Molecular Ecology (1999) **8**, S41–S54

Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers

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Abstract

The harbour porpoise, *Phocoena phocoena*, experiences high levels of nonnatural mortality owing to interactions with commercial fisheries throughout its range. To accurately evaluate the significance of this bycatch, information on population structure is required. We have examined the population structure of this species in the northwest Atlantic Ocean using mitochondrial DNA (mtDNA) sequence and nuclear microsatellite data. Samples from four previously proposed summer breeding populations—the Gulf of Maine, eastern Newfoundland, the Gulf of St Lawrence and West Greenland—were analysed. Control-region sequences revealed a significant partitioning of genetic variation among most of these summer populations, indicating that northwest Atlantic harbour porpoises should not be considered one panmictic population. Analysis of females alone yielded the highest levels of population subdivision, suggesting that females are more philopatric than males. At least three management units may be defined for harbour porpoises in the northwest Atlantic based on these data. Analysis of six microsatellite loci failed to detect significant population subdivision. Male-mediated gene flow may maintain homogeneity among nuclear loci, while female philopatry is sufficient to produce a signal of population subdivision in the maternally inherited mtDNA genome. mtDNA analyses also indicate that winter aggregations of harbour porpoises along the US mid-Atlantic states comprise animals from more than one summer breeding population.

Keywords: bycatch, control region, management, microsatellite, stocks

Received 11 February 1999; revision received 27 May 1999; accepted 27 May 1999

Introduction

Management policies and practices for marine mammals in US waters differ significantly from the management methods based on evolutionarily significant units (Waples 1991) and are more akin to those based on management units (Moritz 1994). In the USA, the Marine Mammal Protection Act of 1972 requires that each population of a marine mammal species present in US waters be maintained at a population size between the maximum net productivity level and carrying capacity (see Wade 1998 for details). The Marine Mammal Protection Act also mandates that 'population stocks' be maintained such that they remain a 'significant functioning element in the ecosystem of which they are a part' where a 'population stock' is defined as: 'a group of marine mammals of the same species or smaller taxa in a common spatial arrangement that interbreed when mature'. Thus, the Marine Mammal Protection Act provides for, and in fact mandates, management of marine mammals at levels below that of the species, but provides only vague direction as to how such management units are to be defined. Determining directly whether individuals in a group of marine mammals are interbreeding is difficult in the wild. Furthermore, we have a limited understanding of what barriers to

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movement and gene flow may be encountered by highly mobile creatures, such as porpoises. Thus, it is difficult to determine where one population boundary ends and the next one begins.

The harbour porpoise, *Phocoena phocoena*, is a small, delphinoid species found throughout north temperate and subarctic waters of the world. This species is primarily restricted to coastal waters, particularly during the breeding season. Throughout its range, this species experiences a high degree of incidental mortality, primarily as a result of entanglement in gillnets (Jefferson & Curry 1994). In US waters of the Gulf of Maine in the northwest Atlantic, the minimum estimated bycatch averaged 1833 animals per year between 1990 and 1995 (Bravington & Bisack 1996; Bisack 1997). This level of bycatch exceeds that allowable under federal law and exceeds the International Whaling Commission's (IWC) maximum recommended removal rate (IWC 1993). The high bycatch rate has raised considerable concern over the sustainability of this Gulf of Maine population. At one time, the US federal government was petitioned to list the population as threatened under the Endangered Species Act. The IWC has also expressed concern and has requested that member states reduce bycatch of this species (IWC 1991, 1992, 1993). In Canada, similar problems exist and the species is listed as threatened in the northwest Atlantic by the Committee on the Status of Endangered Wildlife (Gaskin 1992).

Management efforts for harbour porpoises in the northwest Atlantic are complicated by the fact that the species crosses international boundaries. In addition to US waters of the Gulf of Maine, during summer months harbour porpoises are common in coastal Canadian waters: in the Bay of Fundy, the Gulf of St Lawrence and around eastern Newfoundland, as well as in coastal waters of West Greenland. Bycatch occurs in all of these regions. Gaskin (1984) defined each of these four geographical regions in the northwest Atlantic—the Gulf of Maine/Bay of Fundy, the Gulf of St Lawrence, Newfoundland-Labrador and Greenland (western and southeastern)—as separate 'subpopulations'. These areas contain the highest density of porpoises in the northwest Atlantic during the summer months. The presence of porpoises in these regions is highly seasonal. Breeding is also highly seasonal and occurs during a relatively short period of time in the spring or summer. Female porpoises attain sexual maturity at ≈ 3.5 years and the majority breed every year (Read 1999). Calves remain with their mothers from 8 to 18 months, certainly for their first summer season (Gaskin 1992), and it is unlikely that a juvenile born in the Gulf of Maine, for example, would be found off Newfoundland that same summer. Therefore, biological and ecological evidence suggest that these four regions may serve as core areas in the northwest Atlantic where harbour porpoises forage and reproduce during the summer months. These population subdivisions have formed the basis for the majority of management discussion over the last decade.

The degree of mixing of animals from these four regions is unknown. Satellite telemetry data suggest that porpoises in the Bay of Fundy/Gulf of Maine region are relatively restricted in their movements (Read & Westgate 1997). However, harbour porpoises leave most of the summer breeding areas during winter months and it is unclear where they go. Significant increases in the number of porpoise strandings along the mid-Atlantic states in late winter and early spring (Polachek *et al.* 1995) suggest that at least some animals migrate south along the coast, but whether these are animals from the Gulf of Maine and/or from Canadian waters is unknown. The mid-Atlantic animals also experience incidental mortality in gillnets (Haley & Read 1993). There is a critical need to know from which summer population these animals originate, in order to accurately estimate the level of incidental mortality affecting the population(s).

Mandates to accurately quantify the 'biological significance' of bycatch have led to a critical need for an accurate picture of the population structure of harbour porpoises in the northwest Atlantic. Wang *et al.* (1996) conducted a restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) isolated from harbour porpoises from three putative populations in the northwest Atlantic, including the Gulf of St Lawrence, Newfoundland and the Bay of Fundy. Their results suggest the presence of a weak cline in mtDNA genotype frequencies from the Bay of Fundy north to Newfoundland. The strongest support for population subdivision was present when females were analysed separately, suggesting some degree of philopatry by female porpoises (Wang *et al.* 1996). The purpose of this research was to further examine the validity of the four proposed subpopulations of harbour porpoises in the northwest Atlantic using genetic markers with a higher resolving power than mtDNA RFLP analysis, namely mtDNA control-region sequences and microsatellite markers, with the aim to augment baseline data available for management of the species.

Materials and methods

Samples

Tissue samples from 253 porpoises were collected from the four proposed summer breeding populations in the northwest Atlantic: the Gulf of St Lawrence, eastern Newfoundland, within the Gulf of Maine/Bay of Fundy, and West Greenland; and from a presumed wintering population along the mid-Atlantic United States (New Jersey to North Carolina) (Fig. 1). All summer population samples were collected from incidentally entangled animals, thereby eliminating complications (e.g. dilution of genetic signal)

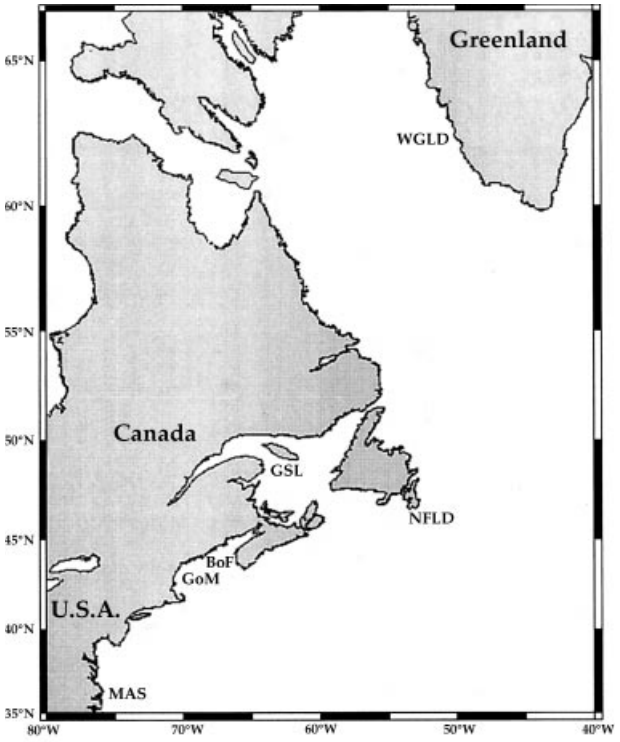

Fig. 1 Northwest Atlantic showing the five areas where harbour porpoise samples were obtained. BoF, Bay of Fundy; GoM, Gulf of Maine; GSL, Gulf of St Lawrence; NFLD, East Newfoundland; WGLD, West Greenland; and MAS, mid-Atlantic states.

arising from samples collected from stranded animals that may have floated in from elsewhere. Samples collected from the mid-Atlantic states were from stranded animals; however, as there is no other known source of animals in this area during winter, we feel it is safe to assume that they are representative of the mid-Atlantic states' aggregation. DNA was extracted following standard proteinase K digestion and phenol–chloroform extraction, as described in Rosel & Block (1996).

Mitochondrial control-region sequences

A 450-bp region of the 5′ end of the highly variable control region and flanking tRNAs was amplified using the polymerase chain reaction (PCR) and the primers L15824 (5′-CCTCACTCCTCCCTAAGACT-3′) and H16265 (5′-GCCCGGTGCGAGAAGAGG-3′) (Rosel *et al.* 1999), with positions defined based on the complete mtDNA sequence of the fin whale (Árnason *et al.* 1991). Genomic DNA (50–250 ng) was added to a 50-μL PCR reaction mix (Saiki *et al.* 1988) containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 15 pmol of each primer, 150 μM of dNTPs and 1.5 U of *Taq* DNA polymerase. The cycling profile consisted of an initial denaturation at 95 °C for 30 s, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and was performed in a Perkin-Elmer thermocycler (model 480). Five microlitres of the product was screened on a 1% agarose gel to determine the quality of the reaction, and the remaining 45 μL of double-stranded product was gel purified and digested with 5–10 U of agarase (Sigma). A sample (3.5–8.5 μL) of this digestion mix was used in a cycle-sequencing reaction using fluorescently labelled dideoxy terminators and Amplitaq FS, according to the manufacturer's recommended conditions (Applied Biosystems) and loaded onto an ABI 373A automated DNA sequencer. All samples were sequenced in both directions with the primers used in the amplification. Alignment of the resultant sequences was performed by eye.

Nucleotide and haplotypic diversity were estimated for all populations (Nei 1987) using the program ARLEQUIN (Schneider *et al.* 1996). An analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was conducted to detect concordance between DNA sequences and geographical location. The AMOVA calculates Φ_{ST}, corresponding to Wright's F_{ST} (Wright 1978), a measure of population subdivision. Φ_{ST} incorporates information on both the degree of genetic distance between haplotypes and the frequencies of haplotypes in each population. A distance matrix of gamma distances (Tamura-Nei model of evolution, $\alpha = 0.5$, as recommended for control-region sequences; Kumar *et al.* 1993) was generated using the computer program MEGA (Kumar *et al.* 1993) for use in ARLEQUIN. The AMOVA was also run using the option of utilizing haplotype frequency data only, i.e. not incorporating the degree of genetic distance between haplotypes, resulting in an estimate of Wright's F_{ST} Recently, O'Corry-Crowe *et al.* (1997) have suggested that this latter method of analysis may be a better estimate of population differentiation in situations where many very closely related haplotypes exist and little phylogeographical structure is observed in the data. These properties are often present in recently separated populations, where sufficient time has not elapsed to allow for sorting of mtDNA lineages into the separate populations. However, haplotype frequencies can respond more quickly to a reduction in genetic exchange, and so haplotype frequencies may differ significantly among populations before phylogeographical partitioning is evident. In these situations, estimates of F_{ST} may more accurately reflect the degree of population subdivision, while Φ_{ST} may be biased downward. In pairwise population comparisons, F_{ST}, rather than Φ_{ST}, is presented.

The AMOVA was first performed excluding the mid-Atlantic States, as this was a winter sample and it is unknown from which summer breeding population or populations it originates. However, to determine whether we could identify the source of these wintering animals, they were included in a subsequent analysis. In all cases, analyses were conducted using both sexes and then repeated on each sex separately. In addition, to determine

whether the sample from the mid-Atlantic states may have been derived from just one of the summer populations, pairwise tests for homogeneity of haplotype frequencies between each summer sample and the winter sample were performed using contingency tests. Monte Carlo methods implemented in the program RXC of Miller (1997) were utilized to determine significance levels (*P*-values) of these tests. Furthermore, under the assumption that the winter sample comprised a mixture of the summer populations, the mtDNA data were analysed using methods of standard likelihood mixture models (Pella & Milner 1987). Relative contributions of each summer stock were estimated using a conditional maximum-likelihood approach with bootstrapping for precision, as implemented in the program CONSQRT (Masuda *et al.* 1991). To test whether the data contained sufficient signal to determine the source of the mid-Atlantic states sample had it come from a single summer population, four sets of summer + winter samples were generated using the program SIMULATR (kindly provided by J. Pella and M. Masuda, and available at ftp://wwwabl.afsc.noaa.gov/sida/mixture-analysis/) and processed using the conditional maximum-likelihood mixture-analysis approach. For each of the four simulations, the simulated data for the winter 'mixture' was taken from just one of the summer populations and sample sizes were identical to those present in the original data.

Finally, a minimum spanning network of mtDNA haplotypes was constructed using the program MINSPNET (Excoffier & Smouse 1994) to visually examine relationships among the haplotypes.

Microsatellite isolation

Harbour porpoise-specific microsatellites were isolated following the procedure of Pulido & Duyk (1994). Genomic DNA from two northwest Atlantic harbour porpoises was pooled and digested with the restriction enzyme *Alu*I. The digested DNA was size selected (300–800 bp), modified with *Bst*XI adapters, ligated into the phagemid cloning vector pJCP1 (provided by G. Duyk, Harvard Medical School) and transformed into the *dut-ung-* *Escherichia coli* strain JMG1 (provided by G. Duyk, Harvard Medical School). This constituted the primary library. The primary library was infected with the M13 helper phage M13K07 (Promega) and single-stranded circular phagemid DNA was recovered. This DNA was used as a template for primer extension using a $(CA)_{10}$ oligonucleotide probe. The double-stranded primer-extension products were transformed into pBluescript (Stratagene). Colony lifts were screened using a ^{32}P end-labelled $(CA)_{10}$ oligonucleotide probe according to standard procedures (Sambrook *et al.* 1989). Plasmid DNA was purified from positive clones using a Wizard miniprep kit (Promega) and was sequenced on an ABI 373A automated sequencer, following the manufacturer's instructions. PCR primers to unique loci containing perfect microsatellites of 13 or more repeat units were designed using the computer program PIPELINE (Resnick & Stein 1995). Primers for each locus were synthesized commercially with a fluorescent phosphoramidite dye attached to the 5′ end of one primer of each pair. Eight of the nine loci were polymorphic.

Microsatellite data collection and analysis

Harbour porpoise samples from the northwest Atlantic were genotyped using these eight loci. Amplifications were conducted in 25-μL reaction volumes containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 150 μM of dNTPs, 7.5 pmol of each primer, 0.75 U of *Taq* DNA polymerase and 10–50 ng of genomic DNA. The cycling profile consisted of an initial denaturation at 95 °C for 30 s, followed by 25 or 27 cycles (see Table 6) of 94 °C for 30 s, 50 °C or 55 °C for 30 s and 72 °C for 30 s, and was performed in a Perkin-Elmer thermocycler (model 480 or 9600). Amplified products were mixed with a size standard (Genescan-500 TAMRA) and loaded onto an ABI 373A automated sequencer (ABI). Sizing of allele fragments using the Genescan Analysis software (ABI) was automated and relied on the use of the internal lane standards (Ziegle *et al.* 1992). Because we isolated a dinucleotide repeat, allele sizes should differ by 2 bp owing to the mutational processes that produce the length variation (Tautz & Renz 1984; Levinson & Gutman 1987). In practice, however, because of the influence of base composition and charge on the mobility of these DNA fragments and the size standard in a gel matrix, 2-bp increments among alleles were not always achieved. As a result, it was necessary to bin fragments into discrete allele categories. This was accomplished by sorting all the alleles at a locus by size. Inspection of this graphical representation of all the alleles clearly showed the cut-off points between each successive allele size.

Genetic diversity was characterized by observed heterozygosity (H_O), expected heterozygosity (H_E) and the number of alleles per locus (A). The analysis package GENEPOP version 3.1 (Raymond & Rousset 1995) was used to perform a variety of statistical tests. Deviations from Hardy–Weinberg equilibrium (HWE) were examined for each population at each locus and for each locus at each population using Fisher's exact test. *P*-values were estimated using a Markov chain model (Guo & Thompson 1992). Tests for differences in genotypic distributions among populations were also performed. GENEPOP default parameters were used for the Markov chain tests (dememorization, batches, iterations). Sequential Bonferroni corrections (Rice 1989) were made to adjust significance levels for

Table 1 Northwest Atlantic harbour porpoise control-region haplotypes by region

Haplotype	GOM	GSL	NFLD	WGLD	MAS	Total
1	1					1
2	3	1	1	2		7
3	31	4	1	4	5	45
4	1					1
5	1					1
6	1			3	1	5
7	1					1
8	1			1		2
9	1		1			2
10	2	1		2	1	6
11	1	3		1	1	6
12	1					1
13	1					1
14	9	5	15	5	8	42
15	1					1
16	1			2	1	4
17		2			1	3
18	1	2	1	4	1	9
19		1			1	2
20	1	2		2	1	6
21	3	2	3	4	3	15
22		1				1
23		1				1
24		2		2		4
25		1				1
26		2	2		1	5
27		1			1	2
28	1	1	1			3
29		1	1	2	1	5
30		2	1	1	1	5
31		1				1
32		1				1
33	2	1				3
34		1			1	2
35		1	1			2
36			1			1
37	1		1			2
38			2	2		4
39			1			1
40			1	1	1	3
41			1			1
42	1		1		1	3
43			1			1
44			1			1
45			1			1
46			1			1
47	1		1			2
48	1		1			2
49	1					1
50	2					2
51	1				1	2
52	1					1
53	1					1
54	1					1
55	1			3		4
56	1					1
57	1					1
58	1					1
59				1		1
60				1		1
61				1		1
62				1		1
63				1		1
64				1	1	2
65				1		1
66				1		1
67				1		1
68					1	1
69					1	1
70					1	1
71					1	1
72					1	1
73					1	1
74					1	1
75					1	1
Total	80	40	42	50	41	253

GOM, Gulf of Maine; GSL, Gulf of St Lawrence; NFLD, Newfoundland; WGLD, West Greenland; MAS, mid-Atlantic states.
The most common haplotypes, 1 and 14, were submitted to GenBank with accession nos AF152570 and AF152571.

multiple tests. The AMOVA was used to test for correlations between geographical collection location and microsatellite DNA diversity. With microsatellite data, the Φ_{ST} estimator incorporates variance in allele size and distribution of alleles in each population. Finally, Slatkin's R_{ST}, an analogue of F_{ST} that assumes a stepwise mutation model rather than an infinite alleles model (Slatkin 1995), was also estimated. Owing to differences in sample sizes among the different populations, Goodman's (1997) unbiased estimate of R_{ST} was obtained using the program RSTCALC 2.2.

Results

Mitochondrial control-region sequences

We resolved 342 bases of the mitochondrial control region from 253 west Atlantic harbour porpoises, including 50 from West Greenland, 42 from Newfoundland, 40 from the Gulf of St Lawrence, 80 from the Gulf of Maine and 41 from the mid-Atlantic states winter sample. There were 61 variable positions defining 75 unique haplotypes (Table 1). Several common haplotypes were shared across

Location	N	Haplotype diversity	Nucleotide diversity
Gulf of Maine	80	0.839 ± 0.039	0.009 ± 0.005
Gulf of St Lawrence	40	0.967 ± 0.014	0.011 ± 0.006
Newfoundland	42	0.872 ± 0.049	0.012 ± 0.007
West Greenland	50	0.967 ± 0.010	0.013 ± 0.007
Mid-Atlantic states	41	0.950 ± 0.023	0.012 ± 0.007

Table 2 Genetic diversity estimates based on mitochondrial DNA (mtDNA) control-region sequences for northwest Atlantic harbour porpoise populations

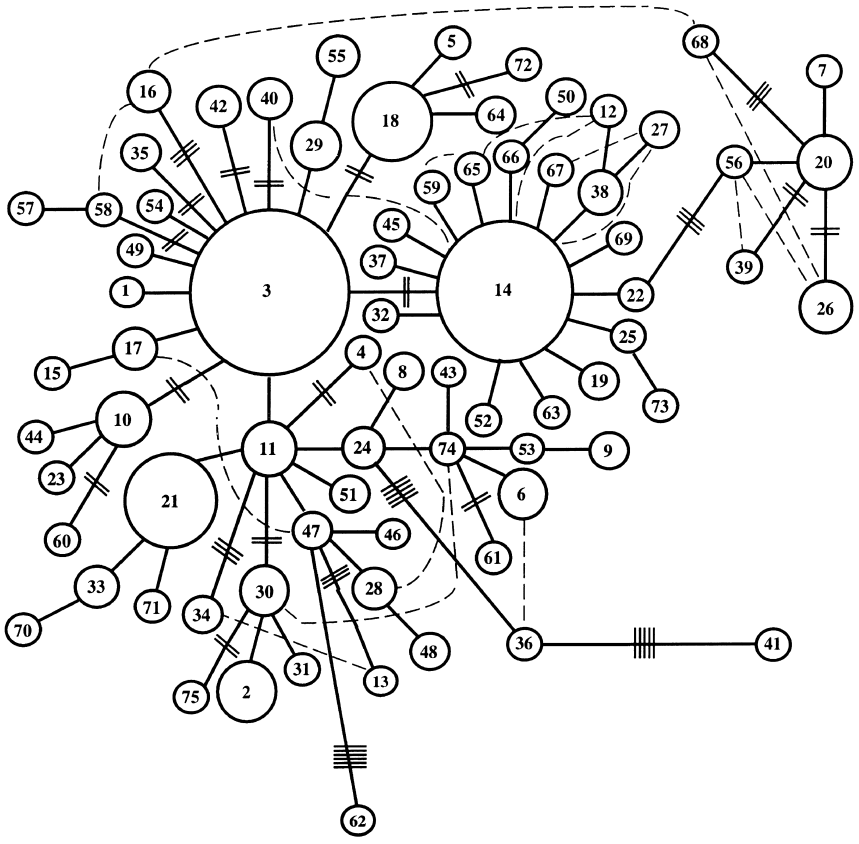

Fig. 2 Haplotype network showing the relationships among 75 harbour porpoise mitochondrial DNA (mtDNA) control-region haplotypes. Haplotype numbers correspond with the numbers in Table 1. The diameter of the circle is approximately proportional to the number of individuals bearing that haplotype. All haplotypes are separated by at least one substitution. Multiple substitutions between haplotypes are indicated by hash marks. Alternative connections between haplotypes (dotted lines) indicate homoplasy in the DNA sequence data.

most populations, but each region was also characterized by rarer, unique haplotypes.

Haplotypic diversity estimates ranged from 0.84 in the Gulf of Maine population to 0.97 in the Gulf of St Lawrence and West Greenland populations, with an overall average of 0.93. Nucleotide diversities ranged from 0.99% in the Gulf of Maine to 1.26% in West Greenland, with an overall average of ≈ 1.1% (Table 2). A minimum-spanning network (Fig. 2) consisted of two common haplotypes from which radiated multiple, rare haplotypes. A clear pattern of haplotype and geographical locale was not detected. The most commonly occurring haplotypes, 3 and 14, were found in all sampling locations, but with differing frequencies, and each has given rise to a number of closely related haplotypes, including five singly occurring haplotypes, unique to West Greenland, originating from haplotype 14, and five singly occurring haplotypes, unique to the Gulf of Maine, arising from haplotype 3. Extensive homoplasy in the data is evident in the large number of possible alternative connections in the network.

The AMOVA results indicated the presence of population subdivision among the summer breeding populations. Whether the AMOVA analysis was performed using genetic distance and frequency information (Φ_{ST}), or using haplotype frequencies alone (F_{ST}), the results using both sexes together indicated that a significant amount of the molecular variance could be accounted for by differences among populations (Table 3). As with previous studies, F_{ST} values were higher and P-values lower when only haplotype frequency information was utilized (O'Corry-Crowe *et al*. 1997). This data indicates that the West Greenland, Gulf of St Lawrence, Newfoundland and Gulf of Maine populations are not panmictic. Analysing the sexes separately produced different patterns. The

Table 3 Results of analysis of molecular variance (AMOVA) on mitochondrial DNA (mtDNA) control-region sequences

	% Variance among populations	% Variance within populations	Φ_{ST}/F_{ST}	*P*-value
A				
Excluding mid-Atlantic states				
Both sexes	1.08	98.92	0.011	≤0.03
Females only	2.30	97.70	0.023	≤0.04
Males only	0.27	99.73	0.0026	≤0.349
Including mid-Atlantic states				
Both sexes	0.54	99.46	0.0054	≤0.13
Females only	1.4	98.60	0.014	≤0.11
Males only	0.0	100.00	0.00	≤0.65
B				
Excluding mid-Atlantic states				
Both sexes	4.57	95.43	0.046	≤0.001
Females only	5.58	94.42	0.056	≤0.001
Males only	4.01	95.99	0.040	≤0.002
Including mid-Atlantic states				
Both sexes	3.41	96.59	0.034	≤0.0001
Females only	4.37	95.63	0.044	≤0.0001
Males only	2.92	97.08	0.029	≤0.003

A, AMOVA using genetic distance and haplotype frequency information; B, AMOVA using haplotype frequencies only.

results for females alone differed significantly from zero using either of the AMOVA analysis methods (Table 3). For males only, the analysis of haplotype frequency alone yielded a significant F_{ST} value (Table 3).

For both sexes pooled together, pairwise population comparisons of F_{ST} values showed a significant partitioning of the molecular variance, after sequential Bonferroni correction, for all pairs except the Gulf of St Lawrence to West Greenland comparison when using haplotype frequency data (Table 4). For females alone, the Gulf of Maine population differed significantly from all other summer breeding populations. For males, four of six pairwise comparisons yielded significant F_{ST} values; the Gulf of Maine to Gulf of St Lawrence and the Gulf of St Lawrence to West Greenland comparisons were not significant.

Inclusion of the mid-Atlantic states samples into the analysis decreased overall Φ_{ST} and F_{ST} values in all three cases: both sexes analysed, females alone and males alone (Table 3). Pairwise comparisons between the mid-Atlantic states and all other populations yielded F_{ST} values that did not differ significantly from zero, except the Gulf of Maine to mid-Atlantic comparison using females. This may stem from the fact that the number of females in the Gulf of Maine sample was very small.

Contingency table analysis rejected homogeneity of haplotype frequencies between the Gulf of Maine and the mid-Atlantic states winter sample ($P < 0.06$), but found no significant differences in haplotype frequencies between the winter sample and any of the remaining summer samples ($0.39 < P < 0.99$). Pooling of haplotypes into six categories to reduce the risk of problems associated with many rare haplotypes did not alter this conclusion. On the other hand, the conditional maximum-likelihood mixture analysis demonstrated that the winter sample

Table 4 Population pairwise F_{ST} and significance values for northwest Atlantic harbour porpoise summer breeding populations estimated from analysis of molecular variance (AMOVA) using mitochondrial DNA (mtDNA) haplotype frequency information

	GOM	GSL	NFLD	WGLD
Both sexes				
GOM	—	0.001	0.001	0.001
GSL	0.042	—	0.020	0.767
NFLD	0.095	0.024	—	0.004
WGLD	0.049	0.000	0.032	—
Females				
GOM	—	0.001	0.001	0.001
GSL	0.115	—	0.379	0.764
NFLD	0.131	0.001	—	0.428
WGLD	0.069	0.00	0.00	—
Males				
GOM	—	0.162	0.005	0.008
GSL	0.011	—	0.007	0.227
NFLD	0.062	0.051	—	0.003
WGLD	0.047	0.008	0.050	—

F_{ST} below diagonal, *P*-values above. *P*-values ≤ 0.008 are significant after Bonferroni correction at $\alpha = 0.05$.
GOM, Gulf of Maine; GSL, Gulf of St Lawrence; NFLD, Newfoundland; WGLD, West Greenland; MAS, mid-Atlantic states.

Table 5 Results of mixture analysis using the conditional maximum-likelihood approach indicating relative contributions of harbour porpoises from the summer populations to the mid-Atlantic winter sample

Population	Point estimate	Standard error	95% Confidence intervals
Gulf of Maine	0.19	0.21	0.00–0.50
Gulf of St Lawrence	0.40	0.34	0.00–0.66
Newfoundland	0.18	0.20	0.00–0.49
West Greenland	0.24	0.25	0.00–0.57

is probably a mixture of more than one of the summer populations as none of the confidence intervals for the contributions of the four summer populations included 100% (Table 5). However, the mtDNA haplotype frequencies of these four summer populations were not sufficiently distinguishing to accurately determine the relative contribution of each population to the winter mid-Atlantic sample (Table 5). Finally, use of the program SIMULATR indicated that the stock mixture analysis could have distinguished the sole summer source of the mid-Atlantic states sample if it existed (data not shown).

Microsatellite isolation

Screening of ≈ 1500 colonies resulted in 265 (18%) positive clones of which 60 were sequenced. The nucleotide sequences were examined for duplicates using the computer program STS PIPELINE (Resnick & Stein 1995), which also simultaneously designs primer pairs for each locus. We found that 23 of 45 (51%) clones were considered to be duplicates by this program. Upon closer examination, we discovered that they were not identical sequences because the repeat region and flanking regions of most of these clones were different. However, they all contained a common sequence of ≈ 100 bp either upstream or downstream of the cloned microsatellite region, causing the computer program to consider them duplicates. When compared with sequences in the GenBank DNA database, this common sequence showed 74% identity with a region of a cosmid-derived microsatellite sequence cloned from the cow, *Bos taurus* (Y. Zhang *et al.* 1995; GenBank accession no. X86815). We chose to eliminate these clones from further analysis because it seemed probable that they might be associated with a larger satellite DNA sequence present in the genome.

Primers designed by STS PIPELINE (Resnick & Stein 1995) were synthesized for nine of the isolated microsatellite markers. Each locus was screened for variation using seven porpoise samples. Eight of the nine loci amplified the appropriately sized DNA fragment were polymorphic (Table 6). However, as we continued genotyping all samples for all alleles, we encountered difficulties in scoring alleles for locus PPHO102. We therefore discontinued use of this microsatellite locus.

Microsatellite analysis

Summary statistics for microsatellite variation are shown in Table 7. As expected, microsatellite variation within populations was high compared with other nuclear markers (e.g. allozymes), with the number of alleles per

Table 6 Characterization of eight harbour porpoise microsatellite loci

Locus	Primer pairs (5′–3′)	Annealing temperature/no. of cycles	Repeat	Fragment size
PPHO104	F: CCTGAGGTGTGTAGTCA R: GACCACTCCTTATTTATGG	57 °C/25x	$(CA)_{19}$	164
PPHO110	F: ATGAGATAAAATTGCATAGA R: ATCATTAACTGGACTGTAGACCTT	50 °C/27x	$(CA)_{22}$	124
PPHO130	F: CAAGCCCTTACACATATG R: TATTGAGTAAAAGCAATTTTG	50 °C/27x	$(CA)_{25}$	192
PPHO131	F: GTTAGGTACCAGCCTCC R: CTAGTTATCATGCAGGGAGT	57 °C/25x	$(CA)_{13}$	186
PPHO133	F: AGGGGTTTCTGAAGTGA R: CCTTAATCACACCTTGG	50 °C/27x	$(CA)_{18}$	186
PPHO137	F: CAGGGCGGCCATGTACAGTTGAT R:GAGTTTGGCTCCCTCTCCAG	57 °C/25x	$(CA)_{26}$	123
PPHO142	F: GAAGGCTCAGGGTATTG R: CAGTTACTTTCCTCGGG	50 °C/27x	$(CA)_{22}$	152
PPHO102	F: CCTATCAACACCCTGGAGTTATGC R: GGGGCTGCACCTGTTCCT	57 °C/27x	$(CA)_{18}$	128

F, forward; R, reverse.
Repeat size and fragment length refer to the original clone from which primers to each locus were designed.
GenBank accession nos of the cloned loci are AF151785-AF151792.

Table 7 Summary statistics for *Phocoena phocoena* microsatellite loci

Region	Locus	PPHO110	PPHO130	PPHO131	PPHO137	PPHO142	PPHO104	PPHO133	Mean all loci	Mean w/o PPHO133
Gulf of Maine										
	N	80	80	79	80	80	80	78	79.57	79.83
	R	101–127	166–200	182–198	102–140	131–161	146–184	173–203		
	A	9	15	9	18	16	17	14	14.00	14.00
	H_E	0.78	0.90	0.81	0.90	0.89	0.90	0.89		
	H_O	0.65	0.88	0.79	0.86	0.86	0.84	0.69*	0.80	0.81
Gulf of St Lawrence										
	N	47	47	47	47	47	47	47	47.0	47.0
	R	107–127	174–200	182–196	104–132	127–159	150–180	173–201		
	A	9	13	8	15	15	15	14	12.71	12.50
	H_E	0.80	0.91	0.83	0.92	0.90	0.87	0.89		
	H_O	0.83	0.87	0.81	0.98	0.83	0.85	0.66*	0.83	0.86
Newfoundland										
	N	48	48	48	48	48	48	48	48.0	48.0
	R	107–127	166–200	182–198	94–132	131–159	134–188	177–199		
	A	10	14	9	15	15	16	11	12.86	13.17
	H_E	0.84	0.89	0.83	0.89	0.87	0.89	0.86		
	H_O	0.85	0.92	0.81	0.94	0.85	0.92	0.54*	0.83	0.88
West Greenland										
	N	50	50	50	49	50	50	49	49.7	49.8
	R	105–125	166–196	182–198	94–128	133–159	148–192	177–201		
	A	9	14	9	15	14	16	12	12.71	12.83
	H_E	0.81	0.91	0.84	0.91	0.86	0.89	0.87		
	H_O	0.76	0.98	0.84	0.96	0.90	0.90	0.65*	0.86	0.89
Mid-Atlantic states										
	N	49	51	50	50	50	50	49	49.9	50.0
	R	101–129	174–202	182–196	104–132	127–157	146–184	173–203		
	A	12	15	8	14	15	16	13	13.29	13.33
	H_E	0.85	0.92	0.83	0.91	0.89	0.90	0.87		
	H_O	0.94	0.96	0.84	0.96	0.88	0.86	0.53*	0.85	0.91

Number of individuals (N), range of allele sizes (R), number of alleles (A), and expected (H_E) and observed (H_O) heterozygosities are given for each locus in each population.
Mean values of N, A and H_O across loci within each population are given for all loci, and for all loci except PPHO133 (see the text).
*Indicates a significant heterozygote deficit ($P < 0.0001$).

locus ranging from eight to 16 and H_O values ranging from 0.53 to 0.96. All but one locus (PPHO133) conformed to HWE in all populations. Significant heterozygote deficiencies were observed in all populations at locus PPHO133. We chose to eliminate this locus from further analysis. For the remaining six loci, H_O values within populations ranged from 0.65 to 0.96.

We examined the microsatellite data for evidence of population structure. An analysis of the distribution of genotypes among populations at each locus revealed significant differences in 10 of 60 pairwise comparisons ($P < 0.05$); however, after a Bonferroni correction for multiple comparisons, only the Newfoundland vs. the Gulf of St Lawrence comparison at locus PPHO104 was significant ($P = 0.002$).

Most of the variation in genetic diversity was found within populations. The analysis of population subdivision attributed less than 0.5% of the genetic variance to among-population variation, which was not significantly greater than 0 ($F_{ST} = 0.18\%$, $P = 0.052$; $R_{ST} = 0.24\%$, $P = 0.181$). Among pairwise population comparisons, only a single value was marginally significant (Gulf of Maine vs. Newfoundland, $F_{ST} = 0.62\%$, $P = 0.005$); all pairwise R_{ST} values were not significant. Estimates of pairwise Nm values ranged from 16.1 to infinity.

Discussion

Genetic diversity

The northwest Atlantic populations of *Phocoena phocoena* show substantially higher levels of mtDNA diversity than populations present in the northeast Atlantic (Tiedemann *et al.* 1996; Walton 1997; Wang & Berggren 1997; Rosel

et al. 1999), and similar or slightly lower levels than those of the northeast Pacific (Rosel *et al.* 1995). Walton (1997) sequenced the homologous section of the control region of 327 harbour porpoises from the northeast Atlantic and found only 24 unique haplotypes. In this study, 253 porpoises from the northwest Atlantic revealed 75 unique haplotypes. Likewise, nucleotide diversity in the northwest Atlantic sample was nearly twice that estimated for the northeast Atlantic. These differences suggest that the northwest and northeast Atlantic populations of harbour porpoises experience limited genetic exchange (Rosel *et al.* 1999).

The average H_O value at six microsatellite loci ranged from 0.81 to 0.91. As expected for these loci with high mutation rates, the values are substantially higher than those estimated from allozyme data. Andersen (1993) surveyed 31 allozyme loci in 262 harbour porpoises from the northeast Atlantic and West Greenland and found that only two were polymorphic. Average H_O values estimated from her data for these two loci were 0.328 and 0.387, respectively. Andersen *et al.* (1997) later collected microsatellite data from three loci in these same populations: two loci were isolated from pilot whales (Schlötterer *et al.* 1991) and one was developed from sequence from cows and pigs (Kirkpatrick 1992). Two of these loci showed significant deviations from HWE in the West Greenland sample, and the authors suggested that this may have resulted from inbreeding or sampling of multiple populations. Our West Greenland samples, collected in the same areas and time periods, showed no evidence of deviation from HWE (with the exception of locus PPHO133, which showed a significant heterozygote deficiency in all populations) and hence do not support the presence of inbreeding or a mixed sample. It may be that a null allele was present at these two loci, as they were derived from evolutionarily divergent taxa.

The mean H_O value for harbour porpoise microsatellites was higher than that found in other cetacean species using loci isolated from the species studied. A world-wide sampling of humpback whales (Valsecchi *et al.* 1997), using three loci, produced a mean H_O value of 0.79 with an average of 15.3 alleles per locus. Using five microsatellite loci, Richard *et al.* (1996) found an average of 10.2 alleles per locus and a mean H_O of 0.79 in sperm whales, *Physeter macrocephalus*. A survey of 15 loci from beluga whales, *Delphinapterus leucas*, revealed the lowest diversity levels, with an average of 8.6 alleles per locus and a mean H_O of 0.65 (Buchanan *et al.* 1996).

Geographic variation

Examination of harbour porpoise control-region sequences revealed small, but significant, differences in the spatial distribution of genetic variation among summer breeding populations in the northwest Atlantic. Analyses based both on mtDNA control-region haplotype frequencies alone (F_{ST}) and haplotype frequencies coupled with the degree of genetic divergence between haplotypes (Φ_{ST}), indicated that there is significant partitioning of genetic variability among the four populations. The Gulf of Maine population was differentiated from all other summer populations. Not surprisingly, this population is the most geographically isolated of the four populations. Satellite telemetry data gathered from nine porpoises tagged in the Bay of Fundy/Gulf of Maine also indicate that this population is disjunct. None of the tagged porpoises left the area, leading the authors to conclude that this population is restricted in their movements (Read & Westgate 1997). The Newfoundland population also showed significant differentiation from the other populations. The habitat used by porpoises off eastern Newfoundland is also fairly well isolated from the other areas by both intervening land masses and deep water. Finally, the Gulf of St Lawrence and West Greenland populations could not be discriminated from one another. These results are congruent with an analysis of organochlorine contaminant levels in juvenile harbour porpoises, which revealed significant geographical variation in contaminant levels among the Gulf of St Lawrence, Newfoundland and Bay of Fundy/Gulf of Maine populations (Westgate & Tolley 1999).

Although the degree of partitioning of genetic variability among the four summer populations was small, it differed significantly from zero, indicating that these four populations are not panmictic. Many of the present day summer feeding areas, including the Gulf of St Lawrence, the Bay of Fundy/Gulf of Maine, western Greenland and at least the coastal waters around eastern Newfoundland, were covered with ice during the last glacial periods 17 000–21 000 years (Williams *et al.* 1998) and hence did not provide suitable habitat for harbour porpoises. Thus, the Gulf of St Lawrence, West Greenland and Newfoundland summer habitats contain relatively young populations and it is probable that there has not been sufficient time to effect significant mtDNA lineage sorting among them. The estimates of genetic exchange rates may thus be biased upwards, a signature of evolutionarily recent fragmentation of a refugial population, rather than of gene flow among ancient populations.

Harbour porpoises are small and difficult to see in the water, and they tend to avoid boats. Individuals bear few marks that could be used for individual identification as can be done, for example, with humpback whales and bottlenose dolphins. These characteristics make the study of behaviour in wild populations very difficult. However, one study of porpoises in the Bay of Fundy suggested that some females return to the area annually (Gaskin & Watson 1985). Whether site fidelity was a behaviour

common in harbour porpoises or unique to these particular females remains to be tested. Wang *et al.* (1996) published the first genetic study of harbour porpoises in the region. Using mtDNA RFLP analysis, they concluded that female porpoises are more philopatric than males, supporting the previous study. To test this hypothesis using higher resolution mtDNA control-region sequences, we subdivided our samples into males and females and reanalysed the data set using an AMOVA. Analysis of females alone produced the highest levels of genetic variance attributable to between-population comparisons, while analysis of males alone produced a lower overall F_{ST} value and a correspondingly higher estimate of *Nm*, although, interestingly, significant population subdivision was detected among males when haplotype frequencies only were used in the analysis. These data support the hypothesis that throughout the northwest Atlantic, females show stronger site fidelity than males, a behaviour that would be difficult to quantify in the field.

However, males may also show site fidelity to a lesser degree. Although the overall degree of population subdivision measured in males was lower than in females (Table 3), suggesting that male movement dilutes the differentiation of populations, several pairwise population comparisons using males differed significantly from zero (Table 4). Analyses of contaminant levels in male porpoises from the Gulf of St Lawrence and Newfoundland also revealed significant differences between these two populations, suggesting that movement of males between these areas is limited (Westgate & Tolley 1999). Analysis of contaminant loads provides information on an ecological timescale, while genetic analyses provide information on an evolutionary timescale. The relatively recent (evolutionarily) separation of these populations, coupled with low levels of male movement between them, may limit the ability of genetic data to differentiate the males, while contaminant analysis was better able to detect the differences.

In contrast to the mtDNA data, the six harbour porpoise-specific microsatellite loci, while highly variable, detected no population differentiation among the northwest Atlantic populations surveyed. Although nonsignificant, the trends seen in the microsatellite data did mirror the results of the mtDNA data, e.g. the Gulf of Maine population differed most from all others. One possible conclusion to draw from this result is that male-mediated gene flow is sufficiently high to maintain near homogeneity among these loci, while stronger female philopatry results in significant geographical heterogeneity in the maternally inherited mtDNA sequences.

Mid-Atlantic states

While the summer range of harbour porpoises in the northwest Atlantic is well defined, where animals spend the winter is not well known. When water temperatures drop and/or ice begins to form in the northern regions, porpoises appear to migrate out of these areas (Gaskin 1992). While the species is common in the Bay of Fundy in the summer, winter abundance is much lower (Gaskin 1992). Stranding records along the mid-Atlantic states (New York to North Carolina) document an increase in harbour porpoises during late winter and early spring (Polachek *et al.* 1995), suggesting that some proportion of the northwest Atlantic populations move south along the US coast during the winter. However, the source of these animals is unknown. Are they simply animals from the Gulf of Maine that have followed the coastline south, or does the mid-Atlantic region provide a wintering area for animals from other summer populations such as the Gulf of St Lawrence, Newfoundland or even West Greenland?

In 1993, 50 harbour porpoises stranded along the mid-Atlantic states, the majority of which were less than 1 year old (Haley & Read 1993). An additional 124 animals were stranded between 1994 and 1996 (inclusive) along the states of Maryland, Virginia and North Carolina (A. Read, personal communication). Some of these animals exhibited signs of human interactions, most commonly entanglement in fishing gear. In order to manage harbour porpoise populations effectively, it is critical that the bycatch be attributed to the correct population stock. Thus, there is a substantial need for determining where these winter animals originate. To analyse this question, we repeated the AMOVA analysis on the mtDNA sequences with the mid-Atlantic states sample included. Inclusion of this sample reduced overall F_{ST} and Φ_{ST} values for comparisons of females, males and both sexes combined. This is the pattern expected if the mid-Atlantic states sample comprises mixed stocks. If the mid-Atlantic states aggregation consisted solely of animals from the Gulf of Maine, we would have expected it to show the same pattern as the Gulf of Maine sample, i.e. significant divergence from all other summer populations. In fact, in pairwise comparisons involving the mid-Atlantic states, the only significant F_{ST} value obtained was that between the Gulf of Maine vs. mid-Atlantic states samples. In addition, haplotype diversity estimates for the Gulf of Maine (0.839 ± 0.04) were significantly lower than any other population, including the mid-Atlantic states (0.95 ± 0.02). Some haplotypes unique to the Gulf of St Lawrence or West Greenland summer populations appeared in the winter mid-Atlantic states sample. In fact, eight of the 28 haplotypes present in the winter sample were unique to that sample, suggesting that either not all source populations were surveyed or, more likely, we did not have sufficiently large sample sizes from the source populations to have surveyed all of the diversity present within them. In order to account for the presence

of other haplotypes, we concluded that the mid-Atlantic states winter aggregation comprised more than just Gulf of Maine animals. The contingency table analysis of mtDNA haplotype frequencies supports this conclusion. However, this test could not reject any of the other populations (Gulf of St Lawrence, Newfoundland, West Greenland) as being the sole source of the mid-Atlantic states sample. This result seems at first to be contradictory to the conditional maximum-likelihood analysis, which indicated that more than one summer population was present in the winter sample. However, because the contingency test analysis could not distinguish between the winter sample and any of the three (non-Gulf of Maine) summer populations, it probably could not distinguish between a winter sample comprising a mixture of the summer populations and any one of the summer populations either. Thus, together these analyses demonstrate that the mid-Atlantic states winter sample probably comprises porpoises from more than one summer population, that any of the four summer populations could contribute to the winter sample and, finally, that the relative contributions of any of the summer populations is very imprecisely determined by the mtDNA data.

Management implications

Genetic data collected globally from harbour porpoises reflects the existence of at least two probable evolutionarily significant units, as defined by Moritz (1994); one in the northeast Pacific and one in the North Atlantic (Rosel *et al.* 1995; Wang *et al.* 1996). These two porpoise populations exhibit reciprocal monophyly in mtDNA sequences, but no nuclear data is available, and so the definition of evolutionarily significant units for these populations has not been fully tested. Within the North Atlantic there is no evidence for reciprocal monophyly or unique diagnosable groups among any populations (Rosel *et al.* 1999). Thus, both the evolutionarily significant unit concept and the phylogenetic species concept (Vogler & DeSalle 1994) would support pooling of all North Atlantic porpoises into one conservation unit. Defining units for management of exploited marine fish and mammal species differs, however, from defining units for conservation of most rare or endangered terrestrial species where the evolutionarily significant unit and phylogenetic species concepts are often applied. Management of exploited marine species must be able to predict and incorporate the effects of harvesting and/or bycatch on the sustainability of any given population stock. Pooling of all North Atlantic populations into one management unit means that the overall quota of allowable porpoise bycatch for the North Atlantic, which could number upwards of 5000 animals, could conceivably occur within one small geographical region. The biological impact of such a removal of porpoises from any given geographical locale is not known; even if there is gene flow into this region from other areas would it be sufficient to maintain the population in the face of substantial incidental mortality? For a risk-averse strategy of management of many cetacean species, the use of evolutionarily significant units or phylogenetic species concept criteria is too restrictive (see also Baker & Palumbi (1997)).

However, in the northwest Atlantic, the mtDNA data do support a significant partitioning of genetic variation among the four defined summer populations. Thus, can we define management units (Moritz 1994) in this region? The Gulf of Maine population revealed significant divergence in mtDNA sequences and frequencies when compared with the other three populations. This population can then be considered a management unit. The Newfoundland sample was also significantly differentiated from the Gulf of St Lawrence and West Greenland. Thus, the Newfoundland population may also be considered a management unit. This then leaves only the Gulf of St Lawrence–West Greenland pair undifferentiated. The lack of distinction between these two populations could be a result of gene flow between them, or an artefact of insufficient power to detect differences given the relatively recent repopulation of these regions following the retreat of the glaciers. Finally, the genetic analysis suggests that there is probably a mixed stock occurring off the mid-Atlantic states in winter. Allocating winter bycatch to the appropriate summer population is the next step in fulfilling management needs for this species in the northwest Atlantic.

Acknowledgements

We are grateful to the following people who helped to provide tissue samples: A. Evely, P. Fontaine, D. Gaskin, M.-P. Heide-Jorgensen, J. Jensen, M. Kingsley, J. Nicholas, A. Read, S. Richardson, G. Stenson, B. White, members of the Grand Manan Whale and Seabird Research Station, and the Southeast Atlantic Stranding Network. Drs M. Masuda and J. Pella provided significant contributions through the implementation of, and advice with, the mixed-stock analysis. We thank two anonymous reviewers for their constructive comments on the manuscript. This work was funded by the New Hampshire/Maine Seagrant Program, and the National Marine Fisheries Service, Northeast Fisheries Science Center.

References

Andersen LW (1993) The population structure of the harbour porpoise, *Phocoena phocoena*, in Danish waters and part of the North Atlantic. *Marine Biology*, **116**, 1–7.

Andersen LW, Holm L-E, Siegismund HR, Clausen B, Kinze C, Loeschcke V (1997) A combined DNA-microsatellite and isozyme analysis of the population structure of the harbour porpoise in Danish waters and West Greenland. *Heredity*, **78**, 270–276.

Árnason Ú, Gullberg A, Widegren B (1991) The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus*. *Journal of Molecular Evolution*, **33**, 556–568.

Baker CS, Palumbi SR (1997) The genetic structure of whale populations: implications for management. In: *Molecular Genetics of Marine Mammals* (eds Dizon AE, Chivers SJ, Perrin WF), pp. 117–146. Special Publication 3, Society for Marine Mammalogy, Lawrence, KS.

Bisack KD (1997) Harbor porpoise bycatch estimates in the US New England multispecies sink gillnet fishery: 1994 and 1995. *Report of the International Whaling Commission*, **47**, 705–714.

Bravington MV, Bisack KD (1996) Estimates of harbor porpoise bycatch in the Gulf of Maine sink gillnet fishery, 1990–93. *Report of the International Whaling Commission*, **46**, 567–574.

Buchanan FC, Friesen MK, Littlejohn RP, Clayton JW (1996) Microsatellites from the beluga whale *Delphinapterus leucas*. *Molecular Ecology*, **5**, 571–575.

Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. *Genetics*, **136**, 343–359.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Gaskin DE (1984) The harbour porpoise *Phocoena phocoena* (L.): regional populations, status, and information on direct and indirect catches. *Report of the International Whaling Commission*, **34**, 569–586.

Gaskin DE (1992) Status of the harbour porpoise, *Phocoena phocoena*, in Canada. *Canadian Field-Naturalist*, **106**, 36–54.

Gaskin DE, Watson AP (1985) The harbour porpoise, *Phocoena phocoena*, in Fish Harbour, New Brunswick, Canada; occupancy, distribution, and movements. *Fishery Bulletin*, **83**, 427–442.

Goodman SJ (1997) RSTCALC: a collection of computer programs for calculating unbiased estimates of genetic differentiation and gene flow from microsatellite data and determining their significance. *Molecular Ecology*, **6**, 881–885.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Haley NJ, Read AJ (1993) *Summary of the Workshop on Harbor Porpoise Mortalities and Human Interactions*. NOAA Technical Mem. National Marine Fisheries Service/NER-5. August 1993.

IWC (1991) Report of the Scientific Committee. *Report of the International Whaling Commission*, **41**, 51–89.

IWC (1992) Report of the Scientific Committee. *Report of the International Whaling Commission*, **42**, 51–86.

IWC (1993) Report of the Scientific Committee. *Report of the International Whaling Commission*, **43**, 55–92.

Jefferson TA, Curry BE (1994) A global review of porpoise (Cetacea: Phocoenidae) mortality in gillnets. *Biological Conservation*, **67**, 167–183.

Kirkpatrick BW (1992) Identification of a conserved microsatellite site in porcine and bovine insulin-like growth factor-I gene 5′ flank. *Animal Genetics*, **23**, 543–548.

Kumar S, Tamura K, Nei M (1993) *MEGA: molecular evolutionary genetics analysis, version 1.0*. The Pennsylvania State University, University Park, PA.

Levinson G, Gutman GA (1987) Slipped-strand mispairing: a major mechanism for DNA sequence evolution. *Molecular Biology and Evolution*, **4**, 203–221.

Masuda M, Nelson S, Pella J (1991) *User's manual for GIRLSEM, GIRLSYM, and CONSQRT. The computer programs for computing conditional maximum likelihood estimates of stock composition from discrete characters. Person Computer Version*. USA-DOC-NOAA-NMFS, Auke Bay Laboratory. Available at ftp: // wwwabl.afsc.noaa.gov/sida/mixture-analysis/

Miller MP (1997) *Tools for population genetic analyses (TFPGA), version 1.3*. Department of Biological Sciences, Box 5640, Northern Arizona University, Flagstaff, AZ 86011–5640.

Moritz C (1994) Defining 'evolutionarily significant units' for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

O'Corry-Crowe GM, Suydam RS, Rosenberg A, Frost KJ, Dizon AE (1997) Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. *Molecular Ecology*, **6**, 955–970.

Pella JJ, Milner GB (1987) Use of genetic marks in stock composition analysis. In: *Population Genetics and Fisheries Management* (eds Ryman N, Utter F), pp. 247–276. University of Washington Press, Seattle, WA.

Polachek T, Wenzel FW, Early G (1995) What do stranding data say about harbor porpoise (*Phocoena phocoena*)? *Report of the International Whaling Commission* (*Special Issue*, **16**), 169–179.

Pulido JC, Duyk GM (1994) Construction of small insert libraries enriched for short tandem repeat sequences by marker selection. In: *Current Protocols in Human Genetics, Vol. 1*. (eds Dracopoli NC, Haines JL, Korf BR *et al*.). John Wiley, New York.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **96**, 248–250.

Read AJ (1999) Harbour porpoise *Phocoena phocoena* (Linnaeus, 1758). In: *Handbook of Marine Mammals, Vol. 6: the Second Book of Dolphins and the Porpoises* (eds Ridgway SH, Harrison R), pp. 323–355. Academic Press, San Diego.

Read AJ, Westgate AJ (1997) Monitoring the movements of harbour porpoises (*Phocoena phocoena*) with satellite telemetry. *Marine Biology*, **130**, 315–322.

Resnick R, Stein LD (1995) *STS Pipeline 1.2*. Whitehead Institute for Biomedical Research, Cambridge, MA.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Richard KR, Whitehead H, Wright JM (1996) Polymorphic microsatellites from sperm whales and their use in the genetic identification of individuals from naturally sloughed pieces of skin. *Molecular Ecology*, **5**, 313–315.

Rosel PE, Block BA (1996) Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. *Marine Biology*, **125**, 11–22.

Rosel PE, Dizon AE, Haygood MG (1995) Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 1210–1219.

Rosel PE, Tiedemann R, Walton M (1999) Genetic evidence for restricted trans-Atlantic movements of the harbour porpoise, *Phocoena phocoena*. *Marine Biology*, **133**, 583–591.

Saiki RK, Gelfand DH, Stoffel S *et al*. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239**, 487–494.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a*

Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schlötterer C, Amos B, Tautz D (1991) Conservation of polymorphic simple sequence loci in cetacean species. *Nature*, **354**, 63–65.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1996) *ARLEQUIN: a software package for population genetics, version 1.0*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

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

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. *Nucleic Acids Research*, **12**, 4127–4139.

Tiedemann R, Harder J, Gmeiner C, Haase E (1996) Mitochondrial DNA sequence patterns of harbour porpoises (*Phocoena phocoena*) from the North and Baltic Sea. *Zeitschrift für Säugetierkunde*, **61**, 104–111.

Valsecchi E, Palsbøll P, Hale P *et al.* (1997) Microsatellite genetic distances between oceanic populations of the humpback whale (*Megaptera novaeangliae*). *Molecular Biology and Evolution*, **14**, 355–362.

Vogler AP, DeSalle R (1994) Diagnosing units of conservation management. *Conservation Biology*, **8**, 354–363.

Wade PR (1998) Calculating limits to the allowable human-caused mortality of cetaceans and pinnipeds. *Marine Mammal Science*, **14**, 1–37.

Walton MJ (1997) Population structure of harbour porpoises *Phocoena phocoena* in the seas around the UK and adjacent waters. *Proceedings of the Royal Society of London B*, **264**, 89–94.

Wang JY, Berggren P (1997) Mitochondrial DNA analysis of harbour porpoise (*Phocoena phocoena*) in the Baltic Sea, the Kattegat-Skagerrak Seas and off the west coast of Norway. *Marine Biology*, **127**, 531–537.

Wang JY, Gaskin DE, White BN (1996) Mitochondrial DNA analysis of harbour porpoise, *Phocoena phocoena*, subpopulations in North American waters. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 1632–1645.

Waples RS (1991) Pacific salmon, *Oncorhynchus* spp. and the definition of 'species' under the Endangered Species Act. *Marine Fisheries Review*, **53**, 11–22.

Westgate AJ, Tolley KA (1999) Geographical differences in organochlorine contaminants in harbour porpoises *Phocoena phocoena* from the western North Atlantic. *Marine Ecology Progress Series*, **177**, 255–268.

Williams M, Dunkerley D, De Deckker P, Kershaw P, Chappell J (1998) *Quaternary Environments*. Oxford University Press, New York.

Wright S (1978) *Evolution and Genetics of Populations, Vol. IV, Variability Within and Among Natural Populations*. University of Chicago Press, Chicago, IL.

Zhang Y, Redaelli L, Castiglioni B, Mezzelani A, Ferretti L (1995) Five polymorphic bovine microsatellite loci: IDVGA-62A, IDVGA-71, IDVGA-82, IDVGA-88, IDVGA-90. *Animal Genetics*, **26**, 365–366.

Ziegle JA, Su Y, Corcoran KP *et al.* (1992) Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics*, **14**, 102–1031.

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