genetics software for exact tests and ecumencism. J. Hered. **86**: 248–249.

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

Ruzzante, D.E., Taggart, C., Cook, D., and Goddard, S.V. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. **53**: 634–645.

Ruzzante, D.E., Taggart, C.T., and Cook, D. 1999. A review of the evidence for genetic structure of cod (*Gadus morhua*) populations in the NW Atlantic and population affinities of larval cod off Newfoundland and the Gulf of St. Lawrence. Fish. Res. **43**: 79–97.

Ruzzante, D.E., Wroblewski, J.S., Taggart, C.T., Smedbol, R.K., Cook, D., and Goddard, S.V. 2000. Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada. J. Fish Biol. **56**: 431–447.

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

Stewart, D.B., Ratynski, R.A., and Bernier, L.M.J. 1993. A fishery development strategy for the Canadian Beaufort Sea – Amundsen Gulf area. Can. Tech. Rep. Fish. Aquat. Sci. No. 1910. Department of Fisheries and Oceans Canada, Winnipeg, Manitoba.

Swain, D.P., and Sinclair, A.F. 2000. Pelagic fishes and the cod recruitment dilemma in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **57**: 1321–1325.

Taggart, C., Penny, P., Barrowman, N.J., and George, C. 1995. The 1954–1993 Newfoundland cod-tagging data base: statistical summaries and spatial-temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. No. 2042. Department of Fisheries and Oceans Canada, St. John's, Newfoundland.

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

Walters, C., and Kitchell, J.F. 2001. Cultivation/depensation effects on juvenile survival and recruitment: implications for the theory of fishing. Can. J. Fish. Aquat. Sci. **58**: 39–50.

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

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

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