Nuclear and mitochondrial markers reveal distinctiveness of a small population of bottlenose whales (*Hyperoodon ampullatus*) in the western North Atlantic

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Abstract

Small populations at the edge of a species' distribution can represent evolutionary relics left behind after range contractions due to climate change or human exploitation. The distinctiveness and genetic diversity of a small population of bottlenose whales in the Gully, a submarine canyon off Nova Scotia, was quantified by comparison to other North Atlantic populations using 10 microsatellites and mitochondrial DNA (mtDNA) control region sequences (434 bp). Both markers confirmed the distinctiveness of the Gully ($n = 34$) from the next nearest population, off Labrador ($n = 127$; microsatellites – $F_{ST} = 0.0243$, $P < 0.0001$; mtDNA – $\Phi_{ST} = 0.0456$, $P < 0.05$). Maximum likelihood microsatellite estimates suggest that less than two individuals per generation move between these areas, refuting the hypothesis of population links through seasonal migration. Both males and females appear to be philopatric, based on significant differentiation at both genomes and similar levels of structuring among the sexes for microsatellites. mtDNA diversity was very low in all populations ($h = 0.51$, $\pi = 0.14\%$), a pattern which may be due to selective sweeps associated with this species' extreme deep-diving ecology. Whaling had a substantial impact on bottlenose whale abundance, with over 65 000 animals killed before the hunt ceased in the early 1970s. Genetic diversity was similar among all populations, however, and no signal for bottlenecks was detected, suggesting that the Gully is not a relic of a historically wider distribution. Instead, this unique ecosystem appears to have long provided a stable year-round habitat for a distinct population of bottlenose whales.

Keywords: cetacean, gene flow, phylogeography, population structure, whaling, Ziphiidae

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Introduction

The northern bottlenose whale (*Hyperoodon ampullatus*) is a deep-diving teuthophage (squid-eater) with an oceanic distribution in cold–temperate North Atlantic waters (*c*. 38°N to 72°N; Reeves *et al*. 1993). These are the largest toothed whales in the North Atlantic after the sperm whale (*Physeter macrocephalus*), reaching adult lengths of 8.7 m (females) to 9.8 m (males). Unlike other beaked whales (family Ziphiidae), bottlenose whales are often curious and approach boats (Gray 1882). This behaviour facilitated

their exploitation, and over 65 000 animals were killed in a multination, multispecies hunt that operated from *c*. 1850 to the early 1970s (Ohlin 1893; Mitchell 1977; Reeves *et al*. 1993). In numbers, these catches are comparable to those of North Atlantic baleen whales over a similar period (1868–1985), when some 79 000 fin (*Balaenoptera physalus*), 12 000 blue (*Balaenoptera musculus*), 16 000 sei (*Balaenoptera borealis*), and < 10 000 humpback whales (*Megaptera novaeangliae*) were killed (Sigurjónsson 1995). Over 90% of bottlenose catches were made between 1882 and the 1920s in the first era of large-scale commercial whaling for this species (Holt 1977; Christensen 1984). Even with improved technology, catches were substantially lower in the second era, between 1960 and the early 1970s (Christensen 1984). Catch distributions indicated the existence of at least six centres of bottlenose whale abundance, each potentially representing

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a separate stock (Benjaminsen 1972): (i) the Gully, a large submarine canyon at the edge of the Scotian Shelf; (ii) northern Labrador–Davis Strait; (iii) northern Iceland; (iv) and (v) off Andenes and Møre, Norway, respectively; and (vi) around Svalbard, Spitzbergen. At least some of these populations still exist today, but it is not known if they have recovered from past exploitation.

In contrast to the general pattern for baleen whales, many odontocetes (toothed whales) do not undertake seasonal migrations between high-latitude feeding grounds and low-latitude breeding grounds. Odontocetes may travel seasonally between different feeding areas (e.g. Sekiguchi *et al.* 1993), but such movements are generally not well documented. However, whalers were convinced that bottlenose whales undertook annual migrations. Based on somewhat inconsistent accounts, these whales either moved northwards, or were already at the northern-most extent of their distribution, in the spring and early summer, and then moved south again in mid summer (early July) or autumn (e.g. Gray 1882; Ohlin 1893; Mitchell & Kozicki 1975). If such migrations did occur, bottlenose whales encountered at different times in different locations could form part of the same population. Empirical evidence for these migrations is weak, however; and recent studies suggest that the Gully population may not migrate at all (Whitehead *et al.* 1997).

Extensive boat-based surveys have demonstrated that the Gully is the southernmost area of consistent bottlenose whale presence in the western North Atlantic (Wimmer & Whitehead 2004). Several lines of evidence suggest that the Gully Scotian Shelf population is biologically distinct and largely isolated from animals in the only other known population centre in this region, off northern Labrador, approximately 1900 km away. Based on photographic capture-recapture analysis, the Gully Scotian Shelf population is estimated to consist of approximately 163 animals (95% confidence interval (CI), 119–214; Whitehead & Wimmer 2005), with dispersal thought to be less than 10% per year (Whitehead *et al.* 1997). Based on their low abundance and the many threats to cetaceans in this area (anthropogenic sound, petrochemical spills, incidental fisheries takes, marine debris and collisions with ships; Whitehead *et al.* 1997), this population was declared 'endangered' by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2002. Catch records suggest that the Labrador–Davis Strait population was substantially larger than the Gully Scotian Shelf population, at least historically. Though no data are available regarding comparative whaling effort, 87 animals were taken from the Scotian Shelf between 1962 and 1967 (the only recorded catches for this region), while 818 animals were taken in the Labrador–Davis Strait in half the time (1969–1971; Reeves *et al.* 1993).

Breeding schedules for these two populations appear to differ by several months. Based on the most recent available data, bottlenose whales in Labrador mate and give birth in April (Benjaminsen 1972), while newborn calves have been observed in the Gully in August (Whitehead *et al.* 1997). However, most Labrador whaling catches occurred in May and June, and there is little information from later in the season (Benjaminsen 1972). Similarly, most research in the Gully has been conducted in July and August (Whitehead *et al.* 1997) and few observations have been made in early spring. These populations may also differ morphologically. Animals caught off northern Labrador appear to be 0.7 m larger on average than animals measured photographically in the Gully (Whitehead *et al.* 1997).

Here we examine polymorphism at 10 microsatellite loci and sequence divergence at the mitochondrial DNA (mtDNA) control region to first test the hypothesis that the bottlenose whales in the Gully are genetically distinct from other populations in the western and central North Atlantic. Our results are inconsistent with the null hypothesis of panmixia. Bottlenose whales in the Gully comprise a small population at the edge of the species' range that is genetically distinct from other populations in the region. We then examine the impact of whaling on the Gully and Labrador populations and their recovery from exploitation.

Materials and methods

Sample collection

Samples were available from a total of 184 bottlenose whales (84 females, 100 males) from three locations in the North Atlantic: the Gully and Labrador in the west, and Iceland in the east (Fig. 1). For the Gully, 36 tissue biopsy samples (representing 34 unique individuals; see Results) were obtained from free-swimming whales in the summers of 1996, 1997, 2002 and 2003, using a crossbow or modified veterinary capture rifle (see Dalebout *et al.* 2001; Hooker *et al.* 2001a for details). For Labrador (referred to as 'Davis Strait' in Dalebout *et al.* 2001), dried gum tissue was obtained from the teeth of 125 whales killed in the Norwegian hunt for this species in 1971 (Christensen 1973). Three biopsy samples were also obtained from free-swimming whales in this area in summer 2003[1]. For Iceland, dried gum tissue was obtained from the teeth of 23 whales killed in the Norwegian hunt in 1967 (Benjaminsen 1972). See Table 1 for sample summary. Biopsy samples were preserved in 20% salt-saturated dimethyl-sulphoxide (DMSO) or 70% ethanol and stored at 4 °C or –20 °C prior to genetic analysis. For whaling samples, jaws were boiled in water for 2 h to facilitate extraction of the teeth (Christensen 1973). The teeth,

[1]These three biopsies were the only genetic samples obtained during two intensive seasons of fieldwork off northern Labrador in the summers of 2003 and 2004, in which we attempted to locate and study this elusive population of bottlenose whales.
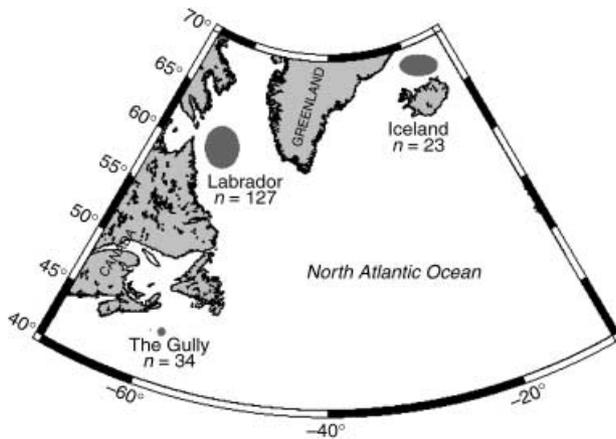
Fig. 1 Bottlenose whale sampling locations in the North Atlantic Ocean. (Map created with OMC, available at http://www.aquarius.geomar.de/.)

Table 1 Number of samples (corrected for replicates from the same individuals) and sampling years for each bottlenose whale population. F, females; M, males

Location	Year	F	M	Total
Gully	1996	0	2	2
	1997	13	4	17
	2002	5	4	9
	2003	2	4	6
				34
Labrador	1971	53	71	124
	2003	1	2	3
				127
Iceland	1967	10	13	**23**
			Total	**184**

with remnant gum tissue attached around the root, were then removed from the jaws and stored unpreserved at room temperature in individually labelled paper envelopes.

Molecular analysis

DNA extraction and molecular sexing. For the majority of samples, whole genomic DNA was extracted using a QIAGEN DNeasy™ tissue kit following manufacturer's recommendations. For whaling samples, dried gum tissue (*c.* 0.01 g) was scraped from the teeth using sterile scalpel blades and collected in sterile Petrie dishes before transfer to 1.5-mL tubes for DNA extraction. For Gully biopsy samples collected in 1996–1997 ($n = 20$), DNA was extracted using the phenol–chloroform method as described in Gowans *et al.* (2000a). This method was also used to extract DNA from a subset of duplicate samples from Labrador ($n = 20$) and northern Iceland ($n = 5$; Dalebout *et al.* 2001), which were used here to assess the possibility of cross-contamination during

the DNA extraction process and to check microsatellite profiles and assess error rates in scoring (see below). For all samples, 4 µL of extracted DNA was electrophoresed on a 1% agarose gel to assess DNA quality. The polymerase chain reaction (PCR)-based SRY plus ZFX-ZFY method of Gilson *et al.* (1998) was used to identify the sex of all individuals for which this information was not known from previous molecular analyses (Gowans *et al.* 2000a). See Table 1 for a summary of sex ratios by population.

Microsatellite genotyping. Ten microsatellite loci (one tetramer and nine dimers; Table 2) isolated from other cetacean species were used to genotype all bottlenose whale samples available for this study. PCR amplifications were carried out using 2 µL of stock DNA (whaling samples) or 2 µL of a 1:10 dilution (biopsies) in 15-µL volumes, with 1.5 µL of 10× buffer, 1.3 µL of 2 mM dNTPs, 0.5 µL of each primer (at 10 µM concentration), 0.1 µL *Taq*, and Mg++ concentrations as shown in Table 2. PCR profiles consisted of a preliminary denaturation step (94 °C, 3 min), 35 three-step cycles of denaturation (92 °C, 30 s), annealing (see Table 2 for T°, 30 s) and extension (72 °C, 30 s) and a final extension step (72 °C, 10 min). PCR products were resolved on 6.5% denaturing acrylamide gels and visualized on a Hitachi FMBIO™ II fluorescent imaging system (MarioBio) by attaching a fluorescent HEX label to one primer of each pair. Reference samples of known allele size were run on each gel to standardize scoring. For each locus, samples were scored manually as they were run, and those that failed to amplify or produced ambiguous bands were re-amplified and run a second or third time if necessary. Scoring was checked again after all samples had been run for all loci, resulting in the correction of typographical errors in approximately 0.7% of scores entered over all samples and loci. To further assess possible inconsistencies in amplification, duplicate extractions representing approximately 30% of whaling samples were run and scored blindly for all 10 loci. Due to lower DNA quality and yield, historical material is more likely to suffer from allelic dropout and ambiguous profiles (Taberlet *et al.* 1999). In the majority of cases (98%), duplicate sample genotypes were identical to those from original samples over all 10 loci. Where, for a particular sample–locus combination, genotype ambiguities could not be resolved, the samples in question were removed from the analysis for that locus ($n = 12/1840$ over all sample–locus combinations; 0.65%). Attempts to sequence at least one homozygote for each locus to confirm the nature of the microsatellite repeats were generally unsuccessful. Clean sequences were, however, obtained for three loci (EV104, DlrFCB11 and MK6) which revealed similar motifs as reported from the source species (Table 2).

Mitochondrial DNA: single-strand conformation polymorphism (SSCP) and sequencing. Four unique mtDNA control region

Table 2 Microsatellite loci used for bottlenose whales in this study

Locus	Source species	Repeat motif*	T_a (°C)	Mg^{++} (mM)†	Size range (bp)	k‡	GenBank Accession No.	Reference
GATA98	humpback whale	(GATA)$_n$	51	2.5	76–84	2	U93892	Palsbøll *et al.* (1997)
GT211	humpback whale	(GT)$_n$	51	2.0	96–106	6	AF309693	Bérubé *et al.* (2000)
EV1	sperm whale	(AC)$_n$(TC)$_n$	55	1.5	184–208	12	G09074	Valsecchi & Amos (1996)
EV37	humpback whale	(AC)$_n$	56	2.5	194–204	8	G09081	Valsecchi & Amos (1996)
EV104	humpback whale	(AC)$_n$(GCAC)$_n$	56	2.0	148–156	5	G09085	Valsecchi & Amos (1996)
DlrFCB1	beluga	(AC)$_n$	56	1.5	105–111	2	G02097	Buchanan *et al.* (1996)
DlrFCB6	beluga	(GT)$_n$	54	2.0	165–187	8	G02101	Buchanan *et al.* (1996)
DlrFCB11	beluga	(AC)$_n$CCC(AC)$_n$	54	2.0	134–154	10	G02104	Buchanan *et al.* (1996)
MK6	bottlenose dolphin	(GT)$_n$	49	2.5	160–170	6	AF237891	Krützen *et al.* (2001)
PPHO130	harbour porpoise	(CA)$_n$	51	2.5	178–194	7	AF151787	Rosel *et al.* (1999)

*Based on the source species.
†Final concentration in each PCR.
‡Observed number of alleles over all samples.

haplotypes (434 bp) were identified previously (Dalebout *et al.* 2001) by direct sequencing from a subset (*n* = 45) of the samples available for the current study. Here, single-strand conformation polymorphism (SSCP) analysis (Orita *et al.* 1989), using a smaller fragment (309 bp), was used to determine the haplotype affinity of the remaining animals. This fragment encompassed the three polymorphic sites that define the four known haplotypes and was amplified via PCR using two fluorescent HEX-labelled primers, Dlp1.5 M-L (5′-CAACACCCAAAGCTGAAATTCTAC-3′) and DlpHam-H (5′-TGGAACGGGCACATGTACG-3′), in 15-μL volumes with 2.5 mM Mg^{++}, annealing temperature 54 °C, and standard conditions (Palumbi 1996). For SSCP, PCR products (4 μL of 1:1 mix with standard formamide loading dye) were denatured for 5 min and snap-cooled on ice for 2 min before electrophoresis on 8% nondenaturing acrylamide gels (37.5:1 acrylamide:bis-acrylamide with 5% glycerol) run at 8 W in a 4 °C cold room for 20–22 h (Sunnucks *et al.* 2000). Multiple representatives of the four known haplotypes were run on each gel as standards and gels were visualized as for microsatellites. The full 434-bp fragment was amplified and sequenced for all samples that appeared to represent rare or new haplotypes using the primers M13Dlp1.5-L and Dlp5-H (Dalebout *et al.* 2001). Sequencing reactions used Beckman dye terminator cycle fluorescent chemistry (DTCS) and were run on a Beckman CEQ™ 8000 automated capillary sequencer.

Statistical analysis

Microsatellite scoring errors and identification of replicate samples. MICRO-CHECKER version 2.2.1 (van Oosterhout *et al.* 2004), was used to assess the potential for large allele dropout and null alleles, and to identify possible scoring errors by comparison of observed and expected homozygote allele frequencies and allele bin step sizes. Replicate samples among the Gully biopsies were identified by matching genotypes using the MS Excel Toolkit version 3.1 (Park 2001). A proportion of the Gully population consists of reliably marked, photo-identifiable individuals (*c.* 66%; Gowans *et al.* 2000b). Efforts were made to obtain biopsy samples from known photo-identified individuals in this population, but this was not always possible. The probability of identity (P_{ID}) was calculated for each locus using the formula of Paetkau *et al.* (1995). Probabilities for each locus were multiplied, on the assumption that these loci are unlinked, to obtain an overall P_{ID} for each population.

Genetic variability within populations. After removal of replicates, indices of microsatellite genetic variation were calculated for each population, including inbreeding coefficients (F_{IS}), and 'allelic richness' (*A*), using FSTAT version 2.9.3.2 (Goudet 1995, 2002). Tests of linkage equilibrium for each locus by population were also conducted using this program (significance of log-likelihood ratio G-statistics tested using 45 000 permutations). Hardy–Weinberg equilibrium (HWE) for each locus by population was assessed using ARLEQUIN version 2.0 (Schneider *et al.* 2000) and an extension of the Guo & Thompson (1992) method. Bonferroni corrections (Rice 1989) were applied to all pairwise test results to adjust for multiple comparisons. SEQUENCHER version 4.2 (Gene Codes Corporation, Inc.) was used to align mtDNA sequences, confirm polymorphic sites and determine haplotype affinity. A median-joining network of haplotypes (Bandelt *et al.* 1999) was inferred using NETWORK version 4.1.0.7. Standard indices of genetic variation (nucleotide diversity, π, and haplotype diversity, *h*) were calculated for each population and over all individuals using ARLEQUIN.

Spatial structure

Genetic differentiation among populations. For microsatellites, analyses of molecular variance (AMOVAS) based on F_{ST} (Weir & Cockerham 1984) were used to investigate differentiation among regions using ARLEQUIN (significance tested by 20 000 permutations). Modified exact tests based on genotype counts (G-tests; Goudet *et al.* 1996) were performed using GENEPOP on the Web (Raymond & Rousset 1994) with significance tested by 10 000 permutations. For mtDNA, frequency- (F_{ST}) and distance-based (Φ_{ST}) AMOVAS (Weir & Cockerham 1984; Excoffier *et al.* 1992) were conducted using ARLEQUIN (significance tested by 20 000 permutations). Kimura 2-parameter corrected distances were used for Φ_{ST} analyses. Exact tests (Raymond & Rousset 1995) were also run using the same program with 20 000 Markov chain steps. Bonferroni corrections were applied to adjust for multiple comparisons.

Bayesian clustering. STRUCTURE version 2.0 (Pritchard *et al.* 2000; Falush *et al.* 2003) was used to test whether our *a priori* definition of populations based on geography was consistent with microsatellite genetic information. This Bayesian clustering method takes a sample of genotypes and uses the assumption of HWE and linkage equilibrium within subpopulations to find the number of populations (*K*) that best fits the data and the individual assignments that minimize Hardy–Weinberg and linkage disequilibrium in those subpopulations. Bayesian methods can be very valuable in complex problems that do not conform naturally to a classical statistical setting, as is often the case with population genetics. In a Bayesian framework, probability is used to assess statistical confidence, but with an expanded definition of probability, such that it is a direct measure of uncertainty (Shoemaker *et al.* 1999). Multiple runs were conducted under a variety of conditions following Pritchard *et al.* (2000); including: ancestry model — with or without admixture; allele frequency model — independent or correlated; burn-in −10 000 or 50 000; number of reps after burn-in −50 000 or 100 000; and values of *K* ranging from 1 to 10.

Influence of 'kin sampling' within populations. In species where closely related individuals are not randomly distributed, sampling may unknowingly target only a few families, leading to potential underestimation of allele frequencies and overestimation of population differentiation (Hansen *et al.* 1997). To identify such individuals within our populations, Ritland's (1996, 2000) method of moments estimator (MME) was used to estimate relatedness coefficients (r) between all pairs of individuals following Ruzzante *et al.* (2001). Pairwise relatedness coefficients were calculated for all $n(n − 1)/2$ potential pairs of individuals in each population, using all individuals from all populations combined to generate the baseline allele frequency distribution. A cut-off value of r = 0.4 was used to identify closely related pairs within each population. One of each pair of individuals with r > 0.4 was subsequently removed from the sample and F_{ST}-based analyses of microsatellite differentiation among regions repeated.

Detection of sex-biased dispersal. The potential for a strong sex-bias in dispersal was tested using microsatellites and FSTAT (Goudet *et al.* 2002), based on the following statistics: (i) F_{ST}, where genetic differentiation between populations is expected to be higher for the more philopatric sex; and (ii) the variance of the assignment index (vAIc). Assignment indices are the probability of assigning an individual's multilocus genotype to each population, while correcting for differences in gene diversity in those populations (Paetkau *et al.* 1995; Favre *et al.* 1997). If dispersal is sex-biased, a sample of the individuals from the dispersing sex in a region will have a higher vAIc than the more philopatric sex, due the presence of both residents (with common genotypes) and immigrants (with rare genotypes). VAIc performs best at low dispersal rates (< 10% per generation), while F_{ST} performs best at higher dispersal rates (> 10% per generation; Goudet *et al.* 2002). Movement patterns of bottlenose whales on the Scotian Shelf suggest that males are most likely to disperse (Wimmer & Whitehead 2004). As such, one-tailed tests were conducted, with 10 000 randomizations.

Dispersal rates and long-term effective population size. MIGRATE versions 2.0.3 and 2.0.6 (Beerli & Felsenstein 1999; 2001) were used to estimate dispersal rates and long-term effective population size (N_e) from the microsatellite data, using a maximum-likelihood (ML) coalescent approach. MIGRATE simultaneously estimates θ, the product of effective population size and mutation rate ($N_e\mu$, where μ is mutation rate/generation), and N_em, effective population size × dispersal rate. A Brownian motion approximation of the stepwise-mutation model was used (Beerli 2002), with default settings for other parameters. Starting estimates for θ were based on F_{ST} calculations, with burn-in = 100 000 trees, 15 short chains with a total of 100 000 genealogies sampled, and three long chains with 1 000 000 genealogies sampled, for each locus. Chain heating was adaptive, with four different temperatures. A constant mutation rate of 10^{-4} (Hedrick 2005) was used to transform estimates of θ into N_e. The values and lower and upper profile likelihood percentiles (0.025 and 0.975) reported are the means from six replicate runs. This approach was considered inappropriate for the mtDNA data set due to the low number of variable sites and large proportion of haplotypes shared among populations (see Results and Beerli 2006). For comparison, analytical estimates of N_em were calculated from

F-statistics, using Wright's (1931) equation, $F_{ST} = 1/(4N_e m + 1)$ for microsatellites, and $F_{ST} = 1/(2N_e m + 1)$ for mtDNA. It is recognized however, that analytical dispersal estimates may be unrealistic due to violation of island model assumptions (Whitlock & McCauley 1999), including that all populations are of equal abundance and that dispersal is symmetrical between areas. ML coalescent-based estimates are not reliant on these unrealistic assumptions and consistently outperform dispersal-rate estimates based on F_{ST} (Beerli 1998).

Effect of temporal differences in population sampling. Given the *c*. 30 years temporal difference in sampling between regions (Iceland and Labrador, 1967–71 vs. Gully, 1996–2003), we considered the possibility that observed genetic structure could result from genetic drift over several generations, if animals in these areas were in fact a single panmictic population. To test this hypothesis, we estimated variance N_e from the microsatellite data using the ML temporal method implemented in the program MLNE version 2.03 (Wang & Whitlock 2003). These analyses were conducted under the following scenario: animals from all three regions were considered to represent a single, closed population with sampling conducted at two time points, such that Iceland–Labrador represented t_0, and the Gully represented t_2 or t_3, where bottlenose whale generation time is approximately 10–15 years (Mead 1984). If the null hypothesis of regional panmixia and genetic drift was correct, we would expect the resulting estimates of variance N_e to be biologically plausible. Conversely, unrealistically small N_e estimates would suggest that observed differentiation among regions is more likely due to real population structure.

Test for genetic bottlenecks. For microsatellites, the potential for a recent bottleneck was tested using M-RATIO (Garza & Williamson 2001). M-RATIO uses $M = k/r$ as its test statistic, where *k* is the number of alleles at a given locus, and *m* is the range in allele sizes in base pairs. The M ratio was calculated across loci for each population with the following parameters (Garza & Williamson 2001): proportion of one-step mutations (*ps*) = 0.9; average size of non-one-step mutations (Δg) = 2.8; and several values of θ due to the lack of data regarding equilibrium/prereduction effective population size. Assuming a constant mutation rate of 10^{-4}, θ values of 2, 0.4, 0.04 and 0.004 correspond to an N_e of 5000, 1000, 100 and 10 individuals, respectively. For mtDNA, Tajima's *D*-test (Tajima 1989a) was used to test for departure from mutation–drift equilibrium. This test was developed to assess selective neutrality but can also be used to test for population expansion following recovery from a genetic bottleneck (Tajima 1989b). This statistic is based on the correlation between the number of segregating sites and the pairwise divergence of haplotypes. If a population is expanding, a higher number of polymorphic sites with low frequency may be observed, and *D* is expected to be significantly negative.

Results

Genetic variability within populations

Microsatellites. All 10 microsatellite loci amplified successfully and were scored unambiguously for the majority of individuals [93% (*n* = 171/184); Table 3]. For Labrador, one individual failed to amplify for most loci and was dropped from the study, leaving a total of 127 animals for this region. For the Gully, calculations indicated that the probability of genotype profiles matching by chance at all 10 loci was less than 1 in 58 million. On this basis, we assumed that samples with matching microsatellite profiles at all loci represented replicate samples from the same individuals. Comparisons of genotypes from the 36 Gully biopsy samples indicated that they were derived from 34 unique individuals (i.e. two pairs of samples had identical genotypes for all 10 loci[2]).

All microsatellites were polymorphic for all three populations, with no evidence for large allele dropout or null alleles. No loci deviated from HWE for any of the populations, and there was no evidence of linkage disequilibrium between pairs of loci after adjustment for multiple comparisons. Levels of microsatellite diversity were similar for each population with average observed heterozygosity ranging from 0.63 in Labrador to 0.66 in Iceland (Table 3). Allelic richness, based on a minimum sample size of 23 individuals, ranged from 5.27 in the Gully to 5.30 in Labrador and Iceland. The majority of the 66 alleles found over the 10 loci screened occurred in all three populations (*n* = 47, 71%). Only a small proportion of alleles (12%) were found in a single population (Gully, *n* = 2; Labrador, *n* = 5; Iceland, *n* = 1), all of which were rare (< 6% of alleles/population).

Mitochondrial DNA. Control region haplotypes were successfully identified for the majority of individuals sampled (*n* = 183) using SSCP and direct sequencing. Only one new haplotype not described previously by Dalebout *et al.* (2001) was found, bringing the total of known unique matrilines for this species to five. The five haplotypes are defined by three polymorphic sites, all transition substitutions, over 434 bp (Table 4), and form a reticulate network (Fig. 2). The most common haplotype (A) was found in all three populations and was represented by 66.7% of

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[2]As expected, these two sample pairs also matched for mtDNA haplotype and sex. Subsequent examination of photo-identification images, taken at the time the biopsies were collected, also supported our conclusions.

Table 3 Microsatellite data for bottlenose whales. Sample size for each region (n = individual whales), number of alleles at each locus (k), allelic richness (A), and observed and expected heterozygosity (H_O, H_E). Loci which differed significantly from Hardy–Weinberg equilibrium in a population are indicated in bold. After correction for multiple comparisons, no loci differed significantly from Hardy–Weinberg equilibrium

	Gully					Labrador					Iceland				
Locus	n	k	A^*	H_O	H_E	n	k	A	H_O	H_E	n	k	A	H_O	H_E
GATA98	34	2	2.00	0.235	0.280	127	2	1.96	0.102	0.119	23	2	2.00	0.217	0.237
GT211	34	5	4.99	0.667	0.733	126	6	5.41	0.627	0.652	23	5	5.00	0.783	0.723
EV1	34	10	9.53	**0.971**	**0.833**	123	10	8.85	**0.943**	**0.833**	23	10	10.00	1.000	0.845
EV37	34	7	6.86	0.765	0.719	119	7	5.27	0.739	0.718	23	5	5.00	0.696	0.788
EV104	34	5	4.68	0.765	0.719	122	5	4.90	0.779	0.745	23	5	5.00	**0.609**	**0.744**
DlrFCB1	34	2	2.00	0.559	0.472	127	2	2.00	0.504	0.490	23	2	2.00	0.522	0.487
DlrFCB6	34	5	4.57	0.647	0.613	123	8	5.38	**0.593**	**0.650**	23	5	5.00	**0.783**	**0.740**
DlrFCB11	34	9	8.54	0.794	0.858	125	10	8.26	0.872	0.840	23	7	7.00	0.826	0.815
MK6	34	5	4.87	0.618	0.555	126	6	4.94	0.524	0.517	23	5	5.00	0.435	0.430
PPHO130	34	5	4.67	0.500	0.524	121	7	6.00	0.653	0.672	23	7	7.00	0.739	0.739
Overall	34	5.50	5.27	0.652	0.631	124	6.30	5.30	0.634	0.624	23	5.30	5.30	0.661	0.655
Probability of identity	1.7×10^{-9}					7.6×10^{-9}					7.4×10^{-9}				
F_{IS} (P value)	−0.039 (0.129)					−0.020 (0.125)					−0.023 (0.305)				

*Based on a minimum sample size of 23 individuals.

Table 4 Frequency and genetic diversity of mitrochondrial DNA (mtDNA) control region haplotypes for bottlenose whales

	Position			Population								
	15	106	213	Gully*	%	Labrador	%	Iceland	%	Total
HapA	G	C	T	25	73.5%	80	63.5%	17	73.9%	122
HapB	.	.	C	4	11.8%	21	16.7%	4	17.4%	29
HapC		T	.	—	—	20	15.9%	2	8.7%	22
HapD	A	.	.	5	14.7%	4	3.2%	—	—	9
HapE	A	.	C	—	_	1	0.8%	—	—	1
			Total	34		126		23		183
			h	0.44 + 0.092		0.55 + 0.042		0.43 + 0.111		0.51 + 0.0379
			π	0.11% + 0.108%		0.15% + 0.128%		0.11% + 0.109%		0.14% + 0.122%

*Sample numbers corrected for replicates as determined from microsatellite genotyping (see text for details).

individuals sampled. The second most common haplotypes (B and C) were represented by 15.8% and 12.2% of individuals, respectively. The former was found in all three populations, while the latter was found only in Labrador and Iceland. Overall, haplotype diversity (h) was 0.516 ± 0.0379 and nucleotide diversity (π) was 0.14% ± 0.122%. At the population level, mtDNA diversity was highest in Labrador (five haplotypes), and lower but similar in the Gully and Iceland (three haplotypes each; Table 4). Sequences representing all haplotypes have been deposited in GenBank (Accession nos AF350437–AF350440, DQ385849).

Spatial structure

Genetic differentiation among populations. For microsatellites, significant population structure was detected with frequency-based statistics over all three regions ($F_{ST} = 0.0159$, $P < 0.0001$), which pairwise comparisons showed to be driven by the Gully (vs. Labrador, $F_{ST} = 0.0243$, vs. Iceland, $F_{ST} = 0.0276$; Table 5). No significant difference was found between Labrador and Iceland ($P = 0.3953$). For mtDNA, significant structure was detected over all three regions with the exact test of haplotype frequencies ($P = 0.0313$), but not with the permutation procedure used for F_{ST} comparisons (Table 5). Pairwise comparisons revealed significant distance-based differentiation only between the Gully and Labrador ($\Phi_{ST} = 0.0455$, $P = 0.0178$). Similar significant results were obtained from pairwise exact tests of haplotype frequencies for these two populations ($P = 0.0043$).

Bayesian clustering. STRUCTURE analyses of the microsatellite data failed to distinguish among the three populations and
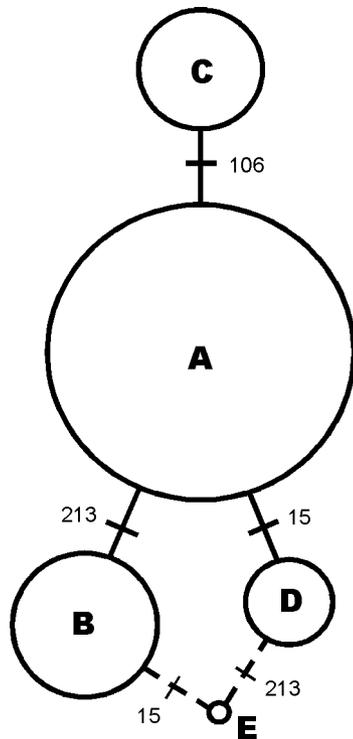
Fig. 2 Median-joining network of relationships among bottlenose whale mtDNA haplotypes. Circles (nodes) represent haplotypes labelled as in Table 4. Haplotype nodes are scaled to overall frequency of occurrence. Crossbars on connections between haplotypes represent single nucleotide substitutions labelled with the position of the polymorphic site involved. Dashed lines represent ambiguous or alternative connections between haplotypes.

the best $-\log \Pr(X|K)$ estimates were found for $K = 1$. Given the relatively low levels of population differentiation observed from AMOVA, this was not unexpected (Manel *et al.* 2005). With increasing values of K, $-\log \Pr(X|K)$ reached a plateau at $K = 3$–4, before decreasing further and seeming to destabilize. As expected in the absence of strong structure (Pritchard *et al.* 2000), the Dirichlet parameter for the degree of admixture (α) varied considerably through the course of each run, and the proportion of samples assigned to each population was close to symmetrical in all cases ($\sim 1/K$ in each population).

Influence of 'kin sampling' within populations. To assess the influence of 'kin sampling' within populations, one individual from each pair with a relatedness coefficient of more than 0.4 was removed to create a reduced dataset (Gully, $n = 26$; Labrador, $n = 109$; Iceland, $n = 23$) for which analyses of population differentiation were repeated. Results from this reduced data set were similar to those from the full data set. Significant structure was found over all three populations ($F_{ST} = 0.0159$, $P < 0.001$), which was driven by the Gully (Table 5). Interestingly, relatedness coefficients
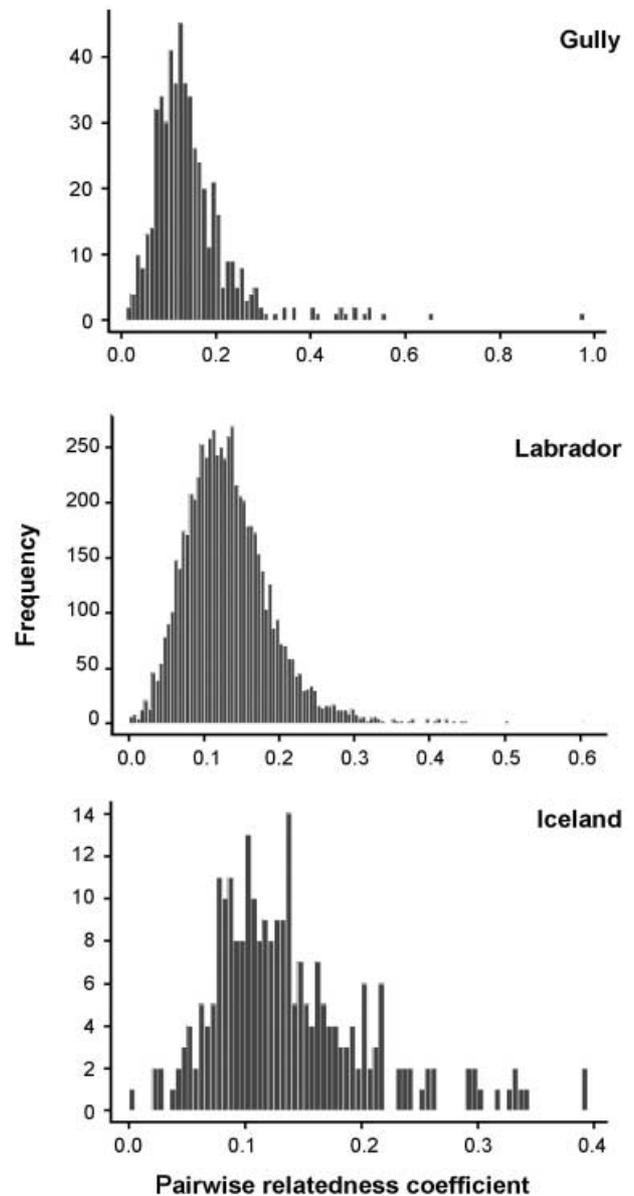
Fig. 3 Frequency distributions of pairwise relatedness coefficients (r) for bottlenose whales in each of the three regions. For each region, the number of pairwise comparisons, $N = (n/n - 1)/2$, where n is the number of individuals sampled. One individual from each of pair of individuals with r > 0.4 was eliminated from each population sample and analyses of population differentiation repeated without the influence of close kin.

of more than 0.5 (as expected on average between full-sibs and parent–offspring pairs) were found only in the Gully (Fig. 3). This is not unanticipated in a small, relatively isolated population with few options for mate choice. However, while higher than those in Labrador or Iceland, F_{IS} scores were not significantly negative, suggesting that inbreeding is unlikely to be a substantial problem in this population at present (Table 3).

Table 5 Analyses of molecular variance (AMOVA) and pairwise comparisons among bottlenose whale populations for microsatellites and mtDNA control region sequences. Significant P values (< 0.05) are shown in bold (an asterisk highlights values that are no longer significant after Bonferroni corrections). ICE, Iceland; LAB, Labrador; n, number of unique individuals sampled (where two values are separated by/ the first value refers to microsatellites, the second to mtDNA). Further analyses of microsatellite variance were conducted using a reduced data set from which the close kin in each population were removed (one from each pair of individuals with a relatedness coefficient > 0.4). Analytical estimates of N_em were calculated from F-statistics following Wright (1931). See text for details

| | | Microsatellites | | | mtDNA | | | | |
| | | Genotype frequency | | | Haplotype frequency | | | Distance-based | |
	n	Variance	F_{ST} probability	Exact test probability	Variance	F_{ST} probability	Exact test probability	Variance	Φ_{ST} probability
All individuals									
All three pops	184/183	F_{ST} = 0.0159	**< 0.0001**	**< 0.0001**	F_{ST} = 0.0128	0.1635	**0.0313***	Φ_{ST} = 0.0228	0.0662
Gully vs. LAB	34, 127/126	0.0243	**< 0.0001**	**< 0.0001**	0.0291	0.0634	**0.0043**	0.0456	**0.0192***
Gully vs. ICE	34, 23	0.0276	**< 0.0001**	**< 0.0001**	0.0007	0.3737	0.0744	0.0315	0.1249
LAB vs. ICE	127/126, 23	0.0000	0.3953	0.1378	−0.0097	0.5553	0.8578	−0.0150	0.7188
Males only									
All three pops	100	0.0171	**0.0004**	**0.0004**	−0.0099	0.5433	0.4634	−0.0019	0.4388
Gully vs. LAB	14, 73	0.0236	**0.0010**	**0.0005**	0.0116	0.2635	0.1699	0.0259	0. 1861
Gully vs. ICE	14, 13	0.0436	**0.0001**	**0.0003**	−0.0332	0.6804	0.6046	0.0047	0.4472
LAB vs. ICE	73, 13	0.0045	0.1699	0.0950	−0.0299	0.8384	0.9337	−0.0341	0.9054
Females only									
All three pops	84/83	0.0218	**0.0003**	**0.0030**	−0.0072	0.5043	0.2521	0.0039	0.3630
Gully vs. LAB	20, 54/53	0.0314	**< 0.0001**	**0.0020**	0.0164	0.1987	0.0731	0.0329	0.1215
Gully vs. ICE	20, 10	0.0193	**0.0373***	**0.0329***	−0.0414	0.8270	0.3810	−0.0105	0.4794
LAB vs. ICE	54/53, 10	0.0027	0.2996	0.3822	−0.0445	0.8270	1.0000	−0.0527	0.9270
Reduced data set — close kin removed									
All three pops	158	0.0168	**< 0.0001**	—	—	—	—	—	—
Gully vs. LAB	26, 109	0.0272	**< 0.0001**	—	—	—	—	—	—
Gully vs. ICE	26, 23	0.0326	**< 0.0001**	—	—	—	—	—	—
LAB vs. ICE	190, 23	0.0009	0.3243	—	—	—	—	—	—
Analytical F_{ST}-based estimates of dispersal (N_em)									
All three pops		16.4				38.56			
Gully vs. LAB		10.0				16.0			
Gully vs. ICE		8.0				6.6			
LAB vs. ICE		n/a†				n/a†			

†Analytical estimates of dispersal between LAB and ICE could not be calculated due to the low, nonsignificant F_{ST} values observed for these comparisons.

Sex-biased dispersal and sex effects. To assess the potential influence of sex-biased dispersal on observed microsatellite differentiation, AMOVAs were conducted, treating males (n = 100) and females (n = 84) separately for each population (Table 5). Similar significant levels of differentiation among populations were observed for both sexes (females, F_{ST} = 0.0218, males, F_{ST} = 0.0171; P < 0.001). No significant difference was observed between the sexes (males vs. females, F_{ST} = −0.0026, P = 0.9703). With FSTAT tests, no evidence for sex-biased dispersal was detected in comparisons of F_{ST} or vAIc scores (all P values > 0.5). For mtDNA, analysis by sex did not reveal significant differences among any of the three regions (all P values > 0.05; Table 5).

Dispersal rates and long-term effective population size

Maximum-likelihood estimates of dispersal to the Gully based on microsatellites were low (approximately 1 individual per generation; Table 6), while estimated dispersal from the Gully ranged from 1 to 3 individuals per generation (to Labrador and Iceland, respectively). In contrast, migration rates between Labrador and Iceland were three to 10-fold higher (10 individuals per generation). Analytical estimates of migration rates derived from F_{ST} values were higher than ML estimates (Table 5) but are likely to be less accurate (Whitlock & McCauley 1999). Analytical estimates of migration rates from mtDNA were generally higher than those from microsatellites (Table 5), but see Discussion.

Table 6 Maximum likelihood estimates of effective dispersal rate ($N_e m$ Males + Females) among regions and long-term effective population size (N_e) based on microsatellite data using MIGRATE. Estimates are averages from six replicate runs. Confidence intervals (95%) derived from likelihood profiles are shown. See Table 5 for comparative analytical F_{ST}-based estimates of dispersal rates

Dispersal per generation	$N_e m$	95% CI
From Gully		
to Labrador	1.5	1.4–1.6
to Iceland	2.9	2.7–3.1
From Labrador		
to Gully	0.8	0.7–0.9
to Iceland	10.4	10.0–10.8
From Iceland		
to Gully	1.5	1.4–1.6
to Labrador	10.1	9.8–10.4

Long-term effective population size		
	N_e	95% CI
Gully	1534	1435–1638
Labrador	3118	2987–3258
Iceland	2709	2496–2947

Estimates of long-term N_e based on microsatellites suggested that the number of breeding adults in the Gully was approximately half that of Labrador and Iceland (Table 6). However, estimates of N_e for the Gully were an order of magnitude higher than the current census estimate (*N*) for this population (Whitehead & Wimmer 2005). N_e is commonly much smaller than *N* in wild populations due to fluctuating population size, high variance in family size and unequal sex ratios (Wright 1969; Frankham 1995). It is extremely unlikely that census estimates based on over a decade of photo-identification studies could err by such a large amount. Similarly, demographic evidence indicates that a much larger N_e is highly improbable for the present-day Gully Scotian Shelf population (Whitehead *et al.* 1997; Whitehead & Wimmer 2005). Instead, these estimates of N_e are likely to be inflated by the effects of low-level interchange with other populations. Estimates of N_e for all three populations may also be somewhat inaccurate due to a combination of small and unequal sample sizes and temporal differences in sampling.

Effect of temporal differences in sampling

In analyses treating all three regions as a single panmictic population with two temporal samples (Labrador–Iceland = t_0, Gully = t_2 or t_3), ML estimates of variance N_e ranged from 77.9 (49.6–152.1) to 91.4 (57.4–174.3). These estimates are implausibly low when compared to estimates of long-term N_e, which are at least an order of magnitude higher for each of the three regions alone (Table 6). Though by no means

conclusive, we feel these results provide good evidence that the observed differentiation of the Gully vs. Labrador and Iceland is due predominantly to isolation and subsequent genetic divergence, and that the effects of the temporal difference in sample collection between populations are likely to be negligible.

Tests for genetic bottlenecks

M ratio values of < 0.7 provide evidence of a genetic bottleneck, while values of > 0.8 are generally representative of equilibrium populations with no history of bottlenecks (Garza & Williamson 2001). For all three populations, microsatellite M ratio values were ≥ 0.75 and no signal for a genetic bottleneck was detected (probability of smaller M ratio if population at equilibrium < 0.05), irrespective of the values used for pre-reduction/equilibrium N_e. For the mtDNA, all Tajima's *D* values were nonsignificant ($P \geq 0.36$).

Discussion

Distinctiveness of the Gully population of bottlenose whales

Our findings are inconsistent with the hypothesis that bottlenose whales from the Gully belong to the same population as those off northern Labrador. Genetic differentiation persisted even after closely related individuals were removed from the analysis, providing strong evidence for the uniqueness of the Gully population. Further, concordant results from both maternal and biparentally inherited markers indicate this is not due solely to female philopatry; male bottlenose whales also do not disperse frequently. The Gully was also found to be highly distinct from the nearest population outside the western North Atlantic, off northern Iceland, based on microsatellites. Mitochondrial DNA differentiation between these regions was less pronounced; a result consistent with low statistical power due to our small sample size for Iceland, which is likely a substantially larger population (NAMMCO 2003). In contrast to comparisons involving the Gully, we did not detect a significant difference between whales from northern Labrador and Iceland.

Studies of several other cetacean species have also revealed the existence of small isolated populations. These include fin whales in the Gulf of California (Bérubé *et al.* 2002), North Pacific minke whales (*Balaenoptera acutorostrata scammoni*) in the Sea of Japan/East Sea (Baker *et al.* 2000), Cuvier's beaked whales (*Ziphius cavirostris*) in the Mediterranean Sea (Dalebout *et al.* 2005) and harbour porpoise (*Phocoena phocoena*) in the Black Sea (Rosel *et al.* 1995). In these cases however, the populations in question inhabit bodies of water that are geographically isolated to some extent from the open ocean. In the Gully, there are no such

obvious barriers to movement. Instead, this population associates specifically with unique bathymetric features (large submarine canyons) on the edge of the continental shelf (Whitehead *et al.* 1997).

Our results are also consistent with a previous mtDNA-based analysis of a small sample of bottlenose whales from the Gully and Labrador (*n* = 20 each; Dalebout *et al.* 2001). This study found low but significant differentiation between these regions but was hampered by the low mtDNA diversity of this species. The possibility that skin biopsies collected from free-swimming animals in the Gully included replicate samples from the same individuals was also of concern. These issues were addressed here through larger sample sizes and the inclusion of highly variable, biparentally inherited markers.

F_{ST} analyses did not reject the null hypothesis of panmixia for bottlenose whales off Labrador and Iceland, suggesting that regular dispersal may occur around Cape Farewell, the southern tip of Greenland. Several other vertebrate species show a similar lack of genetic structure between Labrador and Iceland, including harbour porpoise (Tolley *et al.* 2001), fin whales (Bérubé *et al.* 1998), deepwater redfish (*Sebastes mentella*; Roques *et al.* 2002) and Greenland halibut (*Reinhardtius hippoglossoides*; Vis *et al.* 1997). Water temperature, current systems and bathymetric features likely play an important role in generating and maintaining these patterns. The waters off northern Labrador and Iceland form part of the same sea surface temperature zone (Slutz *et al.* 1985). While water temperature at depths where bottlenose whales feed (> 800 m) will differ from that on the surface, phytoplankton and nektonic prey communities in these areas will nonetheless be comparatively homogenous. Alternatively, it is possible that our failure to reject panmixia between Labrador and Iceland is due to small sample size and lower statistical power when dealing with larger populations. Of the more than 63 000 bottlenose whales taken by Norwegian whalers between 1882 and 1973, most were taken east of Cape Farewell (Reeves *et al.* 1993), and northern Iceland was known as a major centre of exploitation (Benjaminsen 1972). If Iceland's small sample size is the issue, this further strengthens the case for the distinctiveness of the Gully, where sample size is also low.

It is worth noting that the patterns of statistical significance observed in our mtDNA analyses are closely parallel to those described by Hudson *et al.* (1992). In simulations, they showed that (i) distance-based statistics (Φ_{ST}) were more powerful than frequency-based statistics (F_{ST} and χ^2/ exact tests) when dispersal rates ($N_e m$) were > 1; and that (ii) for frequency-based statistics, exact tests had a higher statistical power on average than F_{ST}. These predictions match our results almost perfectly (Table 5).

No evidence for seasonal migration. According to Norwegian whalers, bottlenose whales reached the northernmost areas of their distribution in spring and early summer, and by July had begun to migrate south again (Ohlin 1893; Jonsgård & Øynes 1952). Some sighting and catch data appear to support this idea. For example, the majority of Norwegian catches off Labrador and Iceland took place in May and June (Benjaminsen 1972; Sigurjónsson & Gunnlaugsson 1990). The comparative lack of catches in other months could, however, be simply due to a decrease in, or absence of, whaling activity, but anecdotal reports from fishermen working off northern Labrador today confirm that bottlenose whales are most frequently encountered in early summer and are less common in late July to August. In the Gully, most recent sightings have occurred in July and August, coincident with the majority of research trips, though they are clearly present in early summer as well (Mitchell 1974; Whitehead *et al.* 1997). As such, it is possible that these regions are linked by seasonal migration, with at least some of the whales found in northern waters off Labrador and/or Iceland in spring and early summer, travelling south to the Gully in mid- to late summer.

Our finding of significant differentiation between the Gully-Scotian Shelf and other aggregations refutes this hypothesis and indicates that there is no regular seasonal movement of animals between these areas. The same conclusion was reached by Whitehead *et al.* (1997) based on low estimates of immigration into the Gully from photo-identification data. The low abundance estimate for the Scotian Shelf between June and August (Whitehead & Wimmer 2005) also argues against any substantial migration into the area at this time of year. The large submarine canyons on the Scotian Shelf (the Gully, Haldimand and Shortland) are the only places on the shelf where bottlenose whales are regularly found and are unique to this area (Wimmer & Whitehead 2004). Such bathymetric features are absent off Labrador and Iceland. For the Gully-Scotian Shelf population, local knowledge of these canyons and reliable prey aggregations (Hooker *et al.* 2001b) likely provide a strong disincentive for seasonal migration. It remains possible that some of the animals found off northern Labrador and Iceland in early summer do move south later in the season, but their destination is unknown. One possibility is the Grand Banks–Flemish Cap region off southern Newfoundland, where bottlenose whales are sighted occasionally in mid–late summer (Compton 2004; Wimmer & Whitehead 2004). However, if this region does host a seasonal aggregation, this was not recognized or exploited by whalers (Benjaminsen 1972).

Apparent lack of a strong sex-bias in dispersal. Similar levels of heterogeneity were found at both maternally and biparentally inherited markers. Differentiation was generally not significant for the former (mtDNA) but highly significant for the latter (microsatellites). This might suggest that male bottlenose whales are more philopatric than

females, in contrast to the common mammalian pattern (Greenwood 1980). However, mtDNA diversity in bottlenose whales is very low, possibly as a result of selective sweeps associated with this species' extreme deep-diving ecology (Hooker & Baird 1999; Janik 2001). If so, use of this locus to quantify population structure and gene flow would be invalid. Partitioning of microsatellite variation was, however, very similar among males and females, indicating that both sexes may be philopatric. This conclusion is supported by the lack of significant results from FSTAT tests to detect sex-biased dispersal, although it is recognized that these analyses have limited power unless the dispersal bias is extreme (> fourfold; Goudet *et al.* 2002). If correct, our finding of philopatry for both sexes in the Gully provides an interesting contrast to other odontocetes in which male dispersal is common, including sperm whales (Whitehead 2002), dusky dolphins (*Lagenorhynchus obscurus*; Cassens *et al.* 2005), Dall's porpoise (*Phocoenoides dalli*; Escorza-Trevino & Dizon 2000) and harbour porpoises (Rosel *et al.* 1995). For bottlenose whales, movement within regions may nonetheless be male-biased, as suggested by the photographic tracking of whales between canyons 50 km apart on the Scotian Shelf (Wimmer & Whitehead 2004).

Recovery from whaling and other threats. What effect have over 100 years of documented exploitation and the removal of over 65 000 bottlenose whales had on this species' distribution and abundance? Given these large takes, it could be postulated that the current distribution represents the isolated remnants of a larger oceanic population. This seems unlikely for two reasons. First, catch records clearly indicate relatively discrete areas of bottlenose whale abundance (Benjaminsen 1972; Benjaminsen & Christensen 1979), with catches in the first and second era of whaling showing a similar distribution (Reeves *et al.* 1993). Recent surveys confirm that bottlenose whales still occur in at least some of these regions (Whitehead *et al.* 1997; NAMMCO 2003; Herfst 2004), although accurate estimates of abundance are rarely available. If bottlenose whales were more uniformly and widely distributed historically, catch records would be expected to reflect this. Second, bottlenose whales show a strong preference for shelf-edge waters, generally near or beyond the 1000 m depth contour, where upwellings and bathymetric features act to concentrate nutrients and deep-water nektonic prey, such as *Gonatus* spp. squid on which they rely as their main food source (Sigurjónsson & Gunnlaugsson 1990; Hooker *et al.* 2002). Together, these lines of evidence strongly suggest that current populations are not the fragmented remnants of a historically widespread oceanic population.

Exploitation likely had a significant impact on abundance, even if it did not dramatically modify bottlenose whale distribution. When bottlenose whaling ceased in the early 1970s due a reduction in the value of whale products

(Christensen *et al.* 1977), populations were thought to be depleted (Mitchell 1977). Short-term reduction in abundance and continued small population size will result in the loss of genetic diversity due to the increased effects of genetic drift. No signal for a genetic bottleneck was detected with mtDNA or microsatellites among the populations surveyed here, but such tests are relatively weak (Depaulis *et al.* 2003; Guinand & Scribner 2003). Comparison of past takes to present population sizes could allow some evaluation of recovery, but few estimates of contemporary abundance are available. Recent sighting surveys off Iceland suggest that this stock could consist of several thousand to several tens of thousands of animals, but the long dive times of this species make it difficult to evaluate the accuracy of these estimates (NAMMCO 2003). In contrast to the apparently high numbers of bottlenose whales off Iceland, low sighting rates off Labrador (Compton 2004; Herfst 2004) and Norway (NAMMCO 2003) have not enabled estimation of abundance. Accurate abundance estimates, based on photographic capture–recapture of distinctively marked individuals, are currently available only for the Gully Scotian Shelf population (Whitehead & Wimmer 2005).

Estimates of dispersal rates indicate that it would take at least a century to replace even one-tenth of the small Gully Scotian Shelf population, if it were extirpated. Oil and gas leases almost completely encircle the Gully and cover much of the Scotian Shelf (Canada-Nova Scotia Offshore Petroleum Board 2005). Due to its physical and ecological significance, the presence of bottlenose whales and the threat of petrochemical exploration and mining, the Gully was declared a Marine Protected Area by Canada's Department of Fisheries and Oceans (DFO) in 2004. We have shown that the Gully Scotian Shelf population is genetically distinct and isolated from populations off Labrador and Iceland by low but detectable levels of gene flow. As such, we conclude that the current COSEWIC listing of the Gully Scotian Shelf bottlenose whales as 'endangered' and protection of the Gully canyon are justified. Furthermore, as this is the first comprehensive genetic study of any organism in the Gully MPA, these results could potentially be used to forecast population genetic patterns for other marine fauna resident to this unique area. If such extrapolation is valid, this predicts that populations of many nektonic species (those able to swim against water currents, such as marine mammals, fish, squid, and some crustaceans) found year round in the Gully submarine canyon are also likely to be distinct from populations elsewhere in the western North Atlantic.

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