Biological Conservation 144 (2011) 2479–2489

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Possible cryptic stock structure for minke whales in the North Atlantic: Implications for conservation and management

Pia Anderwald [a,1], Anna K. Daníelsdóttir [b], Tore Haug [c], Finn Larsen [d], Véronique Lesage [e], Robert J. Reid [f], Gísli A. Víkingsson [g], A. Rus Hoelzel [a,*]

[a] School of Biological and Biomedical Sciences, Durham University, South Road, Durham DH1 3LE, United Kingdom
[b] Matis, Skúlagata 4, 101 Reykjavík, Iceland
[c] Institute of Marine Research, Tromsø Branch, P.O. Box 6404, N-9294 Tromsø, Norway
[d] Danish Institute for Fisheries Research, Charlottenlund Castle, 2920 Charlottenlund, Denmark
[e] Fisheries and Oceans Canada, Maurice Lamontagne Institute, P.O. Box 1000, 850 Route de la Mer, Mont-Joli, QC, Canada G5H 3Z4
[f] SAC Veterinary Services, Drummondhill, Stratherrick Rd., Inverness IV2 4JZ, Scotland, United Kingdom
[g] Marine Research Institute, P.O. Box 1390, Skúlagata 4, 121 Reykjavík, Iceland

ARTICLE INFO

Article history:
Received 7 September 2010
Received in revised form 29 June 2011
Accepted 5 July 2011
Available online 27 July 2011

Keywords:
Cetacean
Stock structure
North Atlantic
Dispersal

ABSTRACT

The minke whale is the last of the great whale species to be hunted in significant numbers. Effective management must include an understanding of how genetic diversity is divided and distributed among putative local populations, and as for many migratory species, this is complicated for the minke whale by large-scale seasonal movement among geographic regions. The problem is that the geographic identity of breeding populations is not known, and instead these whales are predictably found and hunted where different breeding stocks may mix on seasonal feeding grounds. Here we use microsatellite DNA and mtDNA markers to investigate minke whale population structure across the species' range in the North Atlantic. We found no evidence of geographic structure comparing putative populations in recognized management areas, though some limited structure had been indicated in earlier studies. However, using individual genotypes and likelihood assignment methods, we identified two putative cryptic stocks distributed across the North Atlantic in similar proportions in different regions. Some differences in the proportional representation of these populations may explain some of the apparent differentiation between regions detected previously. The implication would be that minke whales range extensively across the North Atlantic seasonally, but segregate to some extent on at least two breeding grounds. This means that established stock boundaries in the North Atlantic, currently used for management, should be re-considered to ensure the effective conservation of genetic diversity.

1. Introduction

The evolution of population structure in migratory species is dependent on both temporal and spatial factors. Species that migrate long distances between breeding and feeding grounds may show different patterns of site fidelity, and this impacts on the consequent pattern of genetic diversity. For example, Hoelzel (1998) described various scenarios for migrating baleen whales whereby mixing may occur on either feeding or breeding grounds, and described examples for each. Set migratory routes, e.g. along either side of an ocean basin, can isolate populations and promote genetic differentiation, while multiple breeding populations sharing a single feeding site may show less fidelity to breeding sites, and less differentiation. Understanding the dynamics of these systems is important, since they provide insight into the processes by which biodiversity evolves at the population level in migratory species. Moreover, impacts of conservation concern may affect different parts of the temporal distributional range of species to different extents. There are many avian examples of relevant systems, sometimes affecting populations separated by many thousands of miles (e.g. Northern bald ibis *Geronticus eremita*, white stork *Ciconia ciconia* and black stork *Ciconia nigra*; Berthold et al., 2003), and forming the basis for a number of national conservation regulations and international treaties. Similar examples exist for various other vertebrates and some invertebrate taxa (see review in Robinson et al. (2009)). In this study we focus on the implications for populations of a cetacean species, the minke whale.

* Corresponding author. Address: Molecular Ecology Group, Department of Biological and Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, United Kingdom. Tel.: +44 191334 1325; fax: +44 191334 1201.
E-mail addresses: panderwald@hotmail.com (P. Anderwald), anna@rannis.is (A.K. Daníelsdóttir), tore.haug@imr.no (T. Haug), fl@difres.dk (F. Larsen), LesageV@dfo-mpo.gc.ca (V. Lesage), bob.reid@sac.co.uk (R.J. Reid), gisli@hafro.is (G.A. Víkingsson), a.r.hoelzel@durham.ac.uk (A.R. Hoelzel).
[1] Present address: Coastal and Marine Research Centre, University College Cork, Irish Naval Base, Haulbowline, Cobh, Co. Cork, Ireland.

0006-3207/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.biocon.2011.07.002

For several mysticete cetacean species (see Stevick et al., 2002), the extent and pattern of migration is well known. Perhaps the most familiar is the humpback whale (*Megaptera novaeangliae*), for which two different migration patterns have been identified: separate migration routes within an ocean basin (in the North Pacific), and the distribution of whales from a single breeding population among several feeding grounds (in the North Atlantic; Baker et al., 1986; Clapham et al., 1993). However, other balaenopterid rorqual whale species (all within the genus *Balaenoptera*) are known to be migratory to at least some extent, but the details are not well established (see Stevick et al., 2002). For the minke whale there are data to suggest seasonal movements, e.g. in the northern North Atlantic (Skaug et al., 2004), but also populations that appear to include long-term resident individuals (Dorsey et al., 1990). In the western North Pacific there are two minke whale breeding populations on either side of Japan, that are known to mix on feeding grounds in the Okhotsk Sea (Goto and Pastene, 1997; Pastene et al., 1992; Wada, 1991). However, no breeding populations have yet been identified in the North Atlantic.

For this species, which is still commercially hunted, there is a clear need for the designation of effective units of management for conservation. Based on catch and sighting distributions, biological parameters such as sex and length distribution, mark-recapture data and the general desire to remain in accord with the International Council for the Exploration of the Sea (ICES) boundaries, the International Whaling Commission (IWC) set up four management areas for minke whales in the North Atlantic in 1977 (Donovan, 1991; Rørvik and Jonsgård, 1981): East Canada, West Greenland, Central (East Greenland, Iceland and Jan Mayen) and Northeast (North Sea, Vesterålen/Lofoten, Barents Sea and Svalbard). These were later further subdivided into the "IWC Small Areas" (Anon., 1992; Fig. 1), based on similar principles. Since these boundaries are based on feeding rather than breeding grounds, there is a danger that they do not reflect true population boundaries, but instead temporary mixed assemblages (see Hoelzel, 1991). This concern is reflected in the revised management procedure at the IWC, which has been working towards management implementation through the development of simulation trials. Two major considerations for these trials are the identification of stock structure, and the implications of mixing stocks and temporal patterns for management in locations where whaling takes place (see Butterworth and Punt, 1999; Punt and Donovan, 2007).

Studies of population genetic structure of North Atlantic minke whales have included morphological, biochemical and genetic comparisons (see review in Andersen et al., 2003). Differentiation between management areas has been reported based on allozymes (Daníelsdóttir et al., 1992, 1995), stable isotopes and heavy metals (Born et al., 2003). However, the most extensive study to date was by Andersen et al. (2003), based on 16 microsatellite DNA loci and mtDNA sequence data. While the authors found significant mtDNA differentiation only between West and East Greenland females, microsatellite DNA analyses revealed four sub-populations: West Greenland, Central (East Greenland and Jan Mayen), Northeast (Svalbard, Barents Sea and Vesterålen/Lofoten) and Norwegian North Sea. However, the magnitude of the F_{ST} values (based on the microsatellite DNA loci), though significant, were low (lower than 0.008, except for comparisons with the North Sea), and the sample size from the North Sea region was small ($N = 23$). A general consensus among studies so far has been the lack of sub-division between the IWC Small Areas within the Northeast Atlantic – Svalbard, Barents Sea and Vesterålen/Lofoten. Here we represent those areas with a sample from Svalbard.

In this study we investigate minke whale population structure in the North Atlantic, including regions not previously sampled, and test the hypothesis that migratory behaviour is generating mixed assemblages of breeding populations (as seen for minke whales in the North Pacific) in seasonal northern distributions. Evidence for mixing stocks would become an essential part of simulation trials in support of management for the ongoing fishery, and the proportion of mixing would be one of the key parameters to incorporate into the trials (e.g. Punt and Donovan, 2007). We find that evidence for structure across geographic areas is weak, but that there may be a signal for cryptic structure representing at least two breeding populations and mixing of these stocks across the North Atlantic. This would have further implications about the scale of seasonal movements in minke whales, and the potential for migratory species in general to seasonally re-distribute genetic diversity.

2. Methods

2.1. Sample collection

Samples from the UK were either provided by the Scottish and English strandings co-ordinators (38 samples) or by biopsy-sampling live minke whales in the Hebrides, Scotland, under permit from Scottish Natural Heritage (5 samples). Irish ($n = 4$), Spanish ($n = 3$) and Canadian ($n = 15$) samples were taken exclusively from stranded animals, and Norwegian (North Sea, $n = 36$; Svalbard, $n = 48$; Jan Mayen, $n = 17$), Icelandic ($n = 60$) and West Greenland ($n = 36$) samples from whaling operations (Table 1). No whales were killed in order to provide material for this study. Since minke whales are distributed close to the coast during summer in those areas where samples from fresh strandings were used (accounting for 23% of total samples), it can reasonably be assumed that the stranded animals died close to the locations where they were found and were therefore representative of those geographic locations. For most regions, several years of sampling (ranging from 1980 to 2005; Fig. 1) had to be included in order to get a large enough sample size for statistical analyses. A separate analysis based on year to year differences was therefore not possible. In addition to the North Atlantic samples, 30 individuals from the Sea of Japan were included in the analysis as an outgroup.

2.2. DNA extraction and PCR amplification

Nuclear DNA was extracted by standard protocols, and 10 microsatellite loci were amplified (EV1, EV37 (Valsecchi and Amos, 1996); Igf-1 (Barendse et al., 1994); GATA028, GATA098, GATA417, ACCC392 (Palsbøll et al., 1997); GT509 (Bérubé et al., 2000); KWM2a (Hoelzel et al., 1998); Texvet7 (Rooney et al., 1999). Two thermal profiles were used: Protocol 1) 5 min at 95 °C, 35 cycles of [45 s at 94 °C, 1.5 min at annealing temperature, 1.5 min at 72 °C], 1.5 min at 50 °C, 8 min at 72 °C; Protocol 2) 3 min at 95 °C, 35 cycles of [1 min at 94 °C, 30 s at annealing temperature, 10 s at 72 °C], 15 min at 72 °C. Protocol 2 was used for Igf-1 only. Annealing temperatures were optimized for minke whales and ranged from 45 °C to 62 °C. Amplifications were carried out in 20 μl volumes with the following concentrations: ~10 to 50 ng template DNA, 2 mM of each dNTP, 500 ng/μl primers, 50 ng/μl fluorescent labelled primer (Hex, Fam or Ned), 0.4 units Biotaq DNA polymerase and 0.5–2.5 mM $MgCl_2$. Amplified DNA was subsequently analysed for length variation on an automated ABI PRISM 377 DNA sequencer.

The gender of individuals with unknown sex was determined using specific primers for the ZFY/ZFX gene (Bérubé and Palsbøll, 1996). A 500 bp fragment of the mitochondrial DNA (mtDNA) control region was amplified in the UK, Irish, Canadian and Svalbard samples using light-strand MT4 (5′-CCTCCCTAAGACTCAAGGAAG-3′; Árnason et al., 1993) and modified heavy-strand Dlp5

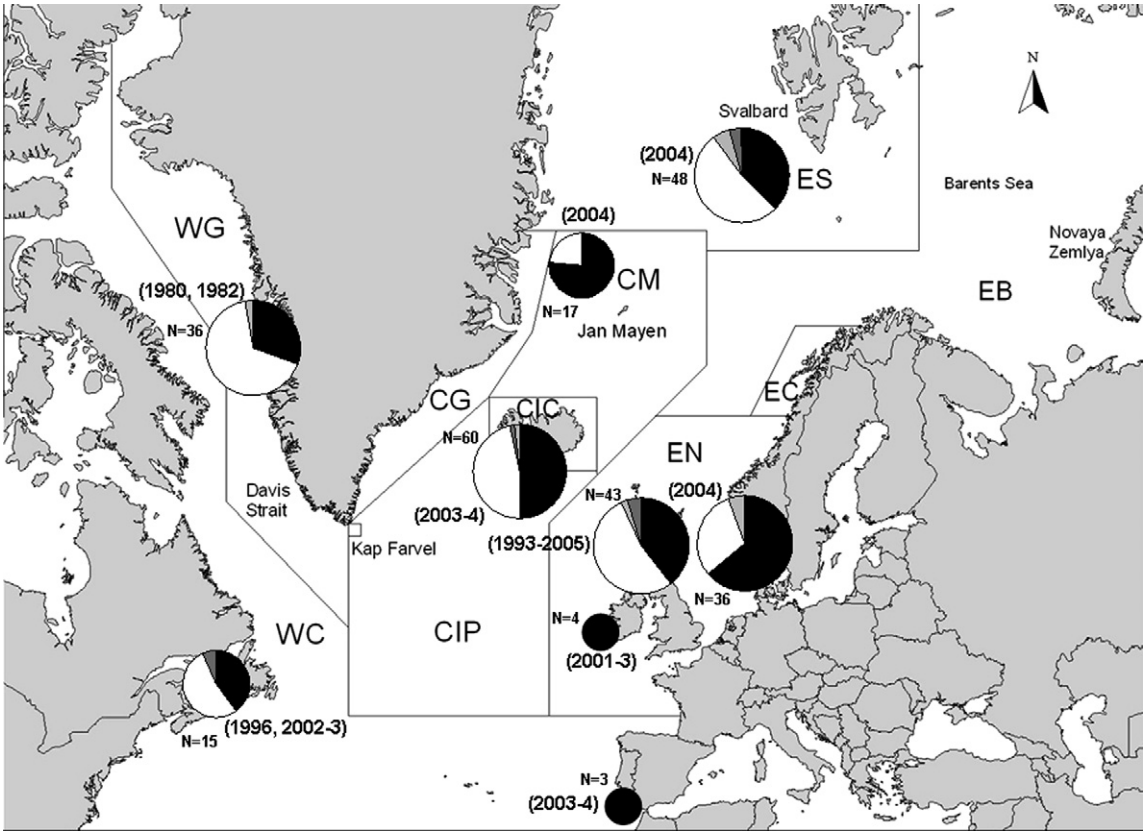

Fig. 1. Sample sites in the North Atlantic within IWC management areas (West Greenland (WG), Central Eastern Greenland (CG), Central Jan Mayen (CM), East Svalbard (ES), East Barents Sea (EB), East Coastal Norway (EC), East North Sea (EN), Central Iceland Coastal (CIC), Central Iceland Pelagic (CIP) and West Canada (WC)). We include one sample site within a given management area, with the exception of EN, for which we include three sample sites: UK, Norway and Ireland. The geographic distribution of PBS1 and PBS2 in the North Atlantic according to GeneClass2 assignments of microsatellite genotypes is shown as pie charts: Black = PBS1, white = PBS2, light grey = putative PBS1 individuals assigned to PBS2, dark grey = putative PBS2 individuals assigned to PBS1. Sizes of pie charts indicate relative sample sizes for different areas (see Section 2.1. for details). Sampling dates are given in parentheses.

Table 1
Number (*N*), sex (f:m) and tissue origin of samples from the different regions.

	N (f:m)	Tissue	Source	Years
UK (UK)	43 (29:14)	Skin	38× strandings, 5× biopsing	1993–2005
West Greenland (GR)	36 (28:8)	Muscle	Whaling	1980 and 1982
Iceland (IC)	60 (27:33)	Skin	Whaling	2003 and 2004
Gulf of St. Lawrence (CN)	15 (13:2)	Skin, 2× muscle	Strandings	1996, 2002 and 2003
Norwegian North Sea (NS)	36 (22:14)	Muscle	Whaling	2004
Svalbard (SV)	48 (47:1)	Muscle	Whaling	2004
Jan Mayen (JM)	17 (17:0)	Muscle	Whaling	2004
Ireland (IR)	4 (3:1)	Skin	Strandings	2001–2003
Spain (SP)	3 (1:1, 1 not sexed)	Skin	Strandings	2003 and 2004

primers (5′-GGATGTCTTATTTAAGRGGAA-3′; Baker et al., 1996). Other geographic regions were omitted due to the extensive database of sequences already available for those regions from earlier studies. The thermal profile for mtDNA amplification was the same as protocol No. 1 used for microsatellite amplification (see above). The annealing temperature was set to 55 °C, and $MgCl_2$-concentration was 1.5 mM. Buffer, dNTP, primer, polymerase and DNA concentrations were the same as used for the microsatellites, but reaction volumes were increased to 50 μl. PCR products were purified with QIAgen PCR purification columns according to the protocol provided by the manufacturer (Qiagen GmbH, Germany) and sequenced using the ABI dye-terminator method.

2.3. Analysis

2.3.1. Microsatellites

All loci in each population (where $N \geqslant 10$) were tested for possible deviations from the Hardy–Weinberg equilibrium (heterozygote deficiency or excess), null alleles and allelic dropout using the program Microchecker 2.2.3 (Van Oosterhout et al., 2004). Bootstrap values for Monte Carlo simulations were set at 1000, and confidence intervals were Bonferroni-corrected. Fisher's exact tests (Rousset and Raymond, 1995) with Markov chain settings of 10,000 dememorization steps, 100 batches and 5000 iterations per batch were performed for all combinations of loci using the program Genepop 3.4 (Raymond and Rousset, 1995) to test for linkage disequilibrium amongst loci. Stringent quality control procedures were implemented (see Morin et al., 2010), and no measurable error detected from 10% blind replicates.

Genetic differentiation among geographic putative population samples (where $N \geqslant 10$) was investigated using F_{ST} (according to Weir and Cockerham, 1984; implemented in the program MSA (Dieringer and Schlötterer, 2002)) and Rho_{ST} (implemented in the program Rst calc (Goodman, 1997)). The number of both permutations and bootstrap values for the test statistics was set at 1000. The population Central Jan Mayen (CM) showed only one allele for the locus Igf-1, and was therefore excluded for the comparison of Rho_{ST} values.

To test for cryptic diversity, the most probable number of putative populations (K) which best explained the pattern of genetic variability was estimated using the program STRUCTURE 2.3.3 (Pritchard et al., 2000; Hubisz et al., 2009). The admixture model with correlated allele frequencies was chosen with a burn-in length of 50,000 and 500,000 Monte–Carlo Markov–Chain steps, and the average admixture coefficient for individuals (α) was inferred with a uniform prior for α (initial value = 1, max = 10, SD = 0.025). For each putative K, four independent runs were performed. All populations were included in the first model ($1 \leq K \leq 14$), then the outgroup Japan was excluded (also $1 \leq K \leq 14$). Further assessment was run including only the three largest populations (the UK, Iceland and Svalbard; $1 \leq K \leq 8$), and for each of these three locations separately ($1 \leq K \leq 5$). To facilitate the further interpretation of the STRUCTURE output, a measure based on the second order rate of change of the likelihood function with respect to K (ΔK) was plotted (after Evanno et al. (2005)).

Based on the highest likelihood run of the full sample set (including Japan) in Structure, all North Atlantic samples were re-assigned to one of two populations according to their highest coefficient of admixture (likelihood assignment). Polymorphism in these two new putative populations, Hardy–Weinberg and linkage equilibrium, as well as F_{ST} and Rho_{ST} were calculated using the same methods as described for the geographical comparisons. In addition, Fisher's exact test for population differentiation was applied, using the program GENEPOP 3.4 (Raymond and Rousset, 1995). Test parameters were set at 10,000 dememorisation steps, 1000 batches and 10,000 iterations per batch. An individual-based assignment test, implemented in the program GeneClass2 (Piry et al., 2004), was performed to provide a second assessment using a Bayesian assignment approach (Rannala and Mountain, 1997). The probability of belonging to each reference population was calculated according to Paetkau et al. (2004), with a minimum of 10,000 simulated individuals and a type 1 error probability set to the default value of 0.01. Finally, a factorial correspondence analysis (FCA) was applied using the software GENETIX (Belkhir et al., 2002) to further assess the distribution of samples by putative population based on individual genotypes.

The effective sizes of the two putative populations, directional estimates of the rate of gene flow between them, and the splitting time were estimated using an isolation with migration model analysis (IMa; Hey and Nielsen, 2007). After several trial runs, parameters were adjusted to a burn-in length of 2,000,000 steps, metropolis coupling of 80 chains, geometric increments of 0.99 and 0.6, generation time of 22 years (Taylor et al., 2007), and a mutation rate of an estimated 10^{-4} (per locus per year). A stepwise mutation model was assumed. Trend lines over the course of the run and update acceptance rates were used to check for sufficient mixing of the Markov chain. The final run included 6,380,375 steps after burn-in and saved 319,019 trees per locus.

2.3.2. Mitochondrial DNA

All sequences were cut to the same length as Bakke et al.'s (1996) published control region sequences (345 bp in the hypervariable 'HVR1' region) and aligned using the program ClustalX1.81 (Thompson et al., 1997). Haplotype names matched earlier studies. Gene and nucleotide diversity (Nei, 1987), Tajima's D (Tajima, 1989), Fu's F_S (Fu, 1997), and conventional F_{ST} from haplotype frequencies (Weir and Cockerham, 1984), ϕ_{ST} (Excoffier et al., 1992) and mismatch distribution were calculated in ARLEQUIN2.0 (Schneider et al., 2000). Estimates of genetic distance for the calculation of nucleotide diversity and ϕ_{ST} used the Tamura–Nei model (Tamura and Nei, 1993) with a gamma correction of $\alpha = 0.293$ (calculated from the data using the software jModelTest 0.1.1; Guindon and Gascuel, 2003; Posada, 2008). The number of permutations for all test statistics was set to 10,000. Due to the small sample size for Ireland ($n = 4$), Irish and British samples were pooled for the geographic comparison. This combined population, together with the Canadian and Svalbard samples, was then compared with data from published studies (Andersen et al., 2003; Bakke et al., 1996) for the remaining North Atlantic regions. Diversity indices, F_{ST} and ϕ_{ST} were calculated for the populations inferred from STRUCTURE in the same way as for the geographic comparisons.

A median-joining network was constructed from all North Atlantic haplotypes (i.e. UK/Ireland, Canada and Svalbard, plus published sequences from Andersen et al., 2003 and Bakke et al., 1996 for all other regions) using the NETWORK software (www.fluxus-engineering.com; Bandelt et al., 1999). The transition to transversion weight was set at 6:1 (as calculated from the data), epsilon set at 10, and deletions were weighted the same as transversions. The MP option (Polzin and Daneschmand, 2003) was enabled to delete redundant links and median vectors. In addition, a Bayesian phylogeny was constructed using the program MRBAYES (Huelsenbeck and Ronquist, 2001). The General Time Reversible method (GTR) was chosen as the evolutionary model, with gamma-shaped mutation rate variation including a proportion of invariable sites. The Monte–Carlo Markov–Chain length was set to 10,000,000 repeats, with a sampling frequency of 100. Parameter values and trees were then summarized using 25% (i.e. 25,000) of the samples, and an unrooted consensus tree drawn using the program TREEVIEW.

3. Results

3.1. Microsatellites

In all sampled regions (see Fig. 1) except Iceland, the sex distribution was biased towards females (Table 1). Significant homozygote excess was detected for two loci (Table 2). Texvet7 had to be excluded from further analysis due to possible null alleles in three regions (Table 2). Although two putative population samples showed homozygote excess for Kwm2a, the exclusion of this locus did not affect the results (data not shown). The locus pairs EV1–EV37 and GATA417-ACCC392 showed significant linkage disequilibrium, but only in the outgroup (Japan). The omission of one of each pair did not change the results, and therefore these loci were retained.

After Bonferroni correction, significant genetic differentiation could only be detected between the North Atlantic and Japan (Table 3). Using the program STRUCTURE 2.3.3, the highest likelihood was indicated for $K = 3$ populations when Japan was included, with Japan clearly separated from the North Atlantic, and two populations within the North Atlantic (Fig. 2, Supplementary Table S1). These two latter populations were independent of their regional origin. However, for all runs excluding the Japan samples, STRUCTURE indicated the presence of only one population (see Table S1 for details), and this remained the case when the 'locprior' option was applied (data not shown).

Although $K = 3$ showed a higher likelihood than $K = 2$ (Table S1) for the model including the outgroup Japan, $\mathrm{Ln}(P(X|K))$ reached a plateau after $K = 2$, before decreasing again, and the plot of ΔK (calculated according to Evanno et al. (2005)) showed a peak at $K = 2$. According to Pritchard and Wen (2003) and Evanno et al. (2005), the true value could therefore be $K = 2$. However, the analysis employing ΔK will detect the highest level when structure is hierarchical (Evanno et al., 2005), which in this case would be the distinction between the North Atlantic and North Pacific ($K = 2$). Further, while artefact signals for structure are possible, the performance of this program decreases with decreasing F_{ST} values (especially if $F_{ST} \leqslant 0.02$; Latch et al., 2006). Therefore, the possibility of two populations within the North Atlantic was investigated

Table 2
Diversity by sampling location and locus. UK = United Kingdom, WG = West Greenland, CIC = central Icelandic coastal, WC = West Canada, NO = Norwegian North Sea, ES = East Svalbard, CM = central Jan Mayen, JP = Sea of Japan (outgroup).

	UK (N = 43)	WG (N = 36)	CIC (N = 60)	WC (N = 15)	NO (N = 36)	ES (N = 48)	CM (N = 17)	JP (N = 30)
Texvet7								
n	5	6	6	5	5	6	5	10 (4)
n_e, r	4.0, 4.9	3.6, 5.3	3.3, 4.7	3.4, 5.0	2.3, 3.8	3.3, 4.7	3.2, 4.9	4.4, 8.0
H_o	0.721	0.556*	0.683	0.867	0.500	0.646	0.471*	0.414*
H_e	0.761	0.734	0.704	0.726	0.580	0.708	0.711	0.786
a_0		0.118					0.163	0.233
EV1								
n	8	8	12 (1) 6.0, 8.7	9 (1)	9	9 (1)	7	17 (8)
n_e, r	5.0, 6.6	5.0, 6.7	0.850	6.2, 9.0	5.6, 7.5	5.4, 7.0	4.0, 6.8	7.1, 12.3
H_o	0.86	0.917	0.841	1	0.861	0.771	0.706	0.900
H_e	0.809	0.811		0.867	0.835	0.823	0.774	0.873
Kwm2a								
n	7	7	6	7	7	7	5	8 (2)
n_e, r	3.6, 6.2	2.6, 6.1	3.0, 5.7	4.0, 7.0	2.9, 6.4	3.5, 6.3	2.7, 5.0	4.0, 6.4
H_o	0.814	0.722	0.600	0.667	0.583	0.729	0.412*	0.600*
H_e	0.727	0.619	0.673	0.775	0.661	0.722	0.647	0.76
GATA028								
n	10	10	9	6	11 (1)	11 (1)	8	9 (2)
n_e, r	5.4, 8.0	4.7, 8.3	4.8, 6.9	3.2, 6.0	5.7, 8.5	6.1, 8.6	4.6, 7.6	5.7, 8.1
H_o	0.837	0.694	0.800	0.733	0.861	0.896	0.765	0.833
H_e	0.823	0.799	0.800	0.713	0.835	0.845	0.804	0.838
GT509								
n	8	10	12 (1)	5	10	12 (1)	9	11
n_e, r	5.1, 6.7	5.3, 7.7	5.4, 8.0	4.4, 5.0	5.5, 7.8	4.9, 8.1	4.6, 8.6	6.1, 8.7
H_o	0.884	0.750	0.800	0.867	0.833	0.833	0.706	0.767
H_e	0.812	0.824	0.822	0.798	0.829	0.805	0.807	0.851
Igf-1								
n	2	2	3	2	3	3	1	5 (3)
n_e, r	1.1, 1.9	1.1, 1.9	1.1, 2.1	1.5, 2.0	1.1, 1.8	1.2, 2.2	1, 1.0	2.8, 4.3
H_o	0.116	0.139	0.133	0.400	0.056	0.167	0	0.567
H_e	0.111	0.131	0.126	0.331	0.055	0.156	0	0.658
GATA417								
n	10	9	10 (1)	8	9	10	9	5 (2)
n_e, r	6.6, 8.5	6.1, 7.5	7.1, 8.3	5.1, 8.0	6.4, 8.3	6.8, 8.4	6.4, 8.7	3.0, 4.7
H_o	0.930	0.943	0.900	0.867	0.833	0.938	0.882	0.500
H_e	0.858	0.848	0.867	0.832	0.856	0.863	0.868	0.682
EV37								
n	7	9 (2)	6	5	8	8 (1)	6	6 (3)
n_e, r	3.4, 5.9	2.9, 6.0	2.6, 5.1	2.3, 5.0	3.4, 6.3	3.3, 6.4	2.5, 5.8	3.2, 4.9
H_o	0.814	0.694	0.717	0.733	0.889	0.792	0.647	0.700
H_e	0.711	0.663	0.623	0.579	0.719	0.703	0.622	0.694
GATA098								
n	7	7	7	4	6	5	4	5
n_e, r	4.3, 6.0	4.1, 5.5	4.1, 5.4	3.1, 4.0	3.8, 5.3	3.7, 4.9	3.3, 4.0	3.2, 4.7
H_o	0.791	0.750	0.750	0.8	0.778	0.646	0.706	0.8
H_e	0.776	0.765	0.762	0.706	0.747	0.735	0.718	0.695
ACCC392								
n	6	5	7 (1)	6	7	6	4	7 (3)
n_e, r	2.7, 5.6	2.3, 4.6	3.3, 5.7	2.1, 6.0	2.9, 5.8	2.5, 5.1	2.5, 4.0	4.0, 6.5
H_o	0.628	0.556	0.683	0.600	0.667	0.604	0.529	0.900
H_e	0.639	0.576	0.705	0.543	0.660	0.605	0.619	0.765

n: number of alleles; (number of private alleles in brackets); n_e: effective number of alleles (Frankham et al., 2002); r: allelic richness (Petit et al., 1998; calculated using FSTAT 2.9.3 (Goudet, 2001)); H_o: observed heterozygosity; H_e: expected heterozygosity; a_0: estimated null allele frequencies.
* Significant heterozygote deficiency. Only regions with $n \geqslant 15$ are included.

Table 3
F_{ST} values for pairwise regional comparisons based on microsatellite DNA loci (F_{ST} values above, significance values below the diagonal). Abbreviations as for Table 2.

	UK	WG	CIC	WC	EN	ES	CM	JP
UK	–	0.00074	−0.00241	0.00911	0.00087	−0.00287	0.00862	0.19172
WG	0.37	–	0.00017	0.01116	0.0063	0.00206	0.01387	0.21049
CIC	0.86	0.43	–	0.00809	−0.00059	−0.00197	0.00498	0.19604
WC	0.06	0.04	0.07	–	0.01066	0.00266	0.01067	0.18945
EN	0.35	0.04	0.55	0.05	–	−0.00279	0.00108	0.19735
ES	0.87	0.21	0.81	0.28	0.84	–	0.00226	0.18681
CM	0.05	0.01	0.13	0.10	0.39	0.28	–	0.20565
JP	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	–

* p-Values which were still significant after Bonferroni correction ($p < 0.0028$).

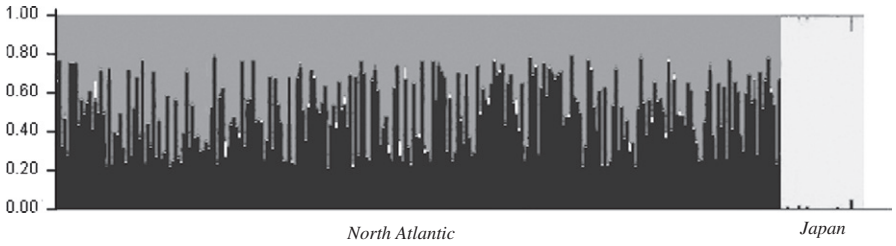

Fig. 2. Estimated proportion of the coefficient of admixture (likelihood assignment) for each individual's microsatellite DNA genotype that originated from population *K* for *K* = 3. Each individual is represented by a column.

further. Based on the likelihood assignment for each individual in the model (from the *K* = 3 outcome that included the outgroup Japan), all North Atlantic samples were thus re-assigned to one of the two populations suggested by STRUCTURE for this model, and the other tests of population differentiation repeated for these new groupings, named 'Putative Breeding Stock' (PBS1) and PBS2, respectively. This was done twice, first simply assigning a sample to a population based on greater than 50% likelihood, and second with a cutoff of 70% (discarding the samples that assigned at lower values).

Based on the fully inclusive dataset, PBS1 showed somewhat lower (significance not implied) average heterozygosities and allelic richness than PBS2 (PBS1: average H_o = 0.675, H_e = 0.668, r = 8.4. PBS2: average H_o = 0.722, H_e = 0.690, r = 8.56). Private alleles were observed between the two populations in all loci except Kwm2a (Table 4). No homozygote excess could be detected, and there were no significant deviations from linkage equilibrium.

By contrast to the regional comparisons, both F_{ST} and Rho_{ST} showed highly significant differentiation between PBS1 and PBS2 (for > 50% assignment: F_{ST} = 0.021, p = 0.0001; Rho_{ST} = 0.025, p < 0.00001; PBS1: n = 133, PBS2 n = 129; for ⩾70% assignment: F_{ST} = 0.045, p = 0.0001; Rho_{ST} = 0.055, p < 0.00001; PBS1: n = 75, PBS2 n = 72). Fisher's exact test also showed a clear separation between PBS1 and PBS2 (χ^2 = "infinity", df = 18, p < 0.00001 for either >50% or ⩾70% assignments). Using the full dataset, assignment of individual genotypes to their most likely source population using the program GeneClass2 (Piry et al., 2004) corroborated these results: 94.7% of individuals were assigned correctly back to PBS1 and PBS2 respectively, based on ranks only (quality index = 85.33%), and 87.4% of individuals were assigned correctly based on probabilities (quality index = 70.15%). A log likelihood plot (Fig. 3a) illustrates the separation between the two population clusters. The factorial correspondence analysis (also based on the full dataset; Fig. 3b) confirmed good separation between these putative populations, though the first two dimensions explained only 5.4% of the total variation. Both putative populations were represented in all areas within the North Atlantic for which sufficient sample sizes were available (Fig. 1). Relative proportions were approximately equal around the UK, Iceland and Svalbard, while PBS1 dominated in the Norwegian North Sea (69%), and PBS2 in West Greenland (67%; comparing these two samples, χ^2 = 9.4, p = 0.0022). Andersen et al. (2003) found their strongest F_{ST} between West Greenland and the North Sea.

Effective population size estimates based on microsatellite loci assessed using IMa (using the full dataset) suggested a smaller size for PBS1 (21; HPD90: 11–56) than for PBS2 (544; HPD90: 204–938). These estimates were stable over multiple runs, and the posterior distributions reasonably tight and non-overlapping. The magnitude of these estimates depends on the estimated mutation rate, which varies over several orders of magnitude for microsatellite loci (see review in Brohede (2003)). The actual rates for these loci in the minke whale are not known, but the average rate assumed (10^{-4}) allows for easy calibration (e.g. if average u = 10^{-5}, estimated Ne for PBS1 would be 210). The HPD estimates for directional migration (migrants per generation) were low in both directions (into PBS1: 0.006; HPD90: 0.0004–0.721; into PBS2: 0.06; HPD90: 0.011–21.23), but the posterior distributions were very broad. The splitting time estimate (20,475; HPD90:

Table 4
Diversity measures by locus for putative populations revealed by Structure.

	PBS1 (N = 133)	PBS2 (N = 129)
EV1		
n	13 (5)	10 (2)
n_e, r	5.4, 12.8	5.7, 10
H_o	0.842	0.845
H_e	0.819	0.828
Kwm2a		
n	7	7
n_e, r	3, 7	3.1, 7
H_o	0.632	0.705
H_e	0.673	0.683
GATA028		
n	12 (3)	9
n_e, r	5.9, 11.9	4.4, 9
H_o	0.820	0.806
H_e	0.833	0.777
GT509		
n	9	14 (5)
n_e, r	4.3, 8.9	5.9, 14
H_o	0.797	0.829
H_e	0.770	0.833
Igf-1		
n	2	3 (1)
n_e, r	1, 2	1.2, 3
H_o	0.045	0.217
H_e	0.044	0.197
GATA417		
n	11 (2)	9
n_e, r	6.9, 11	6.2, 9
H_o	0.887	0.914
H_e	0.857	0.842
EV37		
n	9 (2)	10 (3)
n_e, r	2.6, 9	3.3, 10
H_o	0.692	0.822
H_e	0.616	0.702
GATA098		
n	6	7 (1)
n_e, r	3.8, 6	3.8, 7
H_o	0.729	0.752
H_e	0.741	0.741
ACCC392		
n	7	8 (1)
n_e, r	2.9, 7	2.5, 8
H_o	0.632	0.612
H_e	0.663	0.605

n: number of alleles; (number of private alleles in brackets); n_e: effective number of alleles; r: allelic richness; H_o: observed heterozygosity; H_e: expected heterozygosity.

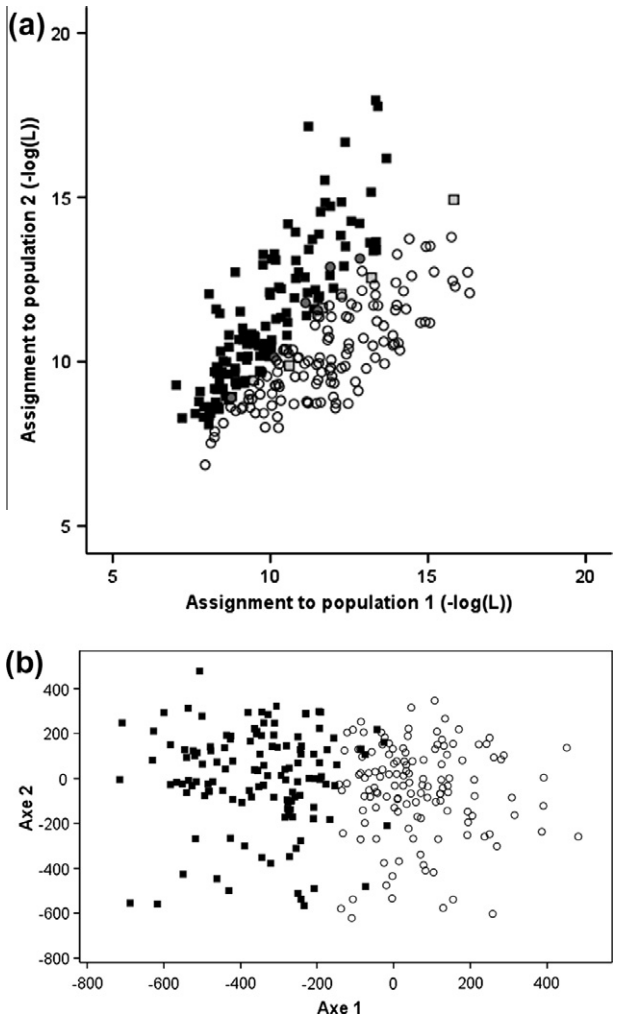

Fig. 3. (a) Log likelihood plot for Geneclass assignment results of PBS1 and PBS2. Correctly assigned individuals based on microsatellite DNA genotypes: filled squares = PBS1, open circles = PBS2. Incorrectly assigned individuals: light grey squares = PBS1 assigned to PBS2, dark grey circles = PBS2 assigned to PBS1. (b) Two-dimensional representation of the factorial correspondence analysis. Individuals are projected on the factor space defined by the similarity of their allelic states.

12,495–29,985) was mostly flat and unresolved across the confidence limit range. Neither migration rate nor splitting time estimates were sufficiently well resolved to provide strong inference.

3.2. Mitochondrial DNA

Given the availability of mtDNA data from earlier studies, we focused on samples from just three regions: the UK and Ireland ($N = 47$), Svalbard ($N = 40$), and Canada ($N = 15$). Among these samples we identified 10 new haplotypes, not observed in earlier studies (accession numbers: JN185219–28). Neither F_{ST} nor ϕ_{ST} detected any population differentiation among our sampled geographic populations ($-0.018 \leq F_{ST} \geq -0.012$, $p > 0.8$; $-0.008 \leq \phi_{ST} \geq 0.002$, $p > 0.3$). Differentiation between the two putative populations PBS1 and PBS2 for mtDNA was assessed for all samples combined in the first instance. In this case only the sample set restricted to 70% or higher assignment approached significance for the comparison ($F_{ST} = 0.017$, $p = 0.09$; PBS1: $n = 26$; PBS2: $n = 30$). We then assessed separately the sites with the largest sample sizes, UK & Ireland and Svalbard (based on assignments of >50%), in case differential male compared to female migration was impacting the pattern of structure (potentially relevant because Svalbard is approximately 2000 km further north). For the UK & Ireland, PBS1 ($n = 22$) and PBS2 ($n = 25$) were significantly differentiated with respect to both F_{ST} ($F_{ST} = 0.044$, $p = 0.017$) and the exact test ($p = 0.032$), although ϕ_{ST} was non-significant ($\phi_{ST} = 0.0035$, $p = 0.356$). The two putative populations were not significantly differentiated in the Svalbard sample (PBS1: $n = 18$; PBS2: $n = 22$). Mitochondrial DNA diversity was higher (significance not implied) in PBS2 ($h = 0.943 \pm 0.016$; $\pi = 0.0089 \pm 0.0052$) than PBS1 ($h = 0.885 \pm 0.041$; $\pi = 0.0075 \pm 0.0045$) for all samples combined.

For both putative populations (all data combined), Tajima's D was non-significant (PBS1: $D = -0.924$, $p = 0.189$; PBS2: $D = -0.568$, $p = 0.304$), but Fu's F_S was large, negative and highly significant (PBS1: $F_S = -11.65$, $p < 0.00001$; PBS2: $F_S = -14.27$, $p < 0.00001$), supporting the possibility of an expansion. A mismatch distribution based on all North Atlantic samples combined (including those published previously; Fig. 4) did not deviate significantly from a model for expansion (SSD = 0.0077; $p = 0.56$), but the distribution was not clearly unimodal, and the confidence limits on tau were broad (tau = 5.86; 95% CI = 2.27–10.12). This would suggest an expansion time of 17,035 YBP (6599–29,419; Rogers and Harpending, 1992; based on a mutation rate of 5×10^{-7} per site per year, after Ho et al. (2007)). The structures of the Bayesian consensus tree and phylogenetic network including all North Atlantic haplotypes (including those published previously; Fig. 5) further support an historical expansion.

4. Discussion

In this study we investigated the possible impact of migratory behaviour on minke whale population structure in the North Atlantic. Our expectation was that the sample sites, all from northern locations in summer, may include mixed assemblages of whales from separate breeding populations (as seen for minke whales during the summer season in the Sea of Okhotsk, north of Japan; Goto and Pastene, 1997; Pastene et al., 1992; Wada, 1991). Consistent with this, neither microsatellite nor mtDNA analyses detected any clear differentiation between any of these regions, though some structure had been indicated in previous studies (one of which included more genetic markers and therefore higher resolution; Andersen et al., 2003). At the same time, the existence of two separate populations independent of geographic origin was suggested from an analysis of individual genotypes. However, this was only the case when all samples including a divergent population from the North Pacific were included in the analysis. When the North Atlantic was considered on its own, the most probable number of populations was estimated to be one, perhaps due to low power. We made no attempt to force $K = 2$ for the sample set restricted to the North Atlantic. Instead we used the dataset that included the outgroup (Japan) where the best

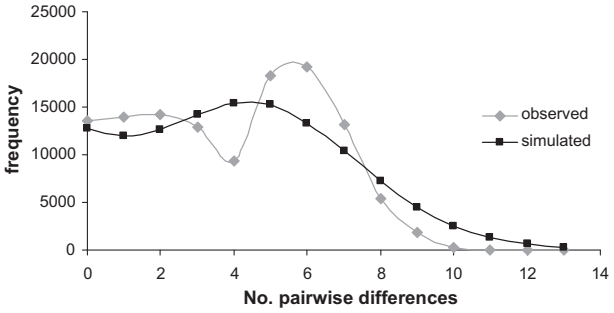

Fig. 4. Mismatch distribution of mtDNA haplotypes (based on all known North Atlantic haplotypes) showing the trace for the observed and simulated distributions.

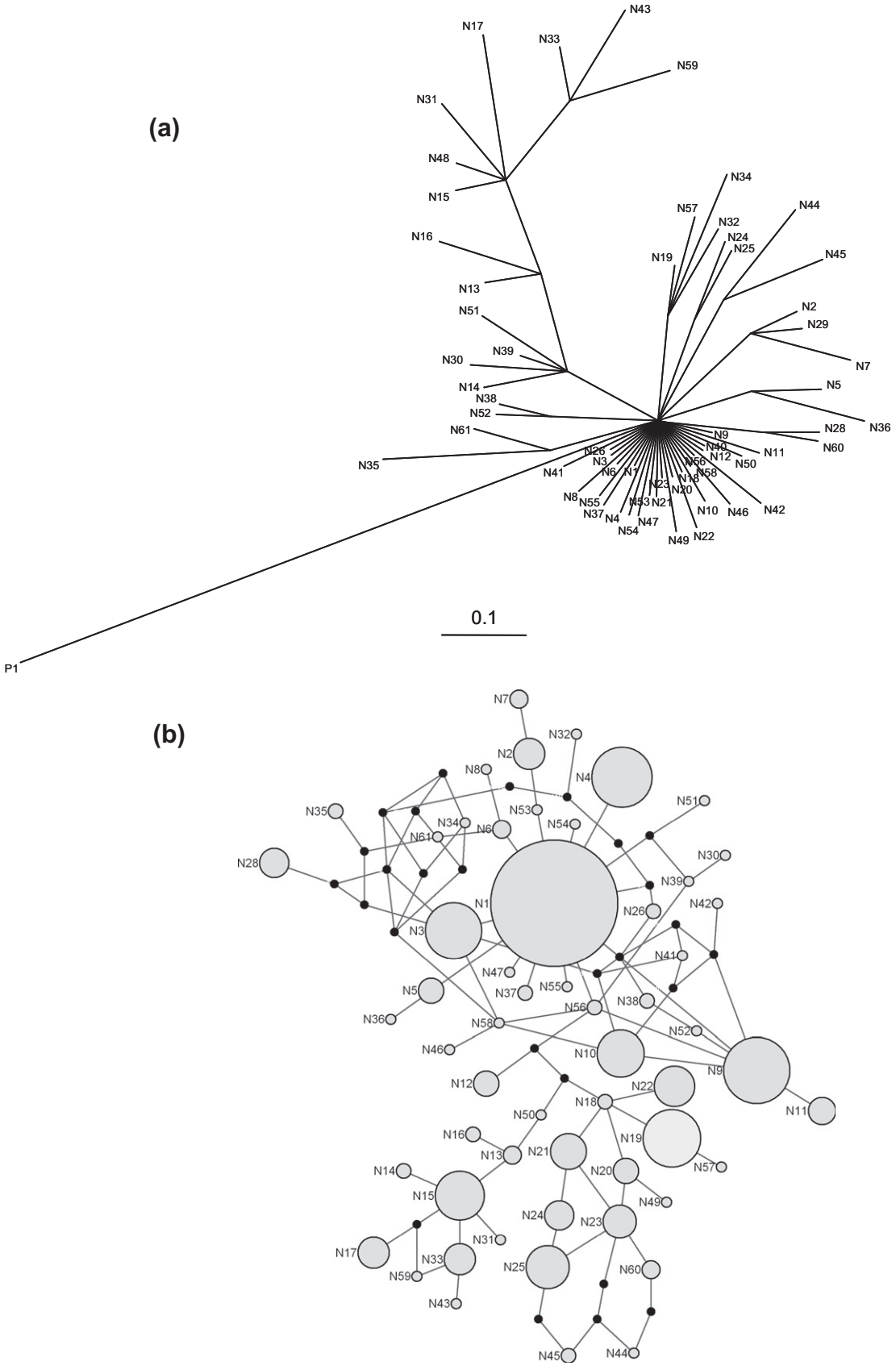

Fig. 5. (a) Bayesian consensus tree inferred from MRBAYES according to the General Time Reversible model. P1 = North Pacific haplotype. (b) Median-joining network using all North Atlantic mtDNA haplotypes. Grey nodes represent the haplotypes, and black nodes represent median vectors. Node sizes are proportional to haplotype frequencies.

supported posterior likelihood was $K = 3$, identifying the outgroup population and two putative North Atlantic populations, the latter without geographic correspondence. The initial separation and comparison of the latter two putative populations suggested that

they were differentiated with an F_{ST} value that is near the boundary of resolution for the STRUCTURE method (Latch et al., 2006). This was taken as a possible reason for why analysis of the North Atlantic on its own may not have revealed this structure, but not as proof that the putative populations were real. For this assessment we undertook further analyses.

The two putative cryptic populations (PBS1 and PBS2) were represented in approximately equal proportions in most areas with large enough sample sizes to assess this, except for the Norwegian North Sea (having a higher proportion of individuals from PBS1) and West Greenland (having a higher proportion of individuals from PBS2). The proportional representation of PBS1 and PBS2 in the two areas was significantly different, and this represented the comparison with the highest F_{ST} in an earlier study (Andersen et al., 2003). It is, therefore, possible that some earlier indications of structure instead reflected differential representations of these two cryptic populations. Unfortunately, samples from West Greenland in our study were not from the same time frame (collected 20 years earlier than those from the North Sea, see Fig. 1), and therefore it is not known if similar proportional differences would be seen comparing contemporary samples. However, Andersen et al. (2003) compared samples from West Greenland collected in 1982, 1996, 1997 and 1998 using both F_{ST} and Chi-square tests. The only comparison showing a significant difference (significant for both tests) was 1997 vs 1998, and the F_{ST} value was low (0.009). This could suggest that the proportional representation of PBS1 and PBS2 in West Greenland was fairly constant over time.

Although it remains possible that the differentiation identified here as putative populations PBS1 and PBS2 is an artefact due to noise from the program STRUCTURE, there are a number of factors arguing against this, including the relationship between the proportional representation of PBS1 and PBS2 and F_{ST} described in the previous paragraph. An artefact would imply the clustering of genetically similar samples that are not actually related by common descent. Both illustrations in Fig. 3, but especially the FCA plot, suggest distinct clusters, rather than two halves of a single cluster as might be expected if there was no real isolation. Furthermore, there should be no genetic hitch-hiking between the nuclear and mitochondrial genomes. A faint signal could be possible in some species through cultural hitch-hiking (with offspring following the migration of maternal kin), but this seems unlikely for minke whales, where offspring are typically weaned prior to arrival on the feeding grounds. Therefore, the fact that a strong differentiation between PBS1 and PBS2 was seen in mtDNA for the UK & Ireland sample set, supports the idea that these clusters do in fact represent clusters of related individuals, and likely breeding populations. The lack of detectable differentiation at mtDNA in the Svalbard sample could be due to noise (not all individuals assigned based on >50% likelihood will have been assigned correctly), or possibly some real difference in the migratory behaviour of males and females. The association based on mtDNA including these samples got stronger when the microsatellite DNA-based assignment likelihoods were restricted to 70% support or higher, consistent with the inclusion of misassigned individuals at the 50% cutoff. This also supports the interpretation of a real association with kinship, since an artificial association of similar microsatellite genotypes should not be reflected in the strength of the signal for differentiation at mtDNA. Further support is provided by the program IMa which gave fairly strong posterior support for different Ne (and by implication, different demographic histories) for the two putative populations. This was supported by lower genetic diversity at all markers for PBS1. There was also no indication of a Wahlund effect for either putative population (while other subdivisions based instead on geography did show some heterozygote deficiencies within putative populations).

As discussed in the introduction and above, minke whales breeding on either side of Japan mix on feeding grounds in the Sea of Okhotsk (Goto and Pastene, 1997; Pastene et al., 1992; Wada, 1991). However, the observed structure in the North Atlantic would suggest a substantial difference in scale. The Sea of Okhotsk is a contained basin measuring approximately 800 km by 800 km. Putative breeding populations are found approximately 1500 km to the southwest, near Japan and further offshore to the east. In the North Atlantic, samples from feeding grounds separated by up to 4000 km shared representatives from each putative breeding population. Although there are no good data on where such populations may breed, the distribution of these genotypes on feeding grounds across the North Atlantic implies either very broad-scale seasonal movements, or perhaps some further complexity to the pattern of population structure (possibly suggested in some of the earlier work; e.g. Andersen et al., 2003).

Some earlier studies had suggested two mtDNA lineages in the North Atlantic (Bakke et al., 1996; Palsbøll, 1990). However, more inclusive phylogenies with greater resolution (Andersen et al., 2003) failed to replicate that structure. Here we find no evidence of separate mtDNA phylogenetic lineages, consistent with Andersen et al. (2003), and therefore no indication that PBS1 and PBS2 correspond to reciprocally monophyletic lineages in a mtDNA tree. The phylogenies (Fig. 5), the mismatch distribution (Fig. 4) and Fu's Fs values are all consistent with an historical expansion in the North Atlantic, possibly associated with the last glacial maximum, consistent with data for other cetacean species including fin whales (*Balaenoptera physalus*; Bérubé et al., 1998) harbour porpoise (*Phocoena phocoena*; Tolley et al., 2001), white-beaked dolphins (*Lagenorhynchus albirostris*; Banguera et al., 2010), as well as earlier data for the minke whale (Pastene et al., 2007).

Various factors including distribution, life history parameters, local conservation threats such as bycatch, pollution, direct human exploitation and competition with fisheries, as well as differences in national legislation, are all taken into account when establishing management areas (e.g. Donovan, 1991). However, biological (breeding) populations must be identified using genetic markers. Most genetic studies on North Atlantic minke whales to date have focused on finding population differentiation between the four proposed North Atlantic management stocks, concentrating particularly on the whaling areas. Our data, indicating instead mixed assemblages of two cryptic breeding populations distributed across the North Atlantic, will have important implications for effective management (e.g. see Donovan, 1991; Hoelzel, 1991, 1998; Butterworth and Punt, 1999; Punt and Donovan, 2007). This is especially true if the level of diversity differs between populations, as is clearly the case in the western North Pacific (Goto and Pastene, 1997; Pastene et al., 1992; Wada, 1991), and is apparently the case for PBS1 compared to PBS2. The risk is that the relatively depauperate stock could be depleted when the proportional representation on whaling grounds is not known. Our estimates suggest a substantial difference in Ne for the two populations, with PBS1 being more than an order of magnitude smaller, and differences in diversity that are consistent with this difference in Ne. This also provides further support for the contention that the putative populations are real, since it would not be expected that clusters of genotypes that are similar by chance should show evidence for very different demographic histories (see above). The Ne estimates are low, but given that we do not know the true mutation rate, could be underestimates. However, even a conservative estimate of mutation at 10^{-5} would suggest relatively low Ne for PBS1 (Ne = 210), and a potential conservation concern. The effective to census population size ratio cannot be estimated here, since no census data could be available for PBS1.

The identification of breeding populations is important towards developing effective conservation and management strategies, and

in particular towards the implementation of management strategies through simulation trials (as promoted by the IWC for their revised management procedure; see Punt and Donovan, 2007). This is complicated in migratory species by large scale seasonal movements, and differential levels of philopatry to breeding populations (and therefore the need to identify mixing proportions in time and space; Punt and Donovan, 2007). The existence of PBS1 and PBS2 would suggest moderate levels of philopatry and unexpectedly large scale seasonal movements in the North Atlantic minke whale. In some cases, as with the humpback whale and grey whale, breeding and feeding grounds occupy locally defined geographic regions (Rice and Wolman, 1971; Clapham, 2009). This is also commonly the case for migratory avian species, and there can be a genetic disposition for specific migratory routes and destinations (as for the blackcap; Berthold et al., 1992). Migration corridors can also be predictable with baleen whales and other marine species, such as the leatherback turtle (*Dermochelys coriacea*), with important consequences for effective management (Shillinger et al., 2008). By contrast, our proposed two population model for the North Atlantic minke whale would imply that this species disperses across the breadth of the ocean from a given breeding population. It also means that their distribution on feeding grounds where they are hunted cannot be easily assigned to breeding population based on geography alone for the purpose of management and conservation. However, mixing estimates obtained so far (based on PBS1 and PBS2; Fig. 1) could be used in initial simulation trials to assess the implications for management. Although a clear signal was not possible using the clustering program STRUCTURE, inference is strong enough to support the need for further research, especially towards the clearer identification of breeding populations. Two appropriate objectives would be (i) the long-term tracking by telemetry of individual whales tagged during the feeding season, and (ii) the collection of genetic samples from southern latitudes during the boreal winter.

Acknowledgements

This project was financially supported by the Basler Stiftung für Biologische Forschung, the British Council, the National Geographic Society, the Swiss Study Foundation, the Sea Watch Foundation, and the Stiftung Dr. Joachim de Giacomi (Switzerland). Samples were kindly provided by R. de Stephanis, S. Murphy, E. Rogan, P. Jepson, R. Deaville, M. Perkins, L. Lindblom, B. Thorgilsson, Y. Morin, P.G.H. Evans and C. Swann. We thank C. de Luna-Lopez, D. Vassilakos, E. Banguera, M. de Bruyn, A. Natoli, C. Nicholson, C. Nichols, M. Pilot and T. White for their help at various stages of the project.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2011.07.002.

References

Andersen, L.W., Born, E.W., Dietz, R., Haug, T., Oien, N., Bendixen, C., 2003. Genetic population structure of minke whales *Balaenoptera acutorostrata* from Greenland, the North East Atlantic and the North Sea probably reflects different ecological regions. Marine Ecology Progress Series 247, 263–280.

Anon., 1992. Annex K, report of the working group on North Atlantic minke trials. Report of the International Whaling Commission 42, 246–251.

Árnason, U., Gullberg, A., Widegren, B., 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whale and two sperm whale species. Molecular Biology and Evolution 10, 960–970.

Baker, C.S., Herman, L.M., Perry, A., Lawton, W.S., Straley, J.M., Wolman, A.A., Kaufman, G.D., Winn, H.E., Hall, J.D., Reinke, J.M., Östman, J., 1986. Migratory movement and population structure of humpback whales (*Megaptera novaeangliae*) in the central and eastern North Pacific. Marine Ecology Progress Series 31, 105–119.

Baker, C.S., Cipriano, F., Palumbi, S.R., 1996. Molecular identification of whale and dolphin products from commercial markets in Korea and Japan. Molecular Ecology 5, 671–685.

Bakke, I., Johansen, S., Bakke, Ø., El-Gewely, M.R., 1996. Lack of population subdivision among the minke whales (*Balaenoptera acutorostrata*) from Icelandic and Norwegian waters based on mitochondrial DNA sequences. Marine Biology 125, 1–9.

Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16, 37–48.

Banguera, E, Bjørge, A., Reid, R.J., Jepson, P., Hoelzel, A.R., 2010. The influence of glacial epochs and habitat dependence on the diversity and phylogeography of a coastal dolphin species: *Lagenorhynchus albirostris*. Conservation Genetics.

Barendse, W., Armitage, S.M., Kossarek, L.M., Shalom, A., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li, L., Neibergs, H.L., Zhang, N., Grosse, W.M., Weiss, J., Creighton, P., McCarthy, F., Ron, M., Teale, A.J., Fries, R., McGraw, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E., Hetzel, D.J.S., 1994. A genetic linkage map of the bovine genome. Nature Genetics 6, 227–235.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2002. GENETIX 4.04, Logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Berthold, P., Helbig, A.J., Mohr, G., Querner, U., 1992. Rapid microevolution of migratory behaviour in a wild bird species. Nature 360, 668–670.

Berthold, P., Gwinner, E., Sonnenschein, E., 2003. Avian Migration. Springer, Dordrecht, Holland.

Bérubé, M., Palsbøll, P., 1996. Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Molecular Ecology 5, 283–287.

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo di Sciara, G., Sears, R., Sigurjónsson, J., Urban, R.J., Palsbøll, P.J., 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758): analysis of mitochondrial and nuclear loci. Molecular Ecology 7, 585–599.

Bérubé, M., Jørgensen, H., McEwing, R., Palsbøll, P.J., 2000. Polymorphic dinucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. Molecular Ecology 9, 2155–2234.

Born, E.W., Outridge, P., Riget, F.F., Hobson, K., Dietz, R., Haug, T., Øien, N., 2003. Stock structure of North Atlantic minke whales (*Balaenoptera acutorostrata*) inferred from regional variation of elemental and stable isotopic signatures in tissues. Journal of Marine Systems 43, 1–17.

Brohede, J., 2003. Rates and Patterns of Mutation in Microsatellite DNA. PhD thesis, Uppsala University, Uppsala, Sweden.

Butterworth, D.S., Punt, A.E., 1999. Experiences in the evaluation and implementation of management procedures. ICES Journal of Marine Science 56, 985–998.

**Clapham, P.J. 2009. Humpback whale. Pp. 582-585. In: *Encyclopedia of marine mammals*.2nd edition. Editors: W.F. Perrin, B. Würsig and J.G.M. Thewissen. Academic Press, San Diego. 1316 pp.

Clapham, P.J., Baraff, L.S., Carlson, C.A., Christian, M.A., Mattila, D.K., Mayo, C.A., Murphy, M.A., Pittman, S., 1993. Seasonal occurrence and annual return of humpback whales in the southern Gulf of Maine. Canadian Journal of Zoology 71, 440–443.

Daníelsdóttir, A.K., Duke, E.J., Árnason, A., 1992. Genetic variation at enzyme loci in North Atlantic minke whales, *Balaenoptera acutorostrata*. Biochemical Genetics 30 (3/4), 189–202.

Daníelsdóttir, A.K., Halldórsson, S.D., Guðlaugsdóttir, S., Árnason, A., 1995. Genetic variation in northeastern Atlantic minke whales (*Balaenoptera acutorostrata*). In: Blix, A.S., Walløe, L., Ulltang, Ø. (Eds.), Whales, seals, fish and man. Elsevier Science, BV, Amsterdam, pp. 105–118.

Dieringer, D., Schlötterer, C., 2002. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3 (1), 167–169.

Donovan, G.P., 1991. A review of IWC stock boundaries. In: Hoelzel, A.R., Donovan, G.P. (Eds.), Genetic Ecology of Whales and Dolphins. Report of the International Whaling Commission, Special Issue 13, Cambridge, UK, pp. 39–68.

Dorsey, E.M., Stern, S.J., Hoelzel, A.R., Jacobsen, J., 1990. Recognition of individual minke whales from the west coast of North America. IWC Special Issue 12, 357–368.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14, 2611–2620.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge University Press. Cambridge, UK, 617pp.

Fu, Y.-X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Goodman, S.J., 1997. RST CALC: a collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. Molecular Ecology 6, 881–885.

Goto, M., Pastene, L.A., 1997. Population Structure of the Western North Pacific Minke Whale based on an RFLP Analysis of the mtDNA Control Region. Report of the International Whaling Commission, Paper SC/48/NP5.

Goudet, J., 2001. FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices (Version 2.9.3). <http://www2.unil.ch/popgen/softwares/fstat.htm>.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. Systematic Biology 52, 696–704.
Hey, J., Nielsen, R., 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. Proceedings of the National Academy of Sciences of the USA 104, 2785–2790.
Ho, S.Y.W., Kolokotronis, S.-O., Allaby, R.G., 2007. Elevated substitution rates estimated from ancient DNA sequences. Biology Letters 3, 702–705.
Hoelzel, A.R., 1991. Whaling in the dark. Nature 352, 481.
Hoelzel, A.R., 1998. Genetic structure of cetacean populations in sympatry, parapatry and mixed assemblages; implications for conservation policy. Journal of Heredity 89, 451–458.
Hoelzel, A.R., Dahlheim, M., Stern, S.J., 1998. Low genetic variation among killer whales (*Orcinus orca*) in the Eastern North Pacific and genetic differentiation between foraging specialists. Journal of Heredity 89, 121–128.
Hubisz, M.J., Falush, D., Stephens, M., Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9, 1322–1332. doi:10.1111/j.1755-0998.2009.02591.x.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Latch, E.K., Dharmarajan, G., Glaubitz, J.C., Rhodes, O.E., 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conservation Genetics 7, 295–302.
Morin, P.A., Martien, K.K., Archer, F.I., Cipriano, F., Steel, D., Jackson, J., Taylor, B.L., 2010. Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. Journal of Heredity 101, 1–10.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY, USA, pp. 180, 257.
Paetkau, D., Slade, R., Burden, M., Estoup, A., 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology 13, 55–65.
Palsbøll, P.J., 1990. Preliminary Results of Restriction Fragment Length Analysis of Mitochondrial DNA in Minke Whales, Balaenoptera Acutorostrata, from the Davis Strait, the Northeast and Central Atlantic. Report of the International Whaling Commission, SC/42/NHMi35.
Palsbøll, P.J., Bérubé, M., Larsen, A.H., Jørgensen, H., 1997. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. Molecular Ecology 6, 893–895.
Pastene, L.A., Kobayashi, T., Fujise, Y., Numachi, K., 1992. Mitochondrial DNA Differentiation in Antarctic Minke Whales taken under Special Permit. Paper SC/44/SHB9 presented to the IWC Scientific Committee, June 1992 (unpublished), 13pp.
Pastene, L.A., Goto, M., Kanda, N., Zerbini, A.N., Kerem, D., Watanabe, K., Bessho, Y., Hasegawa, M., Nielsen, R., Larsen, F., Palsbøll, P.J., 2007. Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata*. Molecular Ecology 16, 1481–1495.
Petit, R.J., El Mousadik, A., Pons, O., 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12, 844–855.
Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., Estoup, A., 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95, 536–539.
Polzin, T., Daneschmand, S.V., 2003. On Steiner trees and minimum spanning trees in hypergraphs. Operations Research Letters 31, 12–20.
Posada, D., 2008. JModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25, 1253–1256.
Pritchard, J.K., Wen, W., 2003. Documentation for structure Software: Version 2. <http://pritch.bsd.uchicago.edu>.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Punt, A.E., Donovan, G.P., 2007. Developing management procedures that are robust to uncertainty: lessons from the International Whaling Commission. ICES Journal of Marine Science 64, 603–612.
Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the USA 94, 9197–9221.
Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86, 248–249.
Rice, D.W. Wolman, A.A., 1971. Life History and Ecology of the Gray Whale (*Eschrichtius robustus*). Am. Soc. Mamm. Spec. Pub. 3.
Robinson, R.A., Crick, H.Q.P., Learmonth, J.A., Maclean, I.M.D., Thomas, C.D., Bairlein, F., Forchhammer, M.C., Francis, C.M., Gill, J.A., Godley, B.J., Harwood, J., Hays, G.C., Huntley, B., Hutson, A.M., Pierce, G.J., Rehfisch, M.M., Sims, D.W., Santos, M.B., Sparks, T.H., Stroud, D.A., Visser, M.E., 2009. Travelling through a warming world: climate change and migratory species. Endangered Species Research 7, 87–99.
Rogers, A.R., Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9, 552–569.
Rooney, A.P., Merritt, D.B., Derr, J.N., 1999. Microsatellite diversity in captive bottlenose dolphins (*Tursiops truncatus*). Journal of Heredity 90, 228–231.
Rørvik, C.J., Jonsgård, Å., 1981. Review of Balaenopterids in the North Atlantic ocean. In: FAO Fish. Ser. (5), Mammals in the Seas 3, pp. 269–286.
Rousset, F., Raymond, M., 1995. Testing heterozygote excess and deficiency. Genetics 140, 1413–1419.
Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin Ver. 2.000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Shillinger, G.L., Palacios, D.M., Bailey, H., Bograd, S.J., Swithenbank, A.M., Gaspar, P., Wallace, B.P., Spotila, J.R., Paladino, F.V., Piedra, R., Eckert, S.A., Block, B.A., 2008. Persistent leatherback turtle migrations present opportunities for conservation. PLoS Biology 6 (7), e171. doi:10.1371/journal.pbio.0060171.
Skaug, H.J., Oien, N., Schweder, T., Bothun, G., 2004. Abundance of minke whales (*Balaenoptera acutorostrata*) in the Northeast Atlantic: variability in time and space. Canadian Journal of Fisheries and Aquatic Sciences 61, 870–886.
Stevick, P.T., McConnell, B.J., Hammond, P.S., 2002. Patterns of Movement. In: Hoelzel, A.R. (Ed.), Marine Mammal Biology: an Evolutionary Approach. Blackwell Publishing, Oxford, UK, pp. 185–216.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10, 512–526.
Taylor, B.L., Chivers, S.J., Larese, J., Perrin, W., 2007. Generation Length and Percent Mature Estimates for IUCN Assessments of Cetaceans. Southwest Fisheries Science Center. Administrative report LJ-07-01.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface. Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24, 4876–4882.
Tolley, K.A., Víkingsson, G.A., Rosel, P.E., 2001. DNA sequence variation and phylogeographic patterns in harbour porpoises *Phocoena phocoena* from the North Atlantic. Conservation Genetics 2, 349–361.
Valsecchi, E., Amos, W., 1996. Microsatellite markers for the study of cetacean populations. Molecular Ecology 5, 151–156.
Van Oosterhout, C., Hutchinson, W.F., Willis, D.P.M., Shipley, P., 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 535–538.
Wada, S., 1991. Genetic Distinction Between Two Minke Whale Stocks in the Okhotsk Sea Coast of Japan. Report of the International Whaling Commission, Paper SC/43/Mi32.
Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38 (6), 1358–1370.