

Agenda Item 6.1

Project Funding through ASCOBANS
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Document 6-07

**Project Report: Genetic structure of
white-sided dolphin (*Lagenorhynchus
acutus*) in the Eastern North Atlantic**

Action Requested

- Take note of the report
- Comment

Submitted by

Secretariat



NOTE:
IN THE INTERESTS OF ECONOMY, DELEGATES ARE KINDLY REMINDED TO BRING THEIR
OWN COPIES OF DOCUMENTS TO THE MEETING

**GENETIC VARIABILITY AND POPULATION STRUCTURE OF
White-sided dolphin (*Lagenorhynchus acutus*, Gray 1828)
IN THE NORTH ATLANTIC**

FINAL REPORT

United Nations Environmental Programme/Agreement on the Conservation of
Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas
(UNEP/ASCOBANS)

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Eulalia Banguera-Hinestroza

Co-authors

***P. Evans, L. Mirimim, R.J. Reid, B. Mikkelsen, B. Couperus, R. Deaville,
E. Rogan, and A.R. Hoelzel.***

©This research is an extension of previous studies by Banguera-Hinestroza (2008), carried out with the support of the Programme Alþan, European Union Programme of high level Scholarship for Latin America (Identification number EO3D17203CO) and the United Nations Environmental Programme/Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas (UNEP/ASCOBANS). No quotation from it should be published without the prior written consent of the author, and information derived from it should be acknowledged.

ABSTRACT

Few studies have been carried out on the two North Atlantic species of the newly proposed genus *Leucopleurus* (Leduc *et al.*, 1999; Banguera-Hinestroza *et al.*, 2010) currently classified within the genus *Lagenorhynchus* (*L. acutus* and *L. albirostris*). Nor has much attention been paid to their conservation status, perhaps due to their perceived abundance, reported as tens of thousands in the North Atlantic. At the same time, recent studies have found evidence for low genetic diversity in both species, which could make isolated or semi-isolated populations more vulnerable to threats such as pollution, loss of coastal habitat, and by-catch. Evolutionary and ecological studies on these species are due, their uniqueness as a new genus restricted to the North Atlantic and its apparent long evolutionary history in this region, could give important insights into the evolution and radiation of the family Delphinidae, and provide a better understanding about the evolutionary processes responsible for the speciation and adaptation of North Atlantic marine fauna.

In this study we provide both new and complementary data about the genetic diversity and population structure of the Atlantic white-sided dolphin (*Lagenorhynchus acutus*, Gray 1828) in order to provide baseline information for its effective conservation, and give insights for a better understanding of its evolutionary history. We analyzed the genetic variation at the mitochondrial (mtDNA) control region for 344 samples from three putative populations in the western North Atlantic and eight different regions in the eastern North Atlantic. The analyses performed showed high haplotypic diversity (H_d) at mtDNA (0.927 ± 0.007), but relatively low nucleotide diversity (0.00891 ± 0.0003). These findings suggest a pattern of genetic diversity congruent with an ancient bottleneck followed by an expansion in range in most *L. acutus* populations that were analyzed.

Population structure analyses showed that samples from the western region of the eastern North Atlantic (West Ireland, Faroe Islands and Northwest British Isles) are similar to samples from the western North Atlantic (USA coasts). However, samples from the North Sea and eastern Scotland did show some degree of differentiation from other populations, from both the eastern and the western North Atlantic.

INTRODUCTION

Distribution

The Atlantic white-sided dolphin (*Lagenorhynchus acutus*, Gray 1828) is a pelagic species, endemic to the North Atlantic, with a preference for temperate and sub-polar waters (Mikkelsen and Lund 1994; Reeves *et al.* 1999; Weinrich *et al.* 2001; Evans *et al.* 2003; Evans & Smeenk, 2008). Its geographic range extends from New England to West Greenland in the western North Atlantic, and from East Greenland, Iceland, British Isles, the North Sea and Norway in the eastern North Atlantic (Gaskin 1992; Evans & Smeenk 2008).

In the western North Atlantic, the species inhabits waters from central West Greenland to North Carolina (about 35°N) (Reeves *et al.* 1999; Hamazaki 2002). In the eastern North Atlantic it is commonly distributed from Iceland and the western Barents Sea, south to the Bay of Biscay, being more abundant from 56°N northwards than south of this latitude (Evans 1992). In the British Isles, its distribution is concentrated around the Hebrides, Northern Isles and northern North Sea in waters 100-500 m deep (Evans *et al.* 2003). It also occurs regularly off western Ireland, but is uncommon in the Irish Sea and is considered rare in the southern North Sea (Evans 1992; Reeves *et al.* 1999; Evans & Smeenk 2008).

Population structure

Knowledge of the distribution and abundance of *L. acutus* is limited in the eastern North Atlantic, where studies have been based mostly on opportunistic surveys and only a few long-term surveys (e.g. Evans 1992; Northridge *et al.* 1997; Hammond *et al.* 2002; Evans *et al.* 2003; Reid *et al.* 2003; Evans and Hammond 2004). Previous genetic studies suggested the existence of at least two populations in this region (Banguera-Hinestroza 2008), and those analyses are extended here.

In the western North Atlantic, studies have mainly been carried out using sightings, strandings and incidental takes. Based in these studies and in the absence of sightings of *L. acutus* between Gulf of Maine and Gulf of St. Lawrence, Palka *et al.* (1997) recommended the division of three population stocks in this region (Gulf of Maine, Gulf of St. Lawrence and

Labrador Sea). However, the existence of a separate stock in the Gulf of Maine is not clear and remains uncertain (See Weinrich *et al.* 2001).

Mikkelsen and Lund (1994) suggested the existence of a single population of this species across its geographic range, based on the lack of phenotypic differences among skulls of 228 specimens from the eastern and western North Atlantic. In contrast to the proposal by Palka *et al.* (1997), these authors did not find evidence to subdivide populations into a northern and southern stock in either of the two areas studied, or into western and eastern North Atlantic stocks. Recent analyses by Banguera-Hinestroza (2008), which are extended in this study, partially agree with Mikkelsen and Lund (1994), but did find genetic differentiation at mtDNA between western North Atlantic and a putative population in the eastern North Atlantic.

Population sizes and main threats in white-sided dolphins

Population sizes of Atlantic white-sided dolphin over large areas are unknown and few estimates of abundance have been published, and when they have, they have mainly been carried out over limited periods (summer months) and in few places (Kingsley and Reeves 1998; Waring *et al.* 2007). A population estimate for a portion of the eastern North Atlantic (North Sea and adjacent waters) recorded around 11,760 (5,867-18,528) dolphins of *Lagenorhynchus* spp., most of which are likely to have been white-beaked dolphins (*L. albirostris*) in July 1994 (Hammond *et al.* 2002). A repeat survey over a wider region in July 2005 revealed too few encounters for a reliable abundance estimate (Hammond 2008). Aerial surveys in the western North Atlantic during July-August 1999, have reported an estimated 51,640 animals (CV=0.38) with a minimum population of 37,904 (Waring *et al.* 2007). However, the total population size of the species in this region is still unknown (Waring *et al.* 2006, 2007).

White-sided dolphins inhabit one of the most intensively fished waters in the world, especially over the continental shelf (Morizur *et al.* 1999), and high by-catch rate in fishing nets and commercial hunting have been reported as the major threats affecting white-sided dolphin populations (Alling and Whitehead 1987; Reeves and Leatherwood 1994; Reeves *et al.* 1999; Waring *et al.* 2006; Evans & Smeenk 2008). In addition, mass strandings (possibly caused by interaction with pelagic trawling) and direct exploitation have also been identified as a major risk for this species. For example, direct catches are common in the Faroe Islands, where more

than 500 dolphins can be killed in the drive fisheries in one year (Bloch & Mikkelsen 2009), and in southwest Greenland, where the annual catch has been estimated at approximately 50 individuals (Reeves *et al.* 1999; Reeves and Leatherwood 1994). It is not known whether these historical harvests pose a threat to the population since, as indicated above, comprehensive population size estimates do not exist for the North Atlantic. Furthermore, little is known about their conservation status, in particular, their effective population size, life history and stock boundaries, all of which are essential for effective management and conservation.

The main aims of this study were to identify potential management units for white-sided dolphins on both sides of the North Atlantic, but with special attention to the eastern North Atlantic; to give insights into the evolutionary history of the species and to provide data and recommendations for further studies and conservation plans for this species. In addition, this study tests hypotheses about possible effects of glaciated epochs on the spatial distribution of lineages, demography history and genetic diversity of *L. acutus* populations.

MATERIALS AND METHODS

Sample Collection

White-sided dolphin samples were collected from throughout its known geographic range. Tissue and teeth samples from the eastern North Atlantic belong to different geographic locations in West Ireland, Northwest British Isles, East Scotland, Shetland Isles, Northern North Atlantic (Faroe Islands & Iceland), Southern North Sea, Denmark, and Southern England. Samples from the western North Atlantic belong to two putative modern populations (Maine and Massachusetts) and one temporally unrelated population from Massachusetts, from the 19th Century.

Bone and teeth samples were collected from the Museum of Natural History Rotterdam, Museum of Natural History Leiden, Fisheries and Maritime Museum Denmark, Museum of Natural History Denmark, Natural History Museum London, Natural History Museum Faroe Islands, and the Smithsonian Natural History Museum. Samples from living and stranded dolphins were collected through cooperative agreements with Stranding Networks from Scotland England & Wales, the National Oceanic and Atmospheric Administration (NOAA) in the USA, the Maritime Institute in Iceland, the Swedish Museum of Natural History

(SMNH) in Stockholm, the Department of Contaminant Research in Stockholm and Imares in Holland. Scientific collaboration was also received from the Marine Research Institute in Ireland; For details of samples and catalogue numbers, refer to Annex 1.

DNA extraction

Total genomic DNA from tissue samples was extracted following the procedure described by Hoelzel and Green (1998). Bone and teeth samples were cleaned with a 10% solution of bleach for two hours; and after being rinsed with water and placed in ethanol for two hours, they were dried overnight and then placed under ultraviolet light for 20 minutes prior to extraction. Teeth or bones were ground using a Mikro-Dismembrator S (© Sartorius). They were then digested in 2 ml lysis solution (85% EDTA (0.5M, pH8 stock), 10% TRIS-HCl (1M pH8 stock), and 5% SDS (1%w/v stock) for 24 h at 55°C. After digestion, the DNA was extracted using a Qiagen PCR purification kit, following the protocol recommended for cleaning PCR products. The samples were diluted in 0.1mM Tris-EDTA buffer and placed at -20°C until use. All extractions were conducted with disposable equipment and extraction controls to detect and minimise any sample contamination.

Mitochondrial control region (mtDNA) amplification and sequencing

The mtDNA control region hypervariable region 1 (HVR1; 322 bp) was sequenced for a total of 344 samples under the following conditions: 20-50 ng of DNA, 10x PCR buffer, 1.5 mM MgCl₂, 50-100 ng of primers, 2.5 mM dNTP and 1U of Taq polymerase (bone samples were amplified using 2U Platinum hot start taq polimerase). Amplifications were conducted in a TCY thermocycler with the following cycle conditions: 94°C 6 min. followed by 35 cycles of 94°C 30 sec (45 cycles in teeth and bone samples), 54°C 45 sec and 72°C 45 sec. A 600 bp fragment was amplified when possible using universal primers MTCRF (5'- TTC CCC GGT CTT GTA AAC C- 3') and MTCR-R (5'-ATT TTC AGT GTC TTG CTT T-3') from Hoelzel and Green (1998). This fragment did not amplify in bone and teeth samples; thus an internal primer was used to amplify the smaller fragment (322bp) (MACR 5' CGGCATGGTGATTAAGCT), and all individuals were compared at this shorter sequence. After amplification, samples were purified using Qiagen columns (Qiagen, Inc.) and were directly sequenced in an ABI 377 automated sequencer. The sequences were aligned using the Clustal X programme (1.83) from Thompson *et al.* (1997), and edited using the programme Chromas Pro (www.technelysium.co.au).

mtDNA analysis

- ***Putative populations***

Samples were initially divided into eight geographic zones in the eastern North Atlantic: western Ireland (WRL; N=80), NW British Isles (NWBI; N=35), East Scotland (ESC; N=31), Shetland Isles (SHT; N=20), Northern North Atlantic (NNA; N=52) Southern North Sea (SNS; N=15), Denmark (DMK; N=14) and Southern England (SGL; N=9). Samples from the western North Atlantic were divided into three putative populations: western ancient (WNAanc; N=26), western Massachusetts (WNAma; N=29) and western Maine (WNAmn; N=33) (Figures 1 and 2). After population structure analyses (see results) the following samples were analyzed together: Western Ireland & NW British Isles (W-ENA; N=115), Denmark & Southern North Sea (North Sea; N=29), WNAma & WNA Maine (WNA; N=62) and East Scotland & Shetland Isles (E-ENA; N=51). Samples from WNAanc and Northern North Atlantic were kept as separated populations. In addition, samples from Southern England were included in the analysis but excluded from most interpretations due to the small number of samples available for this region. Samples that represent join populations and samples from Western North Atlantic origin are mentioned thereafter with their acronyms.

- ***Selection of the best-fit model for mtDNA control region (HVRI)***

The selection of the best-fit models of nucleotide substitution was carried out using jModel test (Posada 2008), the best model was selected using the AKAIKE Information Criterion (AIC) as recommended by the authors (Posada and Buckley 2004).

- ***Genetic diversity and populations differentiation***

The extent of genetic variation in the control region was assessed by examining both haplotype (h) and nucleotide diversity (π), using Arlequin v 3.5.1.2 (Excoffier *et al.* 2009) and DNAsp v 5.10.00 (Rozas *et al.* 2009). The degree of genetic differentiation among geographic samples was determined using the Analysis of Molecular Variance (AMOVA) as implemented in Arlequin v 3.5.1.2 (Excoffier *et al.* 2009). The variance components of gene frequencies were partitioned between geographic regions (groups): western North Atlantic, eastern North

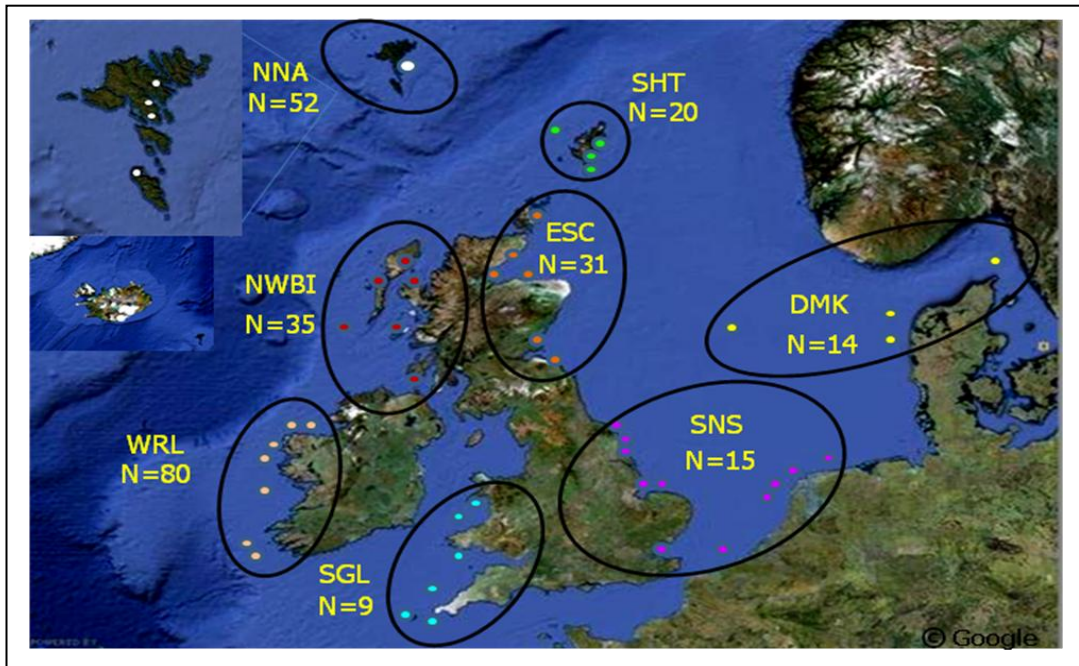


Figure 1. Sample localities for *L. acutus* in the eastern North Atlantic.
N=sample size

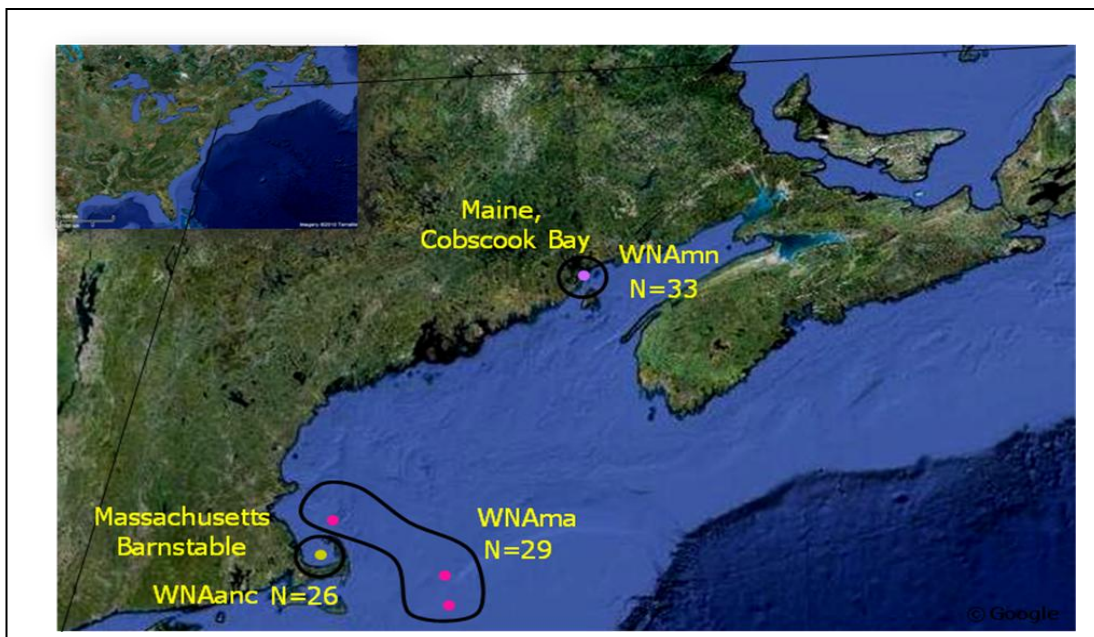


Figure 2. Sample localities for *L. acutus* in the western Atlantic.
N=sample size

Atlantic and North Sea; among populations within groups and within populations. The differentiation between populations was quantified using the F_{st} index (based only on haplotype frequencies), the Φ_{st} index (based on both genetic distances and haplotype frequencies) and the non-differentiation exact test, which tests the hypothesis of a random distribution of different haplotypes among populations (Wright 1951; Excoffier et al. 1992; Raymond and Rousset 1995; Goudet et al. 1996). The statistical significance of fixation statistics was tested using a non-parametric permutation approach with 10,000 permutations, and 100,000 Markov steps were performed in the exact test. Given that transversions and deletions could be of evolutionary importance, these nucleotide changes were given twice the weight of transitions in all analyses. Analyses were also performed using the same weight for all nucleotide changes, obtaining similar results.

- ***Phylogenetic relationships***

The phylogenetic relationships among haplotypes were examined generating a neighbour-joining tree for the complete set of mtDNA haplotypes using the Tamura-Nei substitution model (Tamura and Nei 1993), and the analyses were conducted using MEGA version 4.0 (Tamura *et al.* 2007). In addition, a median-joining network tree was built to infer the ancestral relationships among haplotypes using the programme Network version 4.5.0.0 (Bandelt *et al.* 1999).

- ***Mismatch distribution and Neutrality test***

The distribution of the number of observed differences between pairs of DNA sequences (mismatch distribution; Rogers and Harpending 1992) was used as an assessment of demographic history of the population, using the program Arlequin v 3.5.1.2 (Excoffier *et al.* 2009). Sudden demographic expansion should result in a unimodal distribution of pairwise differences, while multimodal distributions are associated with populations that have been stable over long periods of time (Rogers and Harpending 1992).

The parameters of the demographic expansion were calculated using the method suggested by Schneider and Excoffier (1999). The confidence intervals for these parameters are obtained by a parametric bootstrap approach that assumes that the data are distributed according to the sudden expansion model. The calculations were carried out following the principles explained

by Schneider and Excoffier (1999) and using a coalescent algorithm modified from Hudson (1990). The hypothesis that the observed data fit the sudden expansion model was tested using the sum of square deviations (SSD) (Schneider and Excoffier 1999) and the raggedness index (Harpending 1994).

The coalescence time of expansion in years (t) was calculated using the relationship $\tau=2\upsilon t$, where τ represents the mode of the mismatch distribution (in units of evolutionary time) and υ is the mutation rate for the sequence used ($\mu=5 \times 10^{-7}$; Ho *et al.* 2007). The υ value was calculated as suggested by Rogers and Harpending (1992), using the formula $\upsilon=\mu k$, where μ is the mutation rate per nucleotide and k is the number of nucleotides evaluated.

Fu's test (Fu 1997) and Tajima's test (Tajima 1989b) were used to evaluate the demographic history of *L. acutus* populations. Fu's test (Fu 1997) statistic is very sensitive to population demographic expansion, which leads to large negative F values (Fu 1997). Tajima's D-statistic (Tajima 1989b) tests whether the region is neutral or under selection and whether or not the population is in equilibrium (Tajima 1989a; Tajima 1989b). Positive values of Tajima's D are indicative of either balancing selection or an admixture of two genetically different populations (Pichler 2002), whereas negative values suggest a selective sweep or sudden expansion. Significant D values are often related to factors other than selection such as population bottlenecks and subsequent expansion or heterogeneity of mutation rates (Aris-Brosou and Excoffier 1996). The significance of Tajima's D was determined by generating 1,000 random samples under the assumption of selective neutrality with a coalescent simulation algorithm (Hudson 1990). Both tests were evaluated using Arlequin v 3.5.1.2 (Excoffier *et al.* 2009) and DNAsp v 5.10.00 (Rozas *et al.* 2009).

RESULTS

Model selection

The three best-fit models of nucleotide substitution selected by the AIC criterion were: the Hasegawa-Kishino-Yano model (HKY+I+G and HKY+G); Hasegawa *et al.*, 1985); and the Tamura-Nei model (TN+I+G; Tamura and Nei 1993). However, given that not all population

genetics programs have incorporated the HKY model, the Tamura and Nei model was used in all calculations, with a gamma distribution of 0.28 as suggested by jModeltest (Posada 2008).

Genetic variation at the mtDNA control region

Sixty four haplotypes were found for the 322bp fragment among all geographic regions (eastern and western North Atlantic) defined by 44 polymorphic sites, 26 of which were parsimony informative (Figure 3).

Amongst the eight geographic regions in the eastern North Atlantic (see above), 55 haplotypes were found with unique haplotypes in the Northern North Atlantic (7), Southern North Sea (4), Denmark (1), East Scotland (3), NW British Isles (5) and western Ireland (15). In the western North Atlantic, a total of 24 haplotypes were identified, with 8 unique haplotypes for this region.

Over all samples (excluding western Atlantic ancient samples), haplotypic (gene) diversity (h : 0.927 ± 0.007) was similar to values found in other odontocete populations (see Pichler and Baker 2000). However, relatively low nucleotide diversity values were found (π : 0.00891 ± 0.00028) similar to those found in populations that have gone through historical population bottlenecks (see Hoelzel 2002; Table 1).

Differentiation among populations

The initial analyses performed by regions using F_{st} , ϕ_{st} and exact test suggests no differentiation between Northern North Atlantic, Western Ireland and NW British Isles (See Figures 1 and 2). However some degree of differentiation was found between these regions and samples from East Scotland, Shetland Isles and Southern North Sea (see Table 2). Exact test comparisons of haplotype frequencies are shown in Table 3.

Comparisons based on pooled regions showed that E-ENA samples (East Scotland plus Shetland Isles) are differentiated to some degree from Northern North Atlantic and W-ENA (western Ireland plus NW British Isles; see Table 4). There was no differentiation found between putative populations in the western North Atlantic (WNA) or between western North Atlantic and samples from Western Ireland, NW British Isles and Northern North Atlantic.

Haplotypes	Frequencies	2	3	9	3	4	4	5	6	6	6	6	7	7	9	9	9	0	0	1	1	1	1	2	2	3	3	3	3	3	5	6	6	7	7	9	0	4	4	6	6	6	7	8	8	8	8	9	0	0
Hap 1	2	-	A	G	A	T	T	T	T	C	G	T	A	C	T	C	C	-	-	A	T	G	T	T	T	C	T	T	G	C	-	G	C	C	T	A	T	T	T	T	T	T	T	T	T	T	G			
Hap 2	60	-	T	-	-	G	-	A	T	.	.	C		
Hap 3	9	-	C	T	-	-	G	-	A	T			
Hap 4	22	-	T	-	-	C	.	-	A	T	.	.	C	.	C			
Hap 5	5	-	T	-	-	-	A	T	T				
Hap 6	5	-	T	-	-	C	.	-	A	T	.	.	C			
Hap 7	6	A	C	T	-	-	G	-	A	T			
Hap 8	7	-	T	-	-	T	.	.	-	A	T			
Hap 9	10	-	T	-	-	G	-	A	T	.	G	C				
Hap 10	12	-	T	-	-	G	C	.	-	A	T	.	.	C			
Hap 11	40	-	-	-	-	A			
Hap 12	10	-	T	T	-	-	G	-	A	T				
Hap 13	1	-	T	-	-	C	.	-	A	T	C			
Hap 14	1	-	T	-	-	G	-	T				
Hap 15	15	-	T	C	.	.	-	-	G	-	A	T	C			
Hap 16	2	-	T	-	-	G	-	A	T	.	.	C	.	C				
Hap 17	2	-	-	-	-	A	.	.	C				
Hap 18	15	-	T	-	-	G	-	A	T				
Hap 19	40	-	T	-	-	G	-	A	T				
Hap 20	1	-	.	.	C	.	.	.	T	C	.	.	.	-	-	G	C	-	A	T	C				
Hap 21	13	-	T	-	-	-	A	T				
Hap 22	1	-	T	A	-	-	-	A	T	.	G				
Hap 23	1	A	T	-	-	G	-	A	.	C	C				
Hap 24	1	-	T	-	-	G	-	A	T	.	.	C	C			
Hap 25	2	-	T	-	-	-	A	T	A				
Hap 26	2	-	T	-	-	G	.	.	.	C	.	.	.	-	A	T				
Hap 27	1	-	T	-	-	G	.	.	.	C	.	.	.	-	A	T	C				
Hap 28	9	-	T	-	-	-	A	T	.	.	C	A					
Hap 29	1	-	T	-	-	G	-	A	T	.	.	C				
Hap 30	2	-	T	-	-	-	A	T	.	.	C				
Hap 31	2	-	T	-	-	G	-	A	T	C			

Figure 3. Haplotypes and nucleotide positions in the eastern and western North Atlantic

Haplotypes	Frequencies	2	3	9	3	4	4	5	6	6	6	6	7	7	9	9	9	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3		
Hap 32	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	C	C	-	C	-	-	-	-		
Hap 33	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	C	-	-	A	-	-	-	-		
Hap 34	2	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	-		
Hap 35	2	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	C	-	-	C	-	-	-	-		
Hap 36	1	A	-	-	-	-	-	-	C	T	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	A		
Hap 37	1	-	-	-	-	-	-	C	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	C	-	-	-	-	-	-	-		
Hap 38	1	-	-	-	-	-	-	-	C	T	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	A		
Hap 39	3	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	C	-	-	C	-	-	-	-		
Hap 40	2	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-		
Hap 41	2	-	-	-	-	-	-	-	T	-	-	-	-	-	C	-	-	-	-	G	-	A	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	C	-	-	-	-	
Hap 42	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	C	-	-	-	-	
Hap 43	1	-	-	-	-	-	-	-	T	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	-	-	
Hap 44	2	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	-	-	
Hap 45	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	C	-	-	-	-	
Hap 46	1	-	-	A	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	-	-	-	-	-	
Hap 47	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	C	-	-	-	-	
Hap 48	1	-	-	G	-	-	-	-	T	A	-	-	-	-	C	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	C	-	-	-	-	
Hap 49	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	T	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	
Hap 50	1	-	-	-	-	A	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	-	-	-	-	-	
Hap 51	2	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	C	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	
Hap 52	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	C	A	G	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	-	-	
Hap 53	3	-	-	-	-	-	-	-	T	-	-	C	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	-	-	-	-	-	
Hap 54	1	-	-	-	-	-	-	-	T	-	-	-	-	-	C	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	C	-	-	-	-	
Hap 55	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	G	-	-	-	-	-	-	-	-	
Hap 56	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	T	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	
Hap 57	1	-	-	-	-	-	-	-	C	T	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	-	-	-	-	-	
Hap 58	1	-	-	-	-	-	-	-	T	-	-	-	-	-	T	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	-	-	-	-	-	
Hap 59	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	-	-	-	-	-	
Hap 60	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	
Hap 61	2	A	-	-	-	-	-	-	C	T	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	C	-	-	-	-	
Hap 62	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	A	
Hap 63	1	-	-	-	-	-	-	-	T	A	-	-	-	-	C	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	-	-	-	C	-	-	-	-	-
Hap 64	1	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	A	T	-	-	-	C	-	-	-	-	-	-	-	-

Figure 3. Haplotypes and nucleotide positions in the eastern and western North Atlantic

Table 1. Diversity estimates in the eastern and western North Atlantic

Populations	Sample size	Polimorphic sites	Number of Haplotypes	<i>h</i> : Haplotype diversity	π : Nucleotide diversity
NNA	52	20	21	0.9186 \pm 0.0195	0.0087 \pm 0.0052
WNAma	29	14	14	0.8990 \pm 0.0360	0.0094 \pm 0.0056
WNAmn	33	20	17	0.9280 \pm 0.0266	0.0092 \pm 0.0055
WNA (WNAma + WNAmn)	62	23	22	0.9159 \pm 0.0199	0.0093 \pm 0.0055
WNAanc	26	10	9	0.8862 \pm 0.0362	0.0100 \pm 0.0059
SNS	15	15	12	0.9619 \pm 0.0399	0.0125 \pm 0.0074
DMK	14	13	12	0.9780 \pm 0.0345	0.0116 \pm 0.0070
North Sea (SNS + DMK)	29	20	19	0.9655 \pm 0.0189	0.0119 \pm 0.0069
ESC	31	18	17	0.9462 \pm 0.0210	0.0098 \pm 0.0058
SHT	20	9	8	0.8684 \pm 0.0410	0.0085 \pm 0.0053
E-ENA (ESC+SHT)	51	19	19	0.9286 \pm 0.0165	0.0094 \pm 0.0055
WNBI	35	13	17	0.9092 \pm 0.0329	0.0083 \pm 0.0050
WIR	80	28	33	0.9329 \pm 0.0149	0.0097 \pm 0.0056
W-ENA (WNBI + WIR)	115	31	41	0.9263 \pm 0.0139	0.0092 \pm 0.0054
SEN	9	9	8	0.9722 \pm 0.0640	0.0089 \pm 0.0059
Overall	344	44	64	0.9270 \pm 0.0070*	0.00891 \pm 0.0003

*Over all samples, the genetic diversity and nucleotide diversity values were computed excluding samples from western Ancient origin.

However some degree of differentiation was found between WNA samples and samples from the Southern and Northern North Sea (see Tables 2, 3 and 4). An analysis of Molecular Variance (AMOVA) including all putative populations showed that the 98.98% of the variance could be explained as a result of the differences within populations.

Phylogenetic relationships

No clear association among haplotypes from the different regions was found, either in the neighbour joining tree or in the median-joining network. However, the star-like shape in parts of the network tree suggests one or more sudden expansions of *L. acutus* in the North Atlantic (Figure 4).

Mismatch distribution analysis and neutrality tests

The mismatch distributions show a clear unimodal shape in most populations that were analyzed. The variance (SSD) and the small, non-significant values of the raggedness index (r) suggested that the curves did not differ significantly from that expected under a model of sudden expansion. The exception was Shetland Isles, where the raggedness index was higher than in other populations and significant ($0.1351 P > 0.012$), rejecting the hypothesis of sudden expansion in this region (Figure 5, Table 5).

The sudden expansion in most *L. acutus* populations was also corroborated by the negative values of Tajima's D , with the exception of WNAanc ($0.74768 > 0.8020$) and Shetland Isles ($0.22833 P > 0.622$) (Table 5) where values were positive and not significant. Fu's statistics showed large negative and highly significant values in all populations, except for the WNAanc ($-1.7133 P > 0.197$) and Shetland Isles ($F_s: -1.1814 P > 0.27$) (Table 5), suggesting different demographic histories for these regions. However, when combining samples from Shetland Isles and East Scotland (E-ENA), mismatch distribution graph was unimodal (Figure 5), values of Raggedness index and SSD were small and not significant ($0.00578 P > 0.13$; $0.03489 P > 0.281$ respectively) and Tajima's D and Fu's statistic were negative and significant ($-0.6684 P > 0.04230$; $-8.39077 P > 0.00001$ respectively). It is important to point out that to corroborate whether or not samples from Shetland Isles and East Scotland should be considered as a single population, analysis with nuclear markers should be carry out, especially giving the limitations of uni-parental markers for resolving this question.

Table 2. F_{st} and ϕ_{st} values between populations in the western and eastern North Atlantic.

Populations	NNA	NWBI	WRL	SGL	WNAma	WNAmn	WNAanc	SNS	DMK	ESC	SHT
NNA		-0.0100	-0.0077	-0.0307	-0.0081	-0.0154	0.0068	-0.0024	-0.0103	0.0024	0.0510
NWBI	-0.0051		-0.0061	-0.0178	-0.0055	-0.0119	0.0051	-0.0086	-0.0205	0.0057	0.0529
WRL	-0.0019	0.0013		-0.0299	-0.0019	-0.0091	0.0105	-0.0079	-0.0112	-0.0032	0.0324
SGL	-0.0267	-0.0122	-0.0313		-0.0500	-0.0263	-0.0620	-0.0630	-0.0584	0.0002	0.0501
WNAma	-0.0012	0.0079	0.0079	-0.0322		-0.0038	-0.0132	-0.0316	-0.0128	0.0248	0.0482
WNAmn	0.0024	0.0009	-0.0019	-0.0207	0.0031		0.0098	0.0028	-0.0221	-0.0108	0.0467
WNAanc	0.0102	0.0250	0.0123	-0.0426	0.0000	0.0146		-0.0131	-0.0284	0.0415	0.0518
SNS	0.0156	0.0209	0.0124	-0.0359	-0.0134	0.0222	0.0140		-0.0267	0.0142	0.0350
DMK	0.0026	0.0030	0.0023	-0.0326	0.0015	-0.0112	0.0068	-0.0083		-0.0093	0.0405
ESC	0.0134	0.0200	0.0099	-0.0205	0.0321	0.0127	0.0529	0.0166	-0.0031		0.0396
SHT	0.0358	0.0242	0.0232	-0.0066	0.0344	0.0336	0.0573	0.0344	0.0261	0.0274	

F_{st} values (below diagonal) and ϕ_{st} values (above diagonal) were calculated using 10000 permutations. Values in bold are significant at level 0.05

Populations: NNA=Northern North Atlantic (Faroe Isles plus Iceland); NWBI (Northwest British Isles); WRL (West Ireland); SGL (South England); WNAma (Western North Atlantic, Massachussets); WNAmn (Western North Atlantic, Maine); WNAanc (Western North Atlantic ancient); SNS (Southern North Sea); DMK (Denmark); ESC (East Scotland); SHT (Shetland isles)

Table 3. Non-differentiation exact P-values

	NNA	WNAm	WNAmn	WNAanc	SNS	DMK	ESC	SHT	NWBI	WRL
NNA										
WNAm	2 0.61888±0.0170									
WNAmn	3 0.06321±0.0067	0.34170±0.0113								
WNAanc	4 0.22242±0.0115	0.20787±0.0075	0.19158±0.0061							
SNS	5 0.02409±0.0044	0.58457±0.0109	0.02043±0.0050	0.01977±0.0021						
DMK	6 0.09526±0.0086	0.25031±0.0065	0.50713±0.0039	0.17284±0.0047	0.72868±0.0068					
ESC	7 0.01636±0.0044	0.01492±0.0019	0.05095±0.0046	0.00007±0.0001	0.08473±0.0094	0.34603±0.0161				
SHT	8 0.05617±0.0053	0.06984±0.0052	0.03253±0.0027	0.00677±0.0012	0.06100±0.0066	0.04774±0.0042	0.11390±0.0058			
NWBI	9 0.42599±0.0160	0.33659±0.0096	0.12648±0.0078	0.06888±0.0084	0.07903±0.0055	0.19167±0.0152	0.02511±0.0034	0.25999±0.0103		
WRL	10 0.44394±0.0263	0.56544±0.0248	0.34890±0.0227	0.36896±0.0224	0.14848±0.0170	0.19086±0.0199	0.16317±0.0164	0.71369±0.0161	0.35441±0.0166	
SGL	11 0.78454±0.0104	0.66531±0.0119	0.70961±0.0114	0.58879±0.0100	0.97232±0.0029	0.96882±0.0021	0.76872±0.0082	0.33799±0.0066	0.61567±0.0083	0.94526±0.0078

Values were calculated using 100000 Markov steps. Values in bold are significant at level 0.05

Populations: NNA=Northern North Atlantic (Faeroes isles plus Iceland); WNAm (Western North Atlantic, Massachusetts); WNAmn (Western North Atlantic, Maine); WNAanc(Western North Atlantic ancient); SNS (Southern North Sea); DMK (Denmark); ESC (East Scotland); SHT (Shetland Isles); NWBI (North west British Isles); WRL (West Ireland); SGL (South England).

Table 4. Fst and Non-differentiation exact P-values for join populations

	NNA	W-ENA	SGL	WNA	WNAanc	E-ENA	NORTH SEA
NNA		0.74447±0.0273	0.78177±0.0085	0.19653±0.0210	0.18839±0.0125	0.01194±0.0026	0.04444±0.0046
W-ENA	-0.00316		0.91531±0.0116	0.30050±0.0231	0.48675±0.0132	0.28091±0.0196	0.15319±0.0134
SGL	-0.0267	-0.02563		0.74499±0.0151	0.58186±0.0071	0.78243±0.0061	0.98184±0.0021
WNA	-0.00005	0.00215	-0.02648		0.41640±0.0097	0.00227±0.0010	0.17651±0.0117
WNAanc	0.01015	0.0156	-0.04262	0.00678		0.00017±0.0002	0.18818±0.0089
E-ENA	0.01553	0.01028	-0.02219	0.01913	0.04734		0.04255±0.0038
NORTH SEA	0.0105	0.01019	-0.03192	0.00144	0.01102	0.01114	

Fst values (below diagonal) and Non-differentiation exact P-values (above diagonal). Fst values were calculated using 10000 permutations and Non-differentiation exact P-values were performed using 100000 markov steps. Values in bold are significant at level 0.05.

Populations: NNA (Northern North Atlantic); W-ENA (NW British isles plus Western Ireland); SGL (South England); WNA (Western North Atlantic); WNAanc (Western North Atlantic ancient); E-ENA (Shetland isles plus East Scotland); North Sea (Southern North Sea plus Denmark)

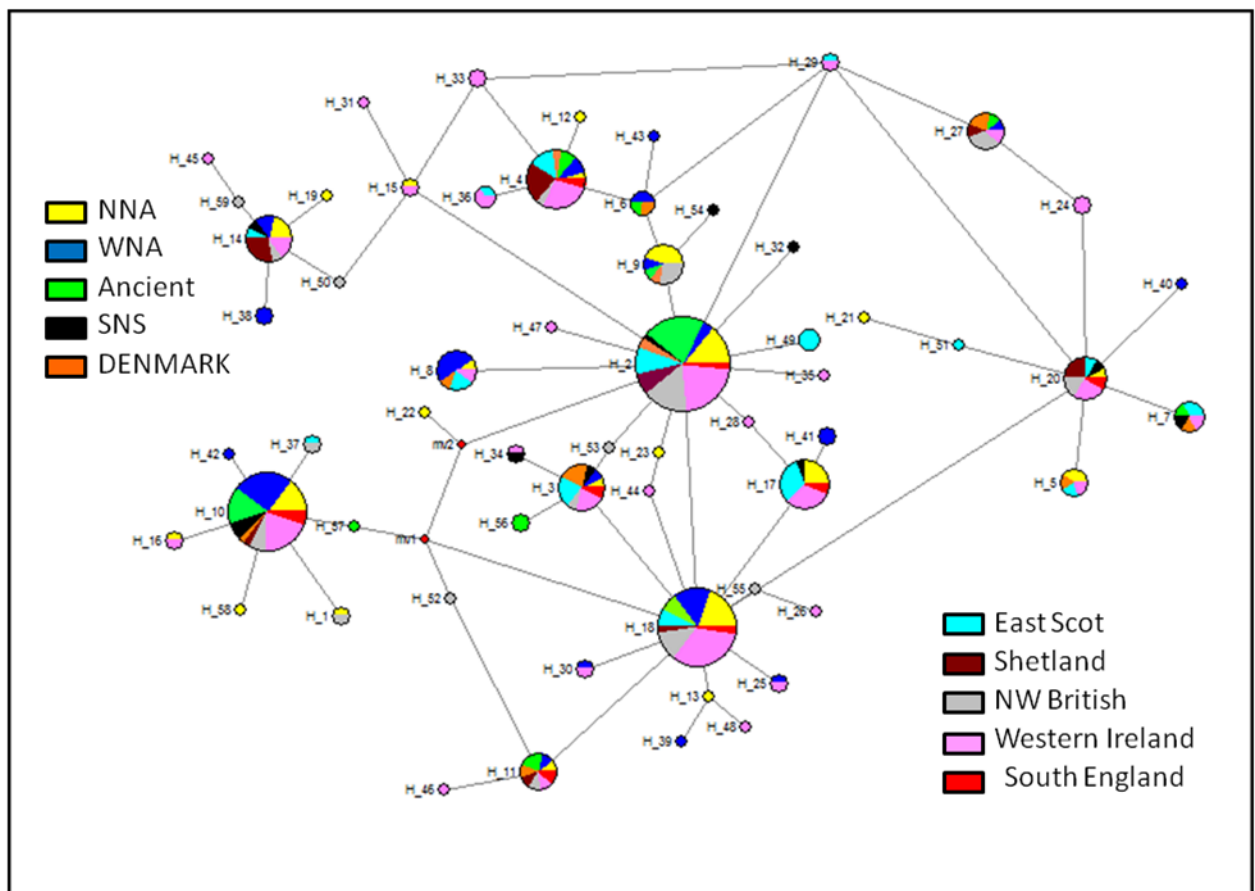


Figure 4. Network tree showing the relationships among haplotypes in the western and eastern North Atlantic. *Line lengths in the network tree are proportional to the number of mutations among haplotypes.

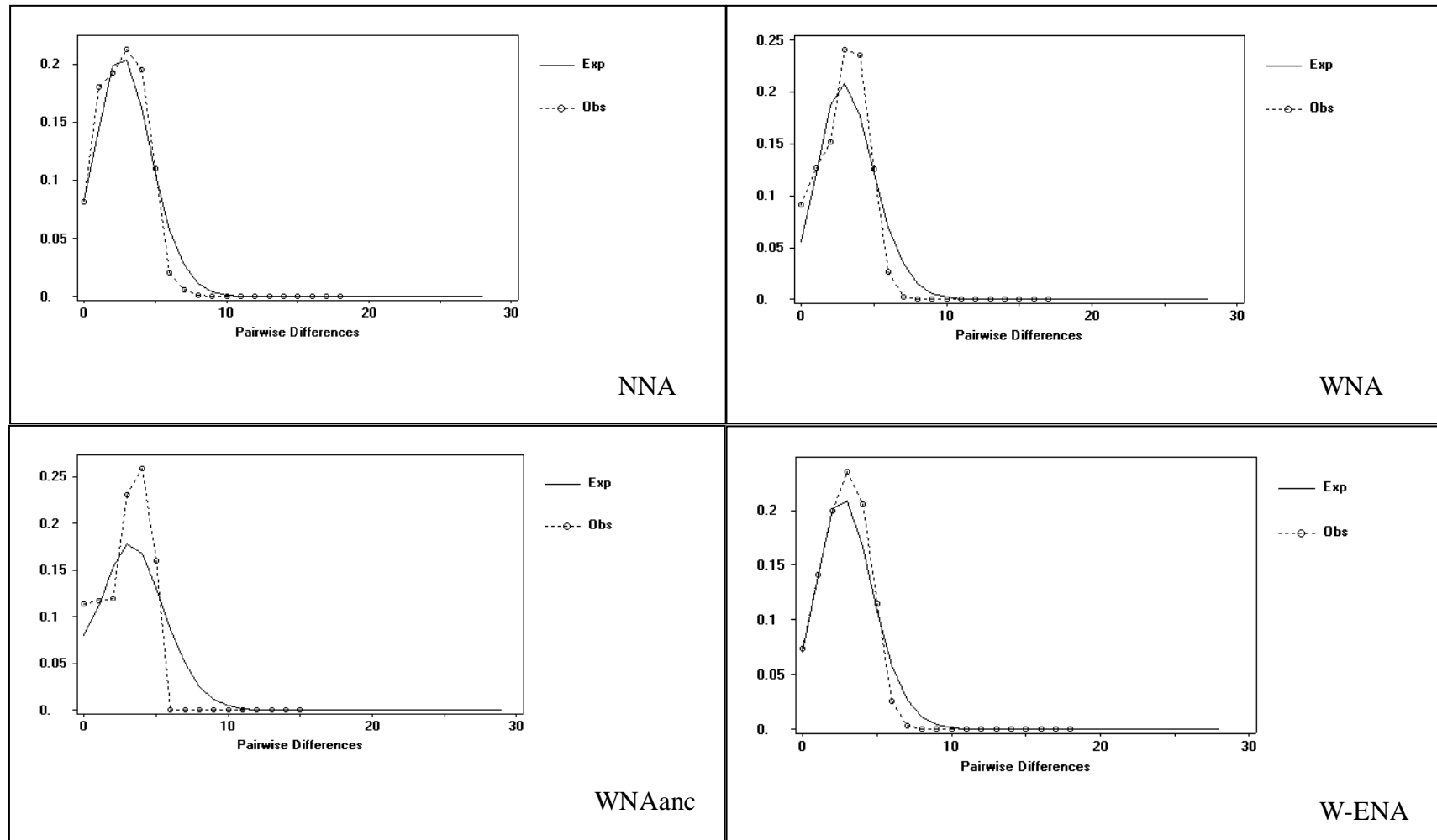


Figure 5. Mismatch distribution graphs for *L. acutus* populations, y axis shows the frequency of the pairwise comparisons.

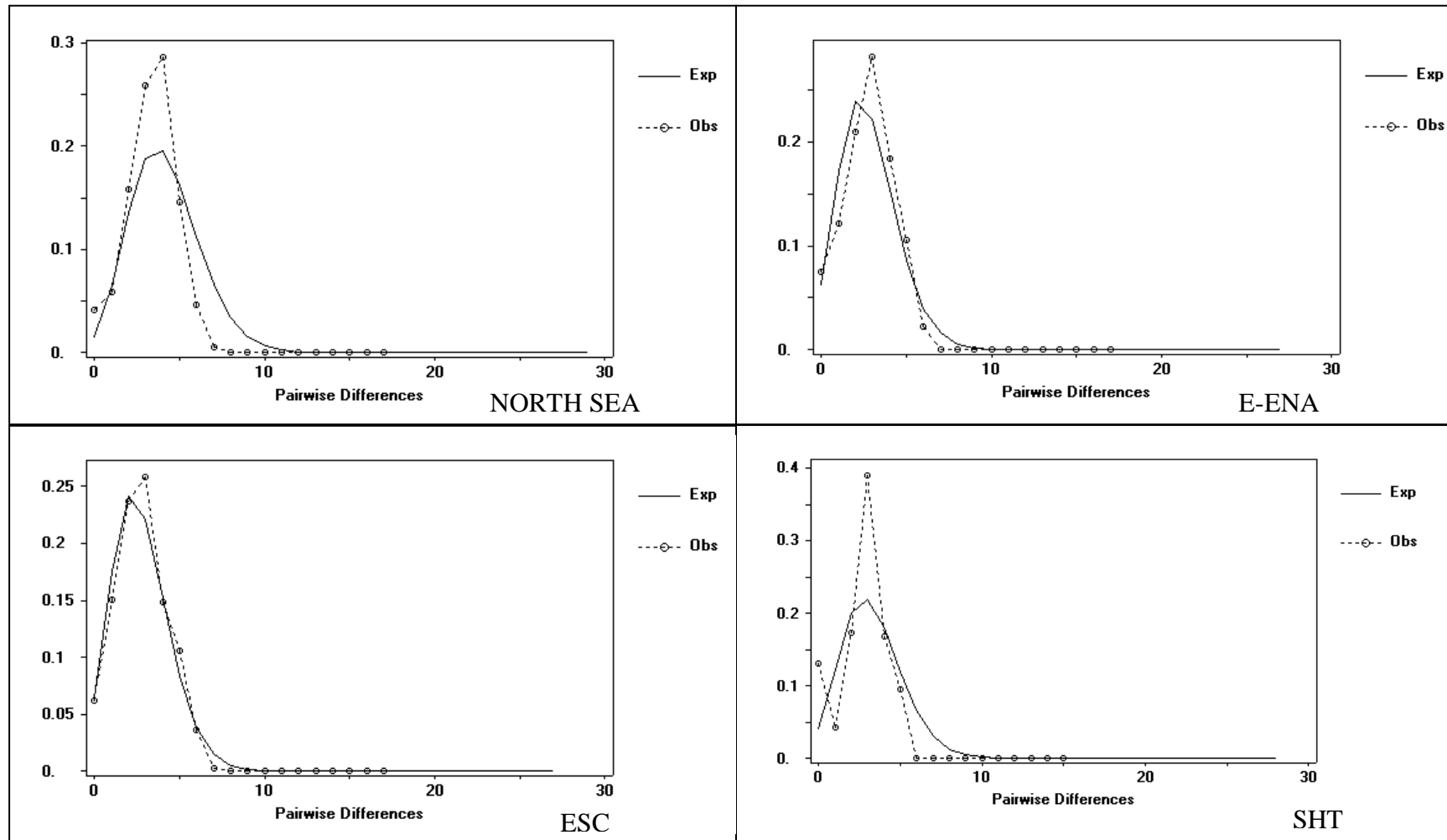


Figure 5. (Cont.). Mismatch distribution graphs for *L. acutus* populations, y axis shows the frequency of the pairwise comparisons

Table 5. Parameters of mismatch distribution for a model of sudden expansion in *L. acutus*, calculation of expansion time and Neutrality tests.

<i>Populations</i>	<i>NNA</i>	<i>WNAma</i>	<i>WNAmn</i>	<i>WNAanc</i>	<i>SNS</i>	<i>DMK</i>	<i>ESC</i>	<i>SHT</i>	<i>NWB</i>	<i>WRL</i>	<i>SGL</i>	<i>Mean</i>	<i>s.d.</i>
<i>Sample size</i>	52	29	33	26	15	14	31	20	35	80	9	31	20
<i>Statistics</i>													
τ	3.1934	3.6367	3.3965	4.0664	4.6406	3.9805	3.2637	3.3125	2.8125	3.4414	3.0293	3.5249	0.5252
τ 5%	1.5371	1.8008	1.7832	1.8789	3.0117	2.3906	1.9141	1.9023	1.3770	1.9492	1.4082	1.9048	0.4637
τ 95%	4.6172	5.0742	4.6426	6.3242	6.0586	5.3613	4.1231	4.4473	4.0273	4.3477	4.6250	4.8771	0.7553
<i>SSD</i>	0.0035	0.0111	0.0068	0.0152	0.0200	0.0053	0.0017	0.0453	0.0010	0.0021	0.0334	0.0132	0.0145
<i>SSD p-value</i>	0.5250	0.1600	0.2520	0.2490	0.1260	0.6210	0.6430	0.0190	0.8670	0.4600	0.2350	0.3779	0.2626
<i>Raggedness index</i>	0.0238	0.0370	0.0311	0.0418	0.0927	0.0326	0.0279	0.1352	0.0222	0.0217	0.1505	0.0560	0.0473
<i>Raggedness p-value</i>	0.6780	0.4280	0.5110	0.4380	0.1080	0.6850	0.6030	0.0220	0.8310	0.6610	0.2010	0.4696	0.2613
<i>*Expansion Time (T)</i>	9917	11294	10548	12629	14412	12362	10136	10287	8734	10688	9408	-	-

<i>Neutrality tests</i>													
Tajima's D	-1.1187	-0.4602	-1.3757	0.7147	-0.5444	-0.6423	-0.8929	0.2283	-0.6275	-1.5408	-0.6645	-0.6295	0.6539
Tajima's D p-value	0.0130	0.0544	0.0033	0.2489	0.0256	0.0222	0.0191	0.1437	0.0420	0.0021	0.0094	0.0531	0.0763
<i>Fu's FS test</i>													
FS	-11.9858	-5.6320	-9.0767	-0.8224	-5.9777	-7.2182	-9.0291	-1.1814	-9.5985	-	-4.5500	-8.2381	6.6944
FS p-value	0.00001	0.00001	0.00001	0.10950	0.00040	0.00001	0.00001	0.05990	0.00001	0.00001	0.00050	0.01548	0.03598

*Expansion time was calculated as ($t = \tau 2\mu$). $\mu = 5 \times 10^{-7}$ (Ho *et al.* 2007)

The expansion time in *L. acutus* populations, calculated from the parameter τ of the mismatch distribution (Table 5), ranged from ~14000 to ~9000 years ago, using a mutation rate of 5×10^7 (Ho *et al.* 2007). These estimates are consistent with a range expansion after the Last Glacial Maxima (LGM) in the Pleistocene (19000 – 14000 YBP; Pedersen 1983).

DISCUSSION

Genetic variability in white-sided dolphins and conservation implications

A total of 344 mitochondrial control DNA sequences were obtained from *L. acutus* throughout its geographic range. The mtDNA genetic diversity found in this species (overall 0.9270 ± 0.0070) was similar to values reported for other delphinids (e.g., Pichler and Baker 2000; Cassens *et al.* 2003; Harlin *et al.* 2003; Hayano *et al.* 2004; Natoli *et al.* 2006; Querouil *et al.* 2007). This haplotypic diversity is in contrast with the relatively low nucleotide diversity found in all the populations that were studied, ranging between 0.0087 ± 0.0052 and 0.0119 ± 0.0069 . Low nucleotide diversity has been reported for several cetacean populations worldwide (e.g. Bérubé *et al.* 1998; Pichler and Baker 2000; Parsons *et al.* 2002; Natoli *et al.* 2006), and has been correlated with factors such as the isolation of a population due to habitat loss and/or disturbance, depletion of population sizes due to overfishing, and other cases of anthropogenic disturbance, as well as with past climatic events (e.g., Pleistocene glaciations) that reduced and isolated populations in several regions, with a pronounced effect in the North Atlantic (see Wares 2002; Hewitt 2000, 2004).

In general a pattern of high genetic diversity and low nucleotide diversity is consistent with population expansion, which creates an excess of haplotypes differing by one or a few mutations (Rogers and Harpending 1992). A rapid expansion from refugial populations may have involved several bottlenecks with progressive loss of allelic diversity among populations in the postglacial colonized regions (see review in Hewitt 2000). Population expansion was corroborated by the Tajima's D statistics, the Fu's statistics, and the mismatch distribution analysis in most populations, indicating a reduction in

population sizes and a strong effect of the LGM in the DNA of this species (expansion time estimates ranged from 9,000 to 14,000 YBP).

It has been recognized that loss of genetic diversity can be the main cause of vulnerability or extinction of wild species (Lehman 1998). Extreme loss of genetic variation could be found in populations that are near to extinction or where population sizes have remained low over a long period of time (Amos 1996). However, after strong bottleneck events, the survival of populations may depend on non-genetic factors and their capacity for recovery in a post-bottleneck phase (e.g. Weber *et al.* 2004). In a pelagic species, such as *L. acutus*, negative effects of low genetic variability could be reduced by maintaining a dynamic genetic flow with other populations, which could be the case between WNA and W-ENA populations (see below). However, isolated populations of this species with reduced genetic diversity and low genetic interchange with oceanic populations, could have less chance to avoid the negative effects of inbreeding, which could have an important influence on population fitness and long term survival.

Population structure in *L. acutus*

- ***Western North Atlantic vs Eastern North Atlantic***

No differentiation was found between the westernmost part of the Eastern North Atlantic and the western North Atlantic: W-ENA vs WNA. These results partially agree with the results reported by Mikkelsen and Lund (1994) who found no craniometrical differences among samples on either side of the North Atlantic. The absence of differentiation between these regions could be explained by the large dispersal capacities in *L. acutus* suggested by the observation of large aggregations of individuals in the mid-Atlantic (e.g. Selzer and Payne 1988). This could prevent differentiation due to ongoing gene flow across populations, as has been suggested in other marine organisms with high dispersal capacities (Bremer *et al.* 2005; Ely *et al.* 2005).

In contrast to the absence of differentiation among Atlantic samples, some degree of differentiation was found between Atlantic populations (WNA, W-ENA and Northern North Atlantic) and putative populations in the easternmost part of the North Atlantic (Shetland Isles, Eastern Scotland and North

Sea) (see Tables 2, 3 and 4). Differentiation between E-ENA vs Northern North Atlantic and E-ENA vs WNA was supported by *F_{st}* and the exact test; whereas differentiation between E-ENA and W-ENA was only supported by *F_{st}*. Differentiation between North Sea (Denmark plus Southern North Sea) vs NNA and North Sea vs E-ENA was only supported by the exact test. When analyzing individual regions, both ϕ_{st} and *F_{st}* showed evidence of differentiation between Shetland and Northern North Atlantic (see Table 2); ϕ_{st} also showed differentiation between Shetland and NW British isles, but this differentiation was not supported by *F_{st}* statistics.

In spite of the differences in population structure indicated among the different statistics, perhaps due to power and sampling effects, it was evident that Eastern North Atlantic populations, especially in Eastern Scotland, show a complex pattern of differentiation as has also been suggested for other species in this region (i.e. *T. truncatus*, Parsons *et al.* 2002). This complex pattern could be explained by the differential effect of glaciated epochs in different regions of the Eastern North Atlantic. For example, it is well known that during the LGM the ice sheet covered most parts of the North Sea, with the exception of the southern North Sea (see Fig 1, in Siegert and Dowdeswell 2004), which could have been the only available habitat for dolphin species in this region. If this is true, then after the retreat of the ice, individuals inhabiting the southern North Sea could have migrated north, colonizing new areas such as East Scotland and Shetland Isles. This may be consistent with the apparent differentiation between *L. acutus* in this region from the rest of the North Atlantic samples, if it means that this region experienced a different demographic history. Although the Shetland sample on its own suggested a more stable population, increasing the sample size, incorporating other regional samples, eliminated that effect and instead suggested expansion as seen for other regions. The level of differentiation suggests that this could have been a separate expansion event, but further data from multiple markers will be required to help resolve this question.

In order to identify the main factors causing the possible isolation of the Shetland Isles, Eastern Scotland and possibly North Sea populations from other eastern regions, to test further hypotheses (such as the existence of offshore and near-shore populations in this species) and to promote effective conservation plans for these regions, a large samples size from some regions will be required (i.e. Shetland Isles and North Sea). In addition, it is necessary to integrate genetic studies with extensive comparative ecological

studies in several regions. These studies will provide the basis not only to understand the evolutionary forces, biotic and a-biotic factors driving the differentiation of populations, but also will give strong support for conservation plans in isolated and unique populations in the eastern North Atlantic.

Conclusions, Conservation Recommendations and Future Studies

This study has provided several insights into the genetic variability, demography history and population structure of *L. acutus*. First, it shows that populations from the easternmost part of the Eastern North Atlantic exhibit a different evolutionary history than populations in the westernmost part of this region. North Sea and Eastern Scotland populations (including Shetland Isles) are possibly isolated from other populations, with the most obvious differences found between Eastern Scotland and Northern North Atlantic populations.

Second, this research showed that the genetic variability of this species has been strongly affected by bottleneck events, perhaps taking place during the LGM. However, more recent events such as habitat loss and anthropogenic disturbance could not be ruled-out as a possible cause of loss of genetic diversity in populations of this species, but this should be addressed in further studies. The level of differentiation found between Shetland Isles and Northern North Atlantic, suggests that special attention should be paid to these two populations, especially to those around the Faroe Islands, given the substantial number of individuals that are killed in subsistence captures in that region each year (Reeves *et al.* 1999; Reeves and Leatherwood 1994). New analysis using bi-parental loci and other nuclear markers will help to clarify the vulnerability of this population.

Finally, our analyses show that conservation plans are particularly required for populations distributed in the easternmost part of the Eastern North Atlantic. However, effective conservation plans for white-sided dolphin, depend greatly upon our understanding of the demographic history of the species, its habitat preferences, and the factors affecting its genetic variability, distribution and population structure. Perhaps the major limitation for the conservation of this species is the absence of studies on habitat needs and basic population data, as well as the fragmentary knowledge of its ecology and behavior, which prevent a fuller interpretation of the genetic findings.

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***GENETIC VARIABILITY AND POPULATION STRUCTURE OF
White-sided dolphin (*Lagenorhynchus acutus*, Gray 1828)
IN THE NORTH ATLANTIC***

FINAL REPORT (ANNEX 1)

***United Nations Environmental Programme/Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas
(UNEP/ASCOBANS)***

April 2010

Eulalia Banguera-Hinestroza

Co-authors

***P. Evans, L. Mirimim, R.J. Reid, B. Mikkelsen, B. Couperus, R. Deaville,
E. Rogan, and A.R. Hoelzel.***

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
<i>Nothern North Atlantic</i>				
Natural History Museum Faroe Islands, Barne Mikkelsen	463/02	NNA	Faroe Islands	Gøta
	315/02	NNA	Faroe Islands	Hvalvík
	456/02	NNA	Faroe Islands	Gøta
	459/02	NNA	Faroe Islands	Gøta
	330/02	NNA	Faroe Islands	Hvalvík
	334/02	NNA	Faroe Islands	Hvalvík
	457/02	NNA	Faroe Islands	Gøta
	471/02	NNA	Faroe Islands	Gøta
	329/02	NNA	Faroe Islands	Hvalvík
	316/02	NNA	Faroe Islands	Hvalvík
	313/02	NNA	Faroe Islands	Hvalvík
	327/02	NNA	Faroe Islands	Hvalvík
	609/03	NNA	Faroe Islands	Hvalvík
	393/03	NNA	Faroe Islands	Tórshavn
	099/03	NNA	Faroe Islands	N/A
	394/03	NNA	Faroe Islands	Tórshavn
	658/03	NNA	Faroe Islands	Hvalvík
	671/03	NNA	Faroe Islands	Hvalvík
	664/03	NNA	Faroe Islands	Hvalvík
	663/03	NNA	Faroe Islands	Hvalvík
	392/03	NNA	Faroe Islands	Tórshavn
	605/03	NNA	Faroe Islands	Hvalvík
	602/03	NNA	Faroe Islands	Hvalvík
	667/03	NNA	Faroe Islands	Hvalvík
	606/03	NNA	Faroe Islands	Hvalvík
	464/02	NNA	Faroe Islands	Gøta
	320/02	NNA	Faroe Islands	Hvalvík

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
	307/02	NNA	Faroe Islands	Hvalvík
	361/02	NNA	Faroe Islands	Hvalba
	325/02	NNA	Faroe Islands	Hvalvík
	333/02	NNA	Faroe Islands	Hvalvík
	304/02	NNA	Faroe Islands	Hvalvík
	460/02	NNA	Faroe Islands	Gøta
	305/02	NNA	Faroe Islands	Hvalvík
	323/02	NNA	Faroe Islands	Hvalvík
	311/02	NNA	Faroe Islands	Hvalvík
	318/02	NNA	Faroe Islands	Hvalvík
	308/02	NNA	Faroe Islands	Hvalvík
	306/02	NNA	Faroe Islands	Hvalvík
	332/02	NNA	Faroe Islands	Hvalvík
	465/02	NNA	Faroe Islands	Gøta
	452/02	NNA	Faroe Islands	Gøta
	363/02	NNA	Faroe Islands	Hvalba
	331/02	NNA	Faroe Islands	Hvalvík
	491/02	NNA	Faroe Islands	Gøta
	328/02	NNA	Faroe Islands	Hvalvík
	467/02	NNA	Faroe Islands	Gøta
	310/02	NNA	Faroe Islands	Hvalvík
	473/02	NNA	Faroe Islands	Gøta
	322/02	NNA	Faroe Islands	Hvalvík
Natural History Museum of Denmark, Mogens Andersen	CN6	NNA	Faroe Islands	N/A
	CN8	NNA	Faroe Islands	N/A
	CN3	NNA	Greenland	N/A
Maritime Institute in Iceland, Gisli Vikingsson and Sverrir D. Halldorsson	50828	NNA	Iceland	Innri-Njarðvík
	50909	NNA	Iceland	Bakkafjara
	51001	NNA	Iceland	Breiðdalsvík

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
	50724	NNA	Iceland	Berufjarðarströnd
NW British Isles	M124/04	NWBI	Western Isles	Carmanish Islands, Harris
Scotish Stranding Network	M069/02	NWBI	Western Isles	Rubha Ardvule, S. Uist
Robert Reid	M020/00	NWBI	Western Isles	Culla Bay, Benbecula
	M0570/99**	NWBI	Western Isles	Stoneybridge, South Uist
	M0572/99	NWBI	Western Isles	Traigh Mhor, Barra
	M027/00	NWBI	Western Isles	Kilpheder, South Uist
	M158/03	NWBI	Western Isles	Bornish, South Uist
	M181/03	NWBI	Western Isles	Daliburgh, S. Uist
	M147/05	NWBI	Western Isles	Newton Ferry, N. Uist
	M203/05	NWBI	Western Isles	Traigh Chuil, Lewis
	M121/06	NWBI	Western Isles	North Boisdale, S. Uist
	M122/06	NWBI	Western Isles	Askernish, S. Uist
	M285/06	NWBI	Western Isles	Borve, Benbecula
	M085/07	NWBI	Western Isles	Aird A' Mhorain, N. Uist
	M011/09	NWBI	Western Isles	Paible, North Uist
	M2408/95	NWBI	Western Isles	Borve Beach, Harris
	M2438/95	NWBI	Western Isles	Nr Aiginis, Lewis
	M0243/93	NWBI	Western Isles	Eochar
	M2117/93	NWBI	Western Isles	Sollas, North Uist
	M1063/97	NWBI	Western Isles	Knockline, North Uist
	M1265/97	NWBI	Western Isles	Stornoway Harbour
	M2788/97	NWBI	Western Isles	Usinish, South Uist
	M1068/98	NWBI	Western Isles	Knockline, N. Uist
	M2055/98	NWBI	Western Isles	Vallary, North Uist
	M2693/98	NWBI	Western Isles	Lochnam Ban, Newton
	M1349/96	NWBI	Western Isles	Frobost, S. Uist
	M0481/97	NWBI	Western Isles	Ormicate, South Uist
	M0575/97	NWBI	Western Isles	Knockintorran, N. Uist

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IMARES, Dr. Bram Couperus	M100/07	NWBI	Strathclyde	Knock, Isle Of Mull
	M635/02	NWBI	Strathclyde	Loch Crinan
	M194/07	NWBI	Strathclyde	Killunaig, Isle Of Mull
	M0089/96	NWBI	Strathclyde	Loch Beg, Isle Of Mull
	M171/07	NWBI	Highland	Gilleann Bay, Isle Of Skye
	M260/03	NWBI	Highland	Dunnet Bay
	M0181/97	NWBI	Highland	Kyle Of Durness
	M0675/97	NWBI	Highland	Mallaig
	M225/08	NWBI	Highland	Ullapool
	M0395/96	NWBI	Highland	East Suisnish, Raasay
	M175/07	NWBI	Dumfries & Galloway	Nr Leffnoll Pt, Stranraer
	LAC 981203-1	NWBI	North Atlantic	57.20N -9.19
	LAC 981203-2	NWBI	North Atlantic	57.20N -9.19
Natural History Museum London, Richard Sabin	SW.1984.22	NWBI	Republic of Ireland	Rathlee, County Sligo, Republic of Ireland
	SW.1916.9	NWBI	Northern Ireland	Portstewart, Londonderry, Northern Ireland.
<i>East Scotland (plus NE England)</i>	M0997/94A	ESC	Orkney	Scapa Beach
Scotish Stranding Network, Robert Reid	M2180/96	ESC	Orkney	Ayre Sound, Sanday
	M2270/96B	ESC	Orkney	South Burray
	M2270/96A	ESC	Orkney	South Burray
	M2270/96C	ESC	Orkney	South Burray
	M2769/97	ESC	Orkney	Points Of The Baits, Scapa
	M205/08	ESC	Orkney	Weyland Bay, Kirkwall
	M074/00	ESC	Orkney	Hellier Holm, Shapinsay
	M1591/98	ESC	Orkney	Newark, Deerness
	M1229/92 or 98?	ESC	Fife	St Andrews
	M0676/94	ESC	Highland	North Kessock
	M2268/94	ESC	Strathclyde	Glen Forsa Bay, Mull
	M2117/97	ESC	Highland	West Beach, Lossiemouth

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
	M2154/97	ESC	Highland	Ardersier
	M2900/97	ESC	Highland	S. Kessock, Inverness
	M0070/98	ESC	Highland	Kiltearn, Evanton
	M1696/98	ESC	Highland	Balnaglack, Ardersier
	M193/00C	ESC	Highland	Brora
	M193/00F	ESC	Highland	Brora
	M193/00B	ESC	Highland	Brora
	M193/00E	ESC	Highland	Brora
	M193/00A	ESC	Highland	Brora
	M193/00D	ESC	Highland	Brora
	M052/01	ESC	Highland	Near Evanton
	M169/04	ESC	Highland	Brora
	M267/05	ESC	Grampian	Burghead
	M066/06	ESC	Grampian	Crovie
	M042/07	ESC	Lothian	Nr Silverknowes
	M057/07	ESC	Fife	West Wemyss
	M222/07	ESC	Highland	Clachnaharry, Inverness
Natural History Museum London, Richard Sabin	SW1990/114	ESC	Northumberland	Beadnell
	ZD.1996.309	ESC	Northumberland	Bamburgh, (South of Castle) Northumberland.
	SW1993/32	ESC	Cleveland	Redcar
Shetland Isles	M002/00	SHT	Shetland Isles	Catfirth Voe
Scotish Stranding Network, Robert Reid	M175/08	SHT	Shetland Isles	Lerwick Marina
	M2821/98	SHT	Shetland Isles	Sand
	M0854/92	SHT	Shetland Isles	Grunna Voe
	M130/00	SHT	Shetland Isles	Otters Wick, Yell
	M187/00	SHT	Shetland Isles	Baltasound, Unst
	M092/01B	SHT	Shetland Isles	Maryfield, Bressay
	M092/01A	SHT	Shetland Isles	Maryfield, Bressay

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
	M126/01	SHT	Shetland Isles	Uyea Sound, Unst
	M261/03B	SHT	Shetland Isles	Weisdale Voe, Mainland
	M261/03A	SHT	Shetland Isles	Weisdale Voe, Mainland
	M261/03C	SHT	Shetland Isles	Weisdale Voe, Mainland
	M293/03B	SHT	Shetland Isles	Weisdale Voe, Mainland
	M293/03A	SHT	Shetland Isles	Weisdale Voe, Mainland
	M304/03	SHT	Shetland Isles	Weisdale Voe
	M098/04	SHT	Shetland Isles	Sullom Voe
	M133/07A	SHT	Shetland Isles	Weisdale Voe
	M061/05	SHT	Shetland Isles	Sand Voe, Mainland
	M105/06	SHT	Shetland Isles	Urafirth, Mainland
	M158/06	SHT	Shetland Isles	Dury Voe
	M0989/97	SHT	Shetland Isles	Whiteness Voe
	M286/06	SHT	Shetland Isles	West Voe, Mainland
	M052/07	SHT	Shetland Isles	Weisdale Voe
Denmark	C260	DMK	Denmark	Vrist
Fisheries and Maritime Museum Denmark, Lasse Fast Jensen	C253	DMK	Denmark	Sidselbjerg strand
	C273	DMK	Denmark	Vrist
	C88	DMK	Denmark	Harboøre
	C248	DMK	Denmark	Husby Klit
	C267	DMK	Denmark	Bøvling klit
Museum of Natural History Denmark, Mogens Andersen	M1087	DMK	Denmark	Between UK and Denmark
	CN23	DMK	Denmark	Krogstrup, near Roskilde Fjord, Zealand; 28.
	CN1297	DMK	Denmark	Roskilde Fjord, Zealand; 1.
	CN895	DMK	Denmark	Klitmoeller, Northern Jutland; 14.

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Swedish Museum of Natural History (SMNH), Peter Nilsson Department of Contaminant Research, Stockholm. Anna Roos	CN19	DMK	Denmark	Frederikssund, Roskilde Fjord, Zealand; 27.
	CN20	DMK	Denmark	Frederikssund, Roskilde Fjord, Zealand; 27.
	CN21	DMK	Denmark	Frederikssund, Roskilde Fjord, Zealand; 27.
	NMR 20095047	DMK	Sweden	Bohuslän, Kungälv, Torsby, Vedhall
	NMR 20085142	DMK	Sweden	56°32'N 12°57'E
	C2005/0537/MG**	DMK	Sweden	Halland, Nom Påarp, Trönninge strand
	C2005/05359/MG**	DMK	Sweden	Halland, Mellbystrand, på stranden, strax S om Lagoaset
South England	SW2002/322	SGL	Ceredigion	Cei Bach
England Stranding Network, Rob Deaville and Paul Jepson	SW2003/90	SGL	Cornwall	Peters Point
	SW2008/123	SGL	Cornwall	Upton Towans
	SW1991/9	SGL	Devon	Saunton Sands
	SW1994/5	SGL	West Glamorgan	Rhossili Beach
	SW1994/54	SGL	Cornwall	Polzeath Beach
Natural History Museum London, Richard Sabin	SW.1928.19	SGL	Porthleven Harbour, Cornwall, England.	
	SW2002/210	SGL	East Sussex	Newhaven Harbour
Wales Stranding network, Rod Penrose	SW2009/406.	SGL	Welsh Coast	Criccieth
Southern North Sea	SW.1981.20	SNS	British Isles	Skegness, Lincolnshire, England.
Natural History Museum London, Richard Sabin	SW.1981.25	SNS	British Isles	Whitby, Yorkshire, England.
	SW.1978.16	SNS	British Isles	Spurn Head, River Humber, Humberside, England.

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Museum of Natural History Leiden, Steven van der Mije	SW.1978.37	SNS	British Isles	Short Sands, Staithes, North Yorkshire, England.
	C.1917.9.5.1	SNS	British Isles	Sutton Bridge, between Kings Lynn and Boston, Norfolk, England. TF 47 85 29 80
	RMNH 41473	SNS	The Netherlands	Balg, Schiermonnikoog
	RMNH 32072	SNS	The Netherlands	Zandvoort, paal 69.5000
	RMNH 34635	SNS	The Netherlands	Spuisluis Lauwersoog, Friesland, Nederland
	RMNH 35581	SNS	The Netherlands	Sint Maartenszee
	RMNH 23587	SNS	The Netherlands	Breskens
England Stranding Network, Rob Deaville and Paul Jepson	RMNH 43472 (SKIN)	SNS	The Netherlands	Castricum
	SW2005/212	SNS	Norfolk	Heacham Dam
	SW2006/277	SNS	Norfolk	Brancaster
	SW2007/83	SNS	Kent	Isle of Sheppey
	SW1994/37	SNS	Humberside	Bridlington
	SW1998/90	SNS	North Yorkshire	Scarborough
Western Ireland	NMR999000001999	WRL	West Ireland	51.20, 11.02
Museum of Natural History Rotterdam, Kees Moeliker and Henry van der Es and IMARES, Bram Couperus	NMR999000001998	WRL	West Ireland	52.19, 11.39
	NMR999000001997	WRL	West Ireland	N/A
	NMR999000001996	WRL	West Ireland	52.19 11.39
	NMR999000001995	WRL	West Ireland	51.45, 11.27
	NMR999000001993	WRL	West Ireland	53.11, 11.47
	NMR999000001489	WRL	West Ireland	51.20, 11.02
	NMR999000001496	WRL	West Ireland	50.56, 11.06

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
Museum of Natural History Leiden, Steven van der Mije	NMR999000000411	WRL	West Ireland	51.20, 11.02
	NMR999000000412	WRL	West Ireland	51°42N-11°29W*
	NMR999000000413	WRL	West Ireland	51°08N-11°34W*
	NMR999000000414	WRL	West Ireland	51°08N-11°34W*
	NMR999000000415	WRL	West Ireland	51.45, 11.27
	NMR999000000416	WRL	West Ireland	51.45, 11.27
	NMR999000000419	WRL	West Ireland	51.20, 11.02
	NMR999000001242	WRL	West Ireland	N/A
	NMR999000001243	WRL	West Ireland	54.10, -11.15
	NMR999000002658	WRL	West Ireland	51.24, -11.24
	NMR999000002660	WRL	West Ireland	51.24, -11.24
	NMR999000001994	WRL	West Ireland	52.10, -11.37
	RMNH 38331	WRL	West Ireland	N/A
	RMNH 38332	WRL	West Ireland	N/A
	RMNH 40040	WRL	West Ireland	N/A
	RMNH 40062	WRL	West Ireland	N/A
	RMNH 40062L (D)	WRL	West Ireland	N/A
	RMNH 40063	WRL	West Ireland	N/A
	RMNH 40064	WRL	West Ireland	N/A
	RMNH 40065	WRL	West Ireland	N/A
	RMNH 40066	WRL	West Ireland	N/A
	RMNH 40067	WRL	West Ireland	N/A
	RMNH 40068	WRL	West Ireland	N/A
	RMNH 40072A	WRL	West Ireland	N/A
	RMNH 40072B	WRL	West Ireland	N/A
	RMNH 40073A	WRL	West Ireland	N/A
	RMNH 40073B	WRL	West Ireland	N/A
	RMNH 40078	WRL	West Ireland	N/A
	RMNH 40061	WRL	West Ireland	N/A

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IMARES Bram Couperus	LAC 940224	WRL	West Ireland	52.09, 11.45
	LAC 941021 (D)	WRL	West Ireland	53.11, 11.47
	LAC 950303	WRL	West Ireland	53.28, 11.33
	LAC 950306 (D)	WRL	West Ireland	52.19, 11.39
	LAC 950322-1 (D)	WRL	West Ireland	50.56, 11.06
	LAC 950322-2	WRL	West Ireland	50.56, 11.06
	LAC 950322-3	WRL	West Ireland	50.56, 11.06
	LAC 950925-1 (D)	WRL	West Ireland	51.45, 11.27
	LAC 950925-2	WRL	West Ireland	51.45, 11.27
	LAC 950925-3	WRL	West Ireland	51.45, 11.27
	LAC 951006-1 (D)	WRL	West Ireland	51.45, 11.27
	LAC 951006-2	WRL	West Ireland	51.45, 11.27
	LAC 961018-1 (D)	WRL	West Ireland	51.20, 11.02
	LAC 961018-2	WRL	West Ireland	51.24, 11.24
	LAC 961018-3 (D)	WRL	West Ireland	51.20, 11.02
	LAC 961024-1 (D)	WRL	West Ireland	51.20, 11.02
	LAC 961024-2 (D)	WRL	West Ireland	51.20, 11.02
	LAC 961024-3 (D)	WRL	West Ireland	51.24, 11.24
	LAC 970327-3	WRL	West Ireland	52.19, 11.39
	LAC 970327-5 (D)	WRL	West Ireland	52.19, 11.39
	(LAC) 6200002**	WRL	West Ireland	50.11, 10.56
Marine Research Institute in Ireland, Emer Rogan and Luca Mirimim	Ross 1	WRL	West Ireland	Killala Bay, County Mayo
	Ross 2	WRL	West Ireland	Killala Bay, County Mayo
	Ross 3	WRL	West Ireland	Killala Bay, County Mayo
	Ross 4	WRL	West Ireland	Killala Bay, County Mayo
	Ross 5	WRL	West Ireland	Killala Bay, County Mayo
	Ross 6	WRL	West Ireland	Killala Bay, County Mayo
	Ross 7	WRL	West Ireland	Killala Bay, County Mayo
	Ross 8	WRL	West Ireland	Killala Bay, County Mayo

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	Ross 9	WRL	West Ireland	Killala Bay, County Mayo
	Ross 10	WRL	West Ireland	Killala Bay, County Mayo
	Ross 11	WRL	West Ireland	Killala Bay, County Mayo
	Ross 12	WRL	West Ireland	Killala Bay, County Mayo
	Ross 13	WRL	West Ireland	Killala Bay, County Mayo
	Ross 14	WRL	West Ireland	Killala Bay, County Mayo
	Ross 15	WRL	West Ireland	Killala Bay, County Mayo
	Ross 16	WRL	West Ireland	Killala Bay, County Mayo
	Ross 17	WRL	West Ireland	Killala Bay, County Mayo
	Ross 18	WRL	West Ireland	Killala Bay, County Mayo
	Ross 19	WRL	West Ireland	Killala Bay, County Mayo
	Claggan 1	WRL	West Ireland	Clew Bay, Co. Mayo
	Claggan 2	WRL	West Ireland	Clew Bay, Co. Mayo
	Claggan 3	WRL	West Ireland	Clew Bay, Co. Mayo
	Claggan 4	WRL	West Ireland	Clew Bay, Co. Mayo
	Claggan 5	WRL	West Ireland	Clew Bay, Co. Mayo
	WSD 8	WRL	West Ireland	N/A
	WSD 10	WRL	West Ireland	N/A
	WSD 15	WRL	West Ireland	N/A
	WSD 16	WRL	West Ireland	N/A
	WSD 1/94	WRL	West Ireland	N/A
	WSD 3/95	WRL	West Ireland	N/A
	WSD T3H4 ⁺	WRL	West Ireland	N/A
	WSD 1/96	WRL	West Ireland	N/A
	WSD 1/97	WRL	West Ireland	N/A
	WSD 2/97	WRL	West Ireland	N/A
	WSD 1/98	WRL	West Ireland	N/A
	WSD 2/98	WRL	West Ireland	N/A
	WSD 3/98	WRL	West Ireland	N/A
	WSD 1/99	WRL	West Ireland	N/A

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	WSD 1/02	WRL	West Ireland	N/A
	WSD 2/02	WRL	West Ireland	N/A
	WSD 3/02	WRL	West Ireland	N/A
	WSD 1/05	WRL	West Ireland	N/A
	WSD 2/05	WRL	West Ireland	N/A
Western North Atlantic (USA coast)	7820	WNama	USA	41°35'N, 68°29'W
National Oceanic and Atmospheric Administration (NOAA), USA. Kelly Robertson	7821**	WNama	USA	41°35'N, 68°29'W
	7822	WNama	USA	41°35'N, 68°29'W
	7823	WNama	USA	41°35'N, 68°29'W
	7824	WNama	USA	41°35'N, 68°29'W
	7825	WNama	USA	41°35'N, 68°29'W
	7826	WNama	USA	41°35'N, 68°29'W
	7827	WNama	USA	41°35'N, 68°29'W
	7828	WNama	USA	41°14'N, 68°43'W
	7829	WNama	USA	41°14'N, 68°43'W
	7830	WNama	USA	41°14'N, 68°43'W
	7831	WNama	USA	41°14'N, 68°43'W
	7836	WNama	USA	41°30'N, 68°43'W
	7838	WNama	USA	42°09'N, 70°14'W
	7839	WNama	USA	42°09'N, 70°14'W
	7840	WNama	USA	42°09'N, 70°14'W
	7841	WNama	USA	42°09'N, 70°14'W
	7842	WNama	USA	42°09'N, 70°14'W
	7843	WNama	USA	42°09'N, 70°14'W
Smithsonian Natural History Museum Charlie Potter	LAC 484923	WNama	USA	Massachusetts-Barnstable
	LAC 484920	WNama	USA	Massachusetts-Barnstable
	LAC 484921	WNama	USA	Massachusetts-Barnstable
	LAC 484919	WNama	USA	Massachusetts-Barnstable
	LAC 484922	WNama	USA	Massachusetts-Barnstable

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
	LAC 484917	WNAma	USA	Massachusetts-Barnstable
	LAC 550469	WNAma	USA	Massachusetts-Barnstable
	LAC 504082	WNAma	USA	Massachusetts-Barnstable
	LAC 504308	WNAma	USA	Massachusetts-Barnstable
	LAC 504199	WNAma	USA	Massachusetts-Barnstable
	LAC 504307	WNAma	USA	Massachusetts-Barnstable
	LAC 504292	WNAma	USA	Massachusetts-Barnstable
	15227	WNAma	Canada East Coast	
<i>Western North Atlantic (USA coast)</i>	LAC 504173	WNAmn	USA	Maine, Cobscook Bay
Smithsonian Natural History Museum Charlie Potter	LAC 504164	WNAmn	USA	Maine, Cobscook Bay
	LAC 504163	WNAmn	USA	Maine, Cobscook Bay
	LAC 504194	WNAmn	USA	Maine, Cobscook Bay
	LAC 504165	WNAmn	USA	Maine, Cobscook Bay
	LAC 504168	WNAmn	USA	Maine, Cobscook Bay
	LAC 504170	WNAmn	USA	Maine, Cobscook Bay
	LAC 504171	WNAmn	USA	Maine, Cobscook Bay
	LAC 504166	WNAmn	USA	Maine, Cobscook Bay
	LAC 504167	WNAmn	USA	Maine, Cobscook Bay
	LAC 504169	WNAmn	USA	Maine, Cobscook Bay
	LAC 504162	WNAmn	USA	Maine, Cobscook Bay
	LAC 504156	WNAmn	USA	Maine, Cobscook Bay
	LAC 504159	WNAmn	USA	Maine, Cobscook Bay
	LAC 504161	WNAmn	USA	Maine, Cobscook Bay
	LAC 504152	WNAmn	USA	Maine, Cobscook Bay
	LAC 504183	WNAmn	USA	Maine, Cobscook Bay
	LAC 504191	WNAmn	USA	Maine, Cobscook Bay
	LAC 504189	WNAmn	USA	Maine, Cobscook Bay
	LAC 504185	WNAmn	USA	Maine, Cobscook Bay
	LAC 504188	WNAmn	USA	Maine, Cobscook Bay

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	LAC 504187	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504172	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504197	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504151	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504149	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504154	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504158	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504157	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC ?4155	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504196	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504178	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504198	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504176	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504179	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504180	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504174	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504193	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504175	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 504182	WNA ^{mn}	USA	Maine, Cobscook Bay
	LAC 572654	WNA	USA	North Caroline
	LAC 572442	WNA	USA	North Caroline
	LAC 572160	WNA	USA	North Caroline
	LAC 572517	WNA	USA	Virginia, Northampton Wreck Island
	LAC 571390	WNA	USA	34° 54'N 72° 26'W
	LAC 571387	WNA	USA	Virginia
	LAC 571327	WNA	USA	39° 55' N 72° 52' W
	LAC 571347	WNA	USA	39° 29' N 72° 51' W
	LAC 571346	WNA	USA	39° 08N 73° 01' W
	LAC 571329	WNA	USA	39° 25' N 72° 52'W

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	LAC 572216	WNA	USA	Virginia
	LAC 550326	WNA	USA	
	LAC 504763	WNA	USA	Virginia
	LAC 550996	WNA	USA	Virginia
	LAC 572468	WNA	USA	New Jersey
	LAC 571987	WNA	USA	Maryland
<i>Western North Atlantic (USA coast)"ancient"</i>	LAC 14232	WNAanc	USA	Massachusetts-Barnstable-Cape cod
Smithsonian Natural History Museum	LAC 14245	WNAanc	USA	Massachusetts-Barnstable-Cape cod
Charlie Potter	LAC 14236	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14234	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14269	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14260	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14250	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14276	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14278	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14275	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14268	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14243	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14229	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14256	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14241	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14270	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14240	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14258	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14254	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14247	WNAanc	USA	Massachusetts-Barnstable-Cape cod

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	LAC 14249	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14233	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14262	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14267	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14259	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14274	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14280	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14230	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14263	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14265	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14257	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 14279	WNAanc	USA	Massachusetts-Barnstable-Cape cod
	LAC 11752		Canada	Nunavot, Canada, Repulse Bay
	LAC 571447		Unknown	Unknown
	LAC 571353		Unknown	Unknown
	LAC 550880		Unknown	Unknown
	LAC 571409		Unknown	Unknown
	LAC 572384		Unknown	Unknown
	LAC 571391		Unknown	Unknown
	LAC 571389		Unknown	Unknown
	LAC 572385		Unknown	Unknown
	LAC 572387		Unknown	Unknown
	LAC 571405		Unknown	Unknown
	LAC 572389		Unknown	Unknown
	LAC 571402		Unknown	Unknown
	LAC 571395		Unknown	Unknown

<u>MUSEUMS AND/OR INSTITUTIONS, CONTACT NAME</u>	<u>INSTITUTION CATALOGUE NUMBER</u>	<u>POPULATION</u>	<u>REGION/ COUNTRY/ OR OCEAN</u>	<u>LOCALITY AND/OR COORDINATES</u>
	LAC 571328		Unknown	Unknown
	LAC 571342		Unknown	Unknown
	LAC 572514		Unknown	Unknown
	LAC 572887		Unknown	Unknown
	LAC 572856		Unknown	Unknown
	LAC 572876		Unknown	Unknown
	LAC 572388		Unknown	Unknown
	LAC 572903		Unknown	Unknown

Annex I

List of the total number of samples obtained for this study, catalogue numbers from each museum and localities.

Samples labelled with one asterisk indicated that perhaps this coordinates should be read in decimal degrees. Samples that were identified as a different species, are labelled with double asterisk. Samples with (D) are duplicated samples from Rotterdam Museum and IMARES.

Note: Not all samples listed in this annex were included in the analysis due to different factors, such as the low quality of DNA for some samples, samples from unknown origin or very old collection dates.