Population structure of harbour porpoises *Phocoena phocoena* in the seas around the UK and adjacent waters

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SUMMARY

The population structure of harbour porpoises from British and adjacent waters was studied by examining variability in a 200 bp (base pair) section of the control region of mitochondrial DNA (mtDNA) extracted from 327 animals. This region contained 20 variable sites giving rise to 24 different haplotypes. Mean nucleotide diversity between all pairs of haplotypes was 0.81% (range 0–4%). The most common haplotype occurred in 63% of the samples and was recorded in all geographical areas; several other haplotypes were present in two or more of the sampling locations. This suggests considerable historical interconnections among populations, probably through gene flow. However, there were significant differences ($p < 0.05$) as determined by AMOVA (Analysis of Molecular Variance, Excoffier *et al.* 1992), between porpoises from the northern and southern North Sea, and between the northern North Sea and the Celtic/Irish Sea. The differences were predominantly due to variation among females. This sex-related difference in population genetic structure suggests that males disperse more than females. This has important consequences for evaluating the consequences of incidental catches of porpoises by fisheries in these seas since there may be a greater impact on local populations than is implied by simple calculations of mortality.

1. INTRODUCTION

The harbour porpoise *Phocoena phocoena* is the smallest and most frequently sighted cetacean in European seas (Klinowska 1991). Because of practical difficulties much remains unknown about its status, movements, and biology. However, it appears that population numbers have declined markedly in areas where it used to be common, such as the southern North Sea, the Baltic and the English Channel (Klinowska 1991). Porpoises are short-lived animals which mainly inhabit coastal areas. One of the major present-day problems is their susceptibility to incidental capture and death by asphyxiation in fishing nets (IWC 1994). The concerns are such that the UK Biodiversity Steering Group (1995) included the porpoise as one of the nine mammalian species on its short list for priority action. Recent comparisons of by-catch levels of porpoises in fisheries operating in the North and Celtic Seas with estimates of abundance in the same areas have indicated that these levels may not be sustainable (IWC 1996). However, any interpretation of the effects of by-catch depends critically on assumptions about the population structure of harbour porpoises. If the porpoises which are observed in a certain geographical area are mistakenly assumed to form a discrete population, then the effect of by-catches in that area will be over-estimated. Conversely if a discrete population in a certain area is not recognized as such, the effect of by-catches will be underestimated.

A worldwide review of porpoise populations was carried out by Gaskin (1984) using available sightings and strandings data. In the seas around the UK he proposed three main discrete populations, namely Ireland/west Britain, the North Sea, and the English Channel. Later work suggested there may be sub-populations within the North Sea (Yurick & Gaskin 1987; Andersen 1993). Genetic methods are now

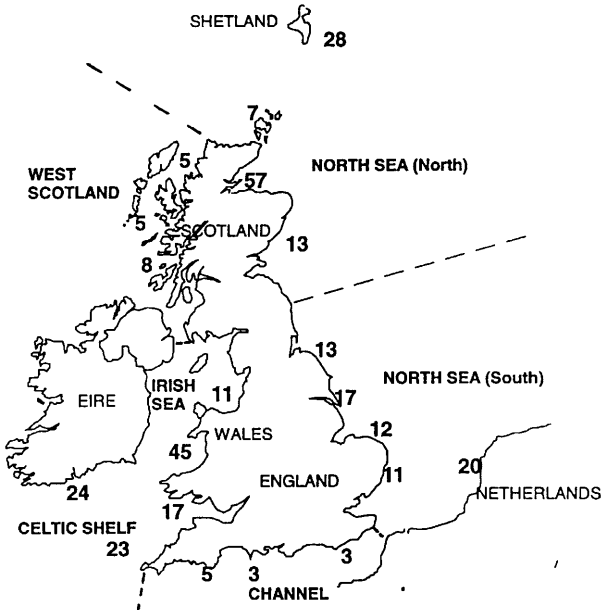

Figure 1. Map showing the areas where samples were collected.

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available to test these proposed population subdivisions. I used direct sequencing of a portion of the control region of mtDNA, obtained via the polymerase chain reaction (PCR, Saiki *et al*. 1988), to study porpoises from around the British Isles and adjacent waters. This method can be used effectively even with small quantities of poor quality DNA as may be obtained from stranded dead animals. MtDNA is a powerful tool in evolutionary biology because of its relatively rapid rate of mutation (5–10 times higher than single copy nuclear genes), although in some cetacean species the substitution rate may be slower than in land mammals (Hoelzel & Dover 1991; Baker *et al*. 1994). Because it is maternally inherited, effective population size for mtDNA is one quarter of that for nuclear genes, leading to a higher rate of local differentiation due to random drift (Baker *et al*. 1994; Moritz 1994). Within the mtDNA genome, the control region is especially variable, and has been widely used in population studies (Avise 1994).

2. METHODS AND MATERIALS

Skin samples were obtained from 291 stranded, and 36 (17 Shetland, 19 Celtic shelf) by-caught porpoises. Information on the sex, weight, length, and pollutant burdens of over 100 of these animals has already been published (Kuiken *et al*. 1994). About 200–500 mg tissue were finely minced and incubated for 24 h at 55 °C in 2 ml digestion buffer (50 mM Tris-HCl pH 7.5, 30 mM EDTA, 50 mM NaCl, 1% SDS, 200 μg proteinase K). Undigested material, lipids and proteins were removed by a modification of the salting out procedure of Bruford *et al*. (1992): 1 vol. chloroform in addition to 0.25 vol. saturated NaCl solution was added to the sample, which was shaken vigorously, left for 10 min and then centrifuged. DNA was precipitated from the aqueous layer by the addition of 2 vol. ice-cold ethanol. Following centrifugation the DNA pellet was taken up in 0.25–0.5 ml TE buffer and stored at −20 °C. The yield was not routinely assayed.

In preliminary studies the primers of Kocher *et al*. (1989), based on conserved sequences in the Thr-tRNA and Phe-tRNA genes which flank the control region, were used for both PCR and sequencing. Later, more specific primers were designed namely ProL (5′-ACCAACACCCAAAGCT) and H506 (5′-TATGTGTGAGCATGGGCTGA) to produce a 569 bp PCR product including 527 bases of the 5′-end of the L-chain. One μl DNA was added to 29 μl of a reaction mix as described by Hoelzel & Green (1992). This was followed by 25–30 cycles of 94 °C for 30 s, 47 °C for 30 s, and 72 °C for 1 min, followed by 3 min at 72 °C. A control blank containing no DNA was included in every machine run to test for contamination. Three μl of the final solution was run on a 1% agarose gel in TAE buffer to check the success of the reaction. If suitable, the remainder of the PCR product was cleaned using a commercial kit (Prep-a-gene, Biorad). The final eluate, in 15 μl water, was divided into 2×6.25 μl portions for sequencing.

The double-stranded PCR product was sequenced with a commercial kit (USB Biochemicals, Sequenase 2) using Winship's (1989) modification of the sequenase protocol. The internal primers H237 (5′-TATAATATGTAAGAGCGT-GC) and H419 (5′-CCTGAAGTAAGAACCAGATG) were used for sequencing. A 500 base sequence of the most commonly occurring haplotype has been submitted to the EMBL database (accession number X91613). The numbers in the primer names refer to the position in this sequence; the ProL primer relates to a sequence in the Pro-tRNA gene which immediately precedes the control region.

The animals were initially divided into five putative 'populations' (figure 1) mainly based on the proposals of Gaskin (1984): West Scotland; North Sea (N); North Sea (S); English Channel; Celtic and Irish Seas. For 14 animals sex was not determined at the time of sampling. In these cases the PCR sex-determination method of Brown *et al*. (1995) was used.

Nucleotide diversity of the haplotypes was measured as percentage difference, using the computer program MEGA (Kumar *et al*. 1993). Other measures of diversity, such as Jukes-Cantor and Tamura-Nei, gave very similar results in the subsequent analyses. The degree of population geographical substructure was tested using the program AMOVA (Excoffier *et al*. 1992). This procedure takes into account both distribution patterns and genetic distances. It calculates standard variance components and an array of haplotype correlation measures referred to as Phi_{ST} statistics, which are analogous to the F_{ST} statistics of Wright (1951). The significance of the variance components is tested using Monte-Carlo resampling methods.

3. RESULTS

The first 360 bases from the 5′ end of the control region L-chain were sequenced in 155 samples. This region contained 28 variable sites which defined 31 distinct haplotypes. Most of the variations occurred in

Table 1. *Distribution and frequency of the 24 haplotypes found in the putative populations tested*

haplotype (base number) 11111111 12455666889011222259 78831357816961624697	population code	1	2	3	4	5	total
gaggcaccggtcagttcctc	A	10	54	56	7	78	205
....t..............	B	0	0	3	0	3	6
.g..t..............	C	0	5	1	1	14	21
.g..t.t...........t	D	0	6	2	0	2	10
..................t	E	0	3	0	1	0	4
.g..t.t........c....	H	1	1	1	0	1	4
.g..t......t........	K	0	0	1	0	0	1
.g..t.............t	L	0	23	4	0	0	27
.g..t...........t...	M	4	0	1	0	0	5
ag..t.t............	O	0	0	0	0	1	1
.g..t...a...g.......	P	0	0	0	1	3	4
.g.atg........c.....	R	0	2	0	0	0	2
.g..t.t............	V	0	7	1	0	8	16
....t.t............	W	0	0	0	0	2	2
aga.t.t............	X	0	0	0	1	5	6
a...t.t......a...t..	Y	1	1	0	0	0	2
..........c.........	AB	0	0	0	0	1	1
............a......	AF	0	0	0	0	1	1
.g..t...a...........	AE	0	0	1	0	1	2
.g.at..............	AG	1	0	0	0	0	1
..................c.	AH	1	1	0	0	0	2
.g..t....a..........	AI	0	1	0	0	0	1
.g..t........a......	AJ	0	0	2	0	0	2
.......t...........	AK	0	1	0	0	0	1
	total	18	105	73	11	120	327

Region 1, west Scotland; Region 2, North Sea (N); Region 3, North Sea (S); Region 4, English Channel; Region 5, Irish/Celtic Seas.

Table 2. *Sample sizes and mean within-population nucleotide diversity for harbour porpoises*

('% diversity' is the mean of all pairwise comparisons within a set of samples.)

	n	males	females	% diversity
west Scotland	18	11	7	1.11
North Sea (N)	105	54	51	0.96
North Sea (S)	73	42	31	0.53
English Channel	11	7	4	0.98
Celtic/Irish Sea	120	64	56	0.73
all animals	327	178	149	0.81
males only	178	178	0	0.84
females only	149	0	149	0.78

the first 200 bases and AMOVA analyses showed very little difference in the results if 200 or 360 base sequences were compared. Therefore, in order to speed up the study, only 200 bases were sequenced in the remaining 172 animals. This region contained 20 variable sites which defined 24 distinct haplotypes, the distribution patterns of which are shown in table 1. Very few of these variable positions were located in, or resulted in, restriction sites, therefore most of the variation present would be undetectable using RFLP analysis. No insertions or deletions were noted. All the nucleotide substitutions were transitions ($A \leftrightarrow G = 13$, $C \leftrightarrow T = 15$); an observation frequently noted in other species (Avise 1994). Haplotype A was detected in 205 (63%) of the samples and was the most common haplotype in all locations. The next two most common haplotypes, L and C, occurred, predominantly in samples from the North Sea (N) and the Celtic/Irish Sea respectively. Seventeen haplotypes occurred five or less times.

Nucleotide diversity between pairs of haplotypes ranged from 0–4%. The mean value within each geographical grouping ranged from 0.53% to 1.11%, with an overall mean of 0.81% (table 2).

The results from the AMOVA are shown in table 3. The overall Phi_{ST} values is 0.042 ($p = 0.002$) indicating that over 95% of total variation is due to within-rather than between-population differences. Two of the ten comparisons (between North Sea (N) and (S) and between North Sea (N) and Celtic/Irish Sea) were statistically significant ($p < 0.05$). Using Phi_{ST} as an analogue for F_{ST}, this yields an estimate of female migrants per generation, calculated from $F_{ST} = 1/(2N_e m+1)$, as 11.4 overall, with 5.3 as the lowest value between a pair of geographical areas.

When the sexes were analysed separately the overall Phi_{ST} values were 0.013 ($p = 0.168$) for males and 0.086 ($p = 0.001$) for females. A significant difference was seen between females of the North Sea (N) and (S) and between both these areas and the Celtic/Irish Sea. For males only the difference between the North Sea (N) and the Celtic/Irish Sea was significant. If only the females are considered, then the estimate of female migrants per generation is 5.3 overall, with 2.2 as the lowest value between a pair of geographical areas.

4. DISCUSSION

The mean nucleotide diversity of 0.81% observed in this study is similar to the values of 0.90% for 81

Table 3. *Comparison of nucleotide diversity between putative populations as determined by the AMOVA program*

(Values below the principle diagonal are estimates of between-population variation (Phi_{ST}), corrected for within-population variation. Values above the diagonals are estimates of the probability that the observed differences between samples will occur by chance, as determined by the AMOVA program using 1000 Monte-Carlo simulations of the data set. An asterisk following a value means that it is statistically significant ($p < 0.05$). If Phi_{ST} is close to zero, then the calculation procedure occasionally produces small negative values; they may be regarded as zero values and as indicating no differentiation between the two populations compared.)

	west Scotland	North Sea (N)	North Sea (S)	Channel	Celtic/ Irish Sea
all animals					
west Scotland	–	0.677	0.118	0.833	0.245
North Sea (N)	−0.016	–	0.000*	0.248	0.002*
North Sea (S)	0.033	0.087	–	0.480	0.066
Channel	−0.039	0.019	−0.013	–	0.829
Celtic/Irish	0.009	0.058	0.018	−0.035	–
males only					
west Scotland	–	0.790	0.156	0.521	0.089
North Sea (N)	−0.036	–	0.085	0.597	0.045*
North Sea (S)	0.047	0.024	–	0.733	0.624
Channel	−0.033	−0.026	−0.045	–	0.690
Celtic/Irish	0.064	0.030	−0.009	−0.039	–
females only					
west Scotland	–	0.106	0.210	0.999	0.496
North Sea (N)	0.098	–	0.002*	0.271	0.002*
North Sea (S)	0.038	0.182	–	0.090	0.019*
Channel	−0.145	0.059	0.105	–	0.751
Celtic/Irish	−0.020	0.079	0.058	−0.080	–

Pacific harbour porpoises and 0.89% for 16 Atlantic porpoises found by Rosel *et al.* (1995) for the same mtDNA region. There is no distinct break in the geographic distribution of the 24 different mtDNA haplotypes found in the present study. Type A was predominant in all areas and no haplotype which occurred more than twice was present exclusively in one area, suggesting considerable historical interconnections among populations. However, type C was more common in the Celtic/Irish Seas and type L in the North Sea (N), indicating some degree of geographical sub-structuring. Cluster analyses of unique haplotypes by UPGMA or nearest-neighbour methods did not indicate that related haplotypes were geographically structured. Rosel *et al.* (1995) found porpoises in all areas of the north-east Pacific from California to Alaska shared some haplotypes, although several distinct haplotype groupings were found. However, they found no haplotypes shared between Pacific and Atlantic porpoise populations.

The overall Phi_{ST} value of 0.042 from the AMOVA analysis indicates that only approximately 4% of the total variance in the pairwise genetic distances is due to inter-population differences, although this is statistically significant ($p = 0.002$). Rosel *et al.* (1994, 1995) found overall Phi_{ST} values of 0.011 in a study on common dolphins and 0.107 ($p = 0.003$) for porpoises in the north-east Pacific. In the latter study values between different pairs of populations were up to 0.194, which is higher than found in the present study in which Phi_{ST} values between areas are generally low, indicating relatively high levels of gene flow. Nevertheless, the significant differences seen between some areas indicates that porpoises around the UK are not panmictic.

There are a number of problems in deciding how samples in studies such as this should be divided for analysis. As there are no apparent geographical barriers to movement around the UK it is difficult, without further information to define, if they exist, geographical limits of discrete populations. Also, because of drifting of dead or sick animals, material collected from stranded animals is not necessarily from the area where the animal lived. Thus, fine-scale geographical divisions are problematical. The main aim of this study was to test the subdivisions proposed by other workers (see Introduction). Thus, the samples were principally divided into Gaskin's (1984) major groupings of Irish/west Britain, North Sea and Channel populations. The North Sea was divided into northern and southern sections, using south-east Scotland where no animals were found as the provisional division line. In addition, these groupings and alternative poolings were compared for maximum heterogeneity by use of both AMOVA (Phi_{ST}) and the Monte-Carlo option (χ^2) of the program REAP (McElroy *et al.* 1992), which utilizes haplotype frequency distribution but not sequence information. Thus, within the Irish Sea/Celtic Sea area no significant differences of heterogeneity were found if this grouping was treated either as a whole or as separate Celtic shelf, Eire and UK populations. Likewise, within the North Sea, no differences were indicated between the Netherlands and English populations, whereas there were significant differences between the northern and southern areas. If the two North Sea groupings were compared to the Irish/Celtic Sea group, then REAP gave a χ^2 value of 56.4 ($p = 0.000$) if the North Sea groups were combined and a value of 102.8 ($p = 0.000$) if they were treated separately. The values changed little if the west Scotland group was added to the Irish/Celtic Sea grouping. The English Channel population was included, as mentioned, to test Gaskin's proposals. No evidence is apparent to indicate a separate Channel population, though the sample size is too small to be certain. In recent years sightings and strandings have become infrequent, and there now appears to be little movement of porpoises through the region; and during a recent summer survey of porpoise numbers in the North Sea and adjacent areas, not a single porpoise was sighted in the Channel (Hammond *et al.* 1995). Thus, it is possible that the Channel population has been extirpated and that the sampled animals had drifted from other areas such as the Celtic shelf or southern North Sea.

Therefore, the results support Gaskin's (1988) broad categorization of harbour porpoises around the British Isles into Irish/west Britain and North Sea populations, with differences also apparent within the North Sea. However, there may be other sub-groupings not apparent from this study, and more data is required to fully describe the relationships within and between the different geographical areas. Avise *et al.* (1987) classified mtDNA phylogeographic patterns into five categories. The present results best fit into his category V, in which there is a continuous genetic divergence pattern with intermediate gene flow and no subdivision by long-term zoogeographic barriers. In this category some haplotypes are widespread whereas others are localized such that the overall pattern is one of a nested series of phylogenetic relationships. It is also possible that there is a patchy continuum of porpoise groupings around the UK with gene flow between groups decreasing as geographic distance increases. In order to investigate this further it would be useful to analyse animals for which more accurate locations are known, e.g. from by-caught, rather than stranded animals, using, for example, the approach of Neigel *et al.* (1991) which provides an estimate of average single-generation dispersal distance from a geographic survey of mtDNA variation.

The results reported here suggest that gene flow and dispersal, as a result of females' movement, is lower than for males. This is consistent with the observation that female mammals often show fidelity to their natal site while juvenile males tend to disperse (Greenwood 1980). This can lead to different distributions according to sex of mtDNA, but the evidence for this may be lost every generation as successive male immigrants leave no male heirs (Baker *et al.* 1994; Avise 1995; Medrano *et al.* 1995). The movement and migratory patterns of porpoises in the north-east Atlantic and North Sea are not well understood, although it has been inferred that Baltic Sea porpoises move into the North Sea during the winter months (Klinowska 1991). On the basis of sightings made during seabird

surveys, Northridge *et al.* (1995) suggested that there is an influx of porpoises into the western sector of the North Sea during the breeding season. Some evidence for genetic differentiation among harbour porpoises from the eastern North Sea has been published. Kinze (1985) used metric and non-metric skull measurements to distinguish between porpoises from the Baltic Sea and the Dutch coast. However, he could not distinguish Dutch animals from other North Sea animals, or North Sea from Baltic Sea animals. Andersen (1993) screened 196 harbour porpoises, mainly from North Sea and Baltic waters, for 23 enzyme systems, representing 31 loci. Two loci were found to be polymorphic, and significant differences in allele frequency were noted between the North Sea and the Baltic porpoises, but not between Greenland and Baltic porpoises. The observed frequency of heterozygous animals was less than expected, suggesting that there is a mixture of several breeding populations in the North Sea. Tiedemann *et al.* (1996) was able to distinguish porpoise populations from the Baltic and German North Sea coasts by sequencing the control region of mtDNA.

There is a substantial by-catch of harbour porpoises in the English and Irish gill-net fishery which operates on the Celtic shelf (Berrow *et al.* 1994), and in the Danish bottom set gill-net fishery which operates in the central North Sea (Vinther 1995). Although these catches are a small fraction of the total estimated harbour porpoise abundance in the North and Celtic Seas (Hammond *et al.* 1995), they may not be sustainable at all, even if there is no structuring (IWC 1996). The results reported here indicate that female porpoises may well form local populations and, if so, would not necessarily rapidly repopulate an area where numbers became depleted. This provides additional support for recent recommendations made by a number of international organizations (ICES 1996; IWC 1996) that there is an urgent need for detailed investigations of the population effects of these by-catches.

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