MOLECULAR ECOLOGY

Molecular Ecology (2014) **23**, 857–874 doi: 10.1111/mec.12653

Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic

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Abstract

Despite no obvious barrier to gene flow, historical environmental processes and ecological specializations can lead to genetic differentiation in highly mobile animals. Ecotypes emerged in several large mammal species as a result of niche specializations and/or social organization. In the North-West Atlantic, two distinct bottlenose dolphin (*Tursiops truncatus*) ecotypes (i.e. 'coastal' and 'pelagic') have been identified. Here, we investigated the genetic population structure of North-East Atlantic (NEA) bottlenose dolphins on a large scale through the analysis of 381 biopsy-sampled or stranded animals using 25 microsatellites and a 682-bp portion of the mitochondrial control region. We shed light on the likely origin of stranded animals using a carcass drift prediction model. We showed, for the first time, that coastal and pelagic bottlenose dolphins were highly differentiated in the NEA. Finer-scale population structure was found within the two groups. We suggest that distinct founding events followed by parallel adaptation may have occurred independently from a large Atlantic pelagic population in the two sides of the basin. Divergence could be maintained by philopatry possibly as a result of foraging specializations and social organization. As coastal environments are under increasing anthropogenic pressures, small and isolated populations might be at risk and require appropriate conservation policies to preserve their habitats. While genetics can be a powerful first step to delineate ecotypes in protected and difficult to

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access taxa, ecotype distinction should be further documented through diet studies and the examination of cranial skull features associated with feeding.

Keywords: cetaceans, conservation, ecotypes, feeding specializations, philopatry, population genetics

Received 27 September 2013; revision received 30 November 2013; accepted 18 December 2013

Introduction

Despite no obvious physical barrier to gene flow and high movement capacities, intraspecific population differentiation in vertebrates can be high at large and small spatial scales (e.g. Natoli *et al.* 2004; Hoffman *et al.* 2005; Sacks *et al.* 2005). Environmental factors, in particular habitat characteristics and past climate changes, have been correlated with population divergence in fishes and mammals (e.g. Bernatchez 1997; Gaggiotti *et al.* 2009; Amaral *et al.* 2012b). The degree of connectivity between populations can also be influenced by an interaction between ecological conditions and behavioural traits. In fishes, natal homing (i.e. site fidelity to natal breeding ground) is suggested as an important factor shaping genetic differentiation among populations through local adaptation to a particular habitat that confers better fitness (e.g. Kawecki & Ebert 2004; Dionne *et al.* 2008). Similarly, despite high mobility, terrestrial carnivores (e.g. wolves and coyotes) can show cryptic population structure linked to individual preferential dispersal towards similar natal area habitats where they will find familiar prey resources (Sacks *et al.* 2005; Pilot *et al.* 2012). Resource specializations may also explain genetic differentiation of killer whales in the Pacific between sympatric fish and marine mammal eating ecotypes (Hoelzel *et al.* 1998a), and in the North-East Atlantic (NEA) among different fish eating populations (Foote *et al.* 2011). Social cohesion and learning of foraging techniques within the matrilineal pod is likely to promote philopatry (Hoelzel *et al.* 1998a).

Niche specializations between genetically different groups of individuals can result in the classification of ecotypes. The term 'ecotype' was first defined in plants following common garden experiments (Turesson 1922a,b) and corresponded to ecological units that arise from genotypical responses to particular habitats. Groups of individuals in distinct environments can become differentiated, resulting in different ecotypes, if heritable variation is sufficient for natural selection to take place and if local adaptation is stronger than gene flow between groups (Begon *et al.* 2006). Since its first appearance, the definition of an ecotype has been controversial (see review in Lowry 2012). We used Lowry's (2012) ecotype definition in this study, that is, groups of populations, which differ across the landscape by genetics (e.g. allele frequencies differences) and ecological and/or physiological traits. Ecotype differentiation can be confirmed using common garden experiments for small animals like Dominican anoles (Thorpe *et al.* 2005). However, for large, highly mobile mammals, these experiments would be impractical and ethically controversial. Molecular, ecological, distribution and behavioural studies are therefore needed. Killer whales in the North-East Pacific were classified into three ecotypes (resident, transient and offshore) from an in-depth knowledge of foraging behaviour, genetics, ranging patterns and morphology (see review in de Bruyn *et al.* 2013). Coastal and pelagic bottlenose dolphin, *Tursiops truncatus*, ecotypes were distinguished through genetics, distribution, diet and skull morphology in the North-West Atlantic (NWA) (Mead & Potter 1995; Hoelzel *et al.* 1998b) and in the Pacific (Walker 1981; Curry & Smith 1998; Perrin *et al.* 2011). The two bottlenose dolphin ecotypes form separate mitochondrial lineages in the NWA, with less genetic diversity in coastal populations. The situation is more complex in the Pacific Ocean and the North-East Atlantic (NEA) (Natoli *et al.* 2004; Tezanos-Pinto *et al.* 2009). In the Pacific, mitochondrial DNA (mtDNA) genetic differentiation between coastal and pelagic bottlenose dolphins is significant, but there is no complete lineage sorting (Segura *et al.* 2006). Tezanos-Pinto *et al.* (2009) suggested that ecotype differentiation in the NWA may not be representative of genetic structuring of bottlenose dolphins worldwide.

In the NEA, bottlenose dolphins are found in coastal waters where they form either discrete small resident groups of tens to hundreds of individuals (e.g. Berrow *et al.* 2012; Cheney *et al.* 2012) or more mobile groups (O'Brien *et al.* 2009). They are transient and/or resident in deep waters near offshore islands (Silva *et al.* 2008), the Gibraltar Strait (de Stephanis *et al.* 2008) and pelagic waters, in particular the shelf-edge of the Bay of Biscay and Celtic Sea with abundance estimates of thousands of individuals (Hammond *et al.* 2009, 2013). In the Mediterranean Sea, resident populations and mobile individuals were also reported (e.g. Gnone *et al.* 2011). There is a distributional hiatus in the NEA, that is, resident coastal populations are mainly observed in shallow waters less than 40 m deep, while the sightings of large-scale surveys are mainly concentrated on the outer shelf, the shelf-edge (depths from 200 to 4000 m)

and oceanic waters. There are also occasional sightings on the rest of the shelf (Certain *et al.* 2008; SAMM aerial campaigns 2011/2012, E. Pettex, personal communication; Hammond *et al.* 2013). Given this shallow coastal vs. deep pelagic habitat distribution, the existence of two distinct ecotypes could be possible. However, no previous study attempted to delineate ecotypes in the NEA. Fine-scale genetic structure was reported locally in Ireland and the Iberian Peninsula where a potential differentiation between pelagic and coastal dolphins was suggested (Fernandez *et al.* 2011; Mirimin *et al.* 2011). In contrast, despite high geographical distance, no differentiation was found between individuals sampled around the pelagic islands of Madeira and the Azores using a relatively small set of 10 microsatellites markers (Quérouil *et al.* 2007). The only large-scale genetic study (Natoli *et al.* 2005) correlated genetic breaks with oceanographic boundaries between Scotland and the NEA (using samples from South England to Gibraltar for the latter) and between West and East Mediterranean Sea. However, despite samples coming from Scotland to the Black Sea, this study was limited by small sample sizes (e.g. 35 samples for the NEA) and the relatively low number of microsatellites used (9). Our understanding of the bottlenose dolphin population structure is therefore extremely fragmented in the NEA. Determining population structure and delineating eventual bottlenose dolphin ecotypes in the NEA is essential for management as anthropogenic pressure can be extremely different in coastal and pelagic environments. The small size of resident coastal populations and the extinction of at least one genetically isolated population in an estuary (Humber Estuary, UK) that has not been repopulated raised conservation concerns for the species in coastal waters (Nichols *et al.* 2007). Moreover, bottlenose dolphins are protected in Europe under the Habitat Directive where they are listed as a species whose conservation requires the designation of Special Areas of Conservation.

In this context, the aim of our study was to determine the population structure of bottlenose dolphins in the NEA. Thanks to a collaborative framework of organizations across Europe, we were able to gather a large sample size (i.e. 405 tissue samples) covering an unprecedentedly wide geographical area encompassing both coastal and pelagic waters. We used a combination of biopsy samples and samples from stranded animals, and interpretation of data from strandings was enhanced by estimating, whenever possible, the most likely area of death of stranded individuals using a drift prediction model (Peltier *et al.* 2012). The most likely area of death is indeed more indicative of the individual living area than stranding location, and the model is a promising and novel approach to improve the reliability of using stranded animals in genetic studies of marine megafauna. We also used a much larger set of independent loci (25 microsatellites and a 682-bp fragment of the mitochondrial control region) than previous studies. In addition, we worked with several clustering methods, which is rarely done in marine mammal population structure studies. The identified populations were characterized in terms of genetic diversity, connectivity and effective population sizes. We placed our work in the broader phylogeographical context of the North Atlantic basin, which raised new hypotheses about the evolutionary history of bottlenose dolphins in this area. Finally, we discussed ecotype delineation, evolutionary scenarios and ecological and behavioural processes driving the population structure of this highly mobile top predator.

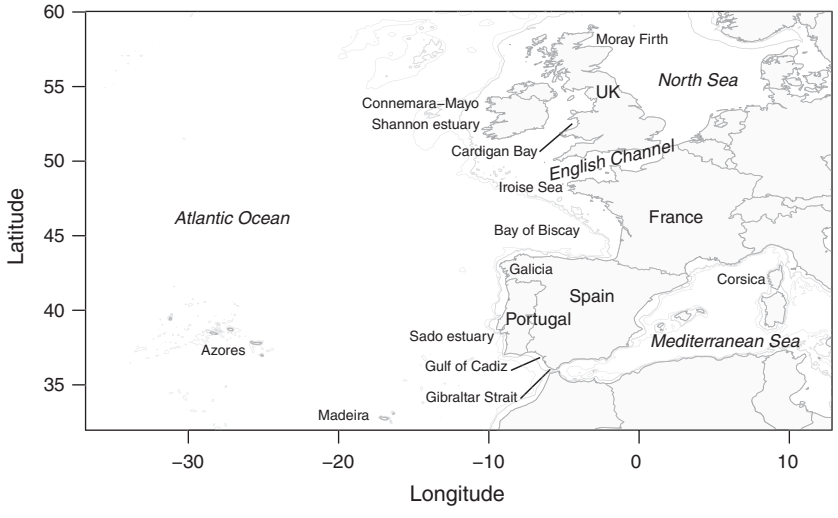

Fig. 1 Map of the study area. −1000 and −200 m isobaths are plotted.

Material and methods

Sample collection, DNA extraction and sexing

A total of 405 bottlenose dolphin samples were obtained from the NEA and the Mediterranean Sea (see studied area in Fig. 1). Samples were collected from free-ranging dolphins by skin biopsy sampling between 2003 and 2012 ($N = 164$) and from skin, muscle or kidney of stranded animals between 1990 and 2012 ($N = 241$). Tissue samples were either frozen or preserved in ethanol or DMSO. DNA was extracted using NucleoSpin Tissue kits (Macherey-Nagel) following the manufacturer's protocol.

After checking for mitochondrial DNA (mtDNA) sequence quality and duplicates (i.e. individuals that were biopsy-sampled more than once), 381 samples (Fig. 2) were kept in the analyses. 343 individuals had both mitochondrial and microsatellite data, 26 only mitochondrial data and 12 only microsatellites, resulting in $N = 355$ for microsatellite and $N = 369$ for mitochondrial data analyses. Samples for which either mitochondrial or microsatellite data were missing came only from stranded individuals and the failure to obtain either mitochondrial or nuclear data is likely linked to decomposition state. Geographical origin was known for 173 samples (biopsy samples: $N = 158$; stranded animals that were previously photo-identified: $N = 15$), while 208 samples came from stranded animals of unknown origin. A drift prediction model which takes into account meteorological conditions (currents, winds and tides), the decomposition state of the carcasses and cetacean body parameters (thickness and floatability) was applied to stranded animals in the Bay of Biscay, English Channel and North Sea (the areas encompassed by the model), in order to estimate their most likely area of death (Appendix S1, Supporting Information, Peltier *et al.* 2012). This could only be estimated when the decomposition state of the carcass was available ($N = 66$). The decomposition state is a proxy of the time after death in terms of intervals of days (Peltier *et al.* 2012). To estimate the most likely area of death, the centroid position of all the drift gps coordinates during the appropriate day interval was calculated for each individual using the geosphere package (Hijmans *et al.* 2012) in R 3.0.0. (R Core Team 2013). All maps were created using the MARMAP package (Pante & Simon-Bouhet 2013).

The gender of the individuals was determined by amplification of the SRY plus ZFX/ZFY fragments as described in Rosel (2003) and/or visually during necropsy.

Microsatellite genotyping and validity

Samples were genotyped at 25 microsatellite loci including 20 published markers and five markers newly developed during this study (see Appendix S2a, Supporting Information for PCR and genotyping conditions and Appendix S2b, Supporting Information for the method of discovery of new microsatellites). To assess genotyping error rate, 28 individuals were randomly selected for re-amplification and scoring at all loci. Thirteen duplicates were also included in error rate calculation. 11.55% of the data set was therefore reprocessed. Individuals were kept in the analyses when at least 12 loci were successfully amplified ($N = 355$), resulting in 1.84% of missing values in the whole data set. Each microsatellite locus was checked for null alleles and scoring errors using Microchecker (Van Oosterhout *et al.* 2004). Departures from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium were tested using

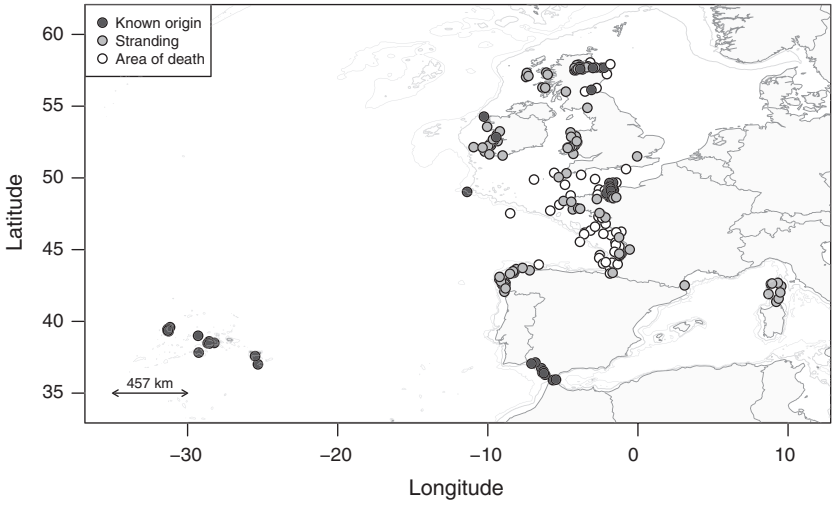

Fig. 2 Sampling locations for individuals of known origin (biopsy samples and stranded individuals previously photo-identified), stranded animals (and their stranding locations) and area of death (stranded animals for which it was possible to apply the drift prediction model). −1000 and −200 m isobaths are plotted.

10 000 iterations in GENEPOP web version 4.2 (Raymond & Rousset 1995). Tests were conducted for the whole data set and for each population identified by the clustering methods. Significance levels were corrected for multiple comparisons using the sequential Bonferroni technique for this test and for all multiple comparisons of the study (Holm 1979).

Mitochondrial DNA sequencing

A 682-base pair (bp) portion of the mitochondrial control region was amplified using primers Dlp1.5 (5′-TCACCCAAAGCTGRARTTCTA-3′) (Baker *et al.* 1998) and Dlp8G (5′-GGAGTACTATGTCCTGTAACCA-3′) (as reported in Dalebout *et al.* 2005; see Appendix S3, Supporting Information for PCR conditions). Consensus sequences were generated and checked for ambiguities with SEQUENCHER 5.0 Demo (Gene Codes Corporation) and manually edited with BioEdit (Hall 1999). Unique haplotypes were identified using DNAsp (Rozas & Rozas 1999).

Population structure

We used three clustering methods to determine the most likely number of populations and assign individuals to these: a multivariate method, the discriminant analysis of principal components (DAPC) (Jombart *et al.* 2010) and two Bayesian methods implemented in STRUCTURE (Pritchard *et al.* 2000) and TESS (Durand *et al.* 2009b). DAPC is a multivariate method that clusters individuals using genetic similarity. It does not rely on any population genetic model and is efficient at detecting hierarchical structure (Jombart *et al.* 2010). STRUCTURE clusters individuals by minimizing Hardy–Weinberg and linkage disequilibria (Pritchard *et al.* 2000). TESS is a spatially explicit Bayesian model, which incorporates individual geographical coordinates as *a priori* information (Durand *et al.* 2009b). These three different approaches were used to ensure the robustness of the inferred results as determining the most likely number of clusters can be challenging (Guillot *et al.* 2009).

DAPC was performed using the package adegenet (Jombart 2008) in R 3.0.0 (Appendix S4, Supporting Information). Membership probabilities were calculated for each individual, and each individual was assigned to a cluster using its maximum membership probability.

In STRUCTURE, the admixture models with correlated and uncorrelated allele frequencies were used, without indicating any *a priori* information on the origin of samples. Ten independent runs for K values set from 1 to 10 were performed using a burn-in period of 50 000 iterations followed by 300 000 Markov chain Monte Carlo (MCMC) steps. The most likely number of clusters was chosen by calculating ΔK (Evanno *et al.* 2005), which is the second-order rate of change of the mean loglikelihood of the data [Ln$P(D)$] between successive K values in STRUCTURE HARVESTER v.0.5 (Earl & Vonholdt 2012). As this method cannot identify $K = 1$, we confirmed the results by plotting Ln$P(D)$ (Pritchard *et al.* 2000), examining individual membership proportion plots and consistency across runs. The Evanno method can reveal hierarchical structure by detecting the upper level of genetic differentiation (Evanno *et al.* 2005); therefore, STRUCTURE was re-run in each of the identified clusters. When K was defined, the run with the highest Ln$P(D)$ value was selected and individuals were assigned to clusters based on maximum membership proportions.

The conditional autoregressive (CAR) admixture model was run in TESS using a burn-in of 20 000 steps followed by 120 000 MCMC steps. The number of clusters (K) to test was set from 2 to 10, with 10 replicate runs for each K. The spatial interaction parameter was set to 0.6 and the degree of trend to linear (which are the default parameters). To exclude land masses from the analysis, nine dummy points were added along French and Spanish coasts (Durand *et al.* 2009a). The most likely number of clusters was selected by plotting Deviance Information Criterion (DIC) values against K and by examining plots of individual assignment probabilities. Consistency of the runs was checked. When K was defined, the run with the lowest DIC was used and individuals were assigned to clusters based on maximum assignment probabilities.

As results were highly consistent between analyses in terms of both the most likely number of clusters and individual assignments (which were identical for 93.53% of individuals across the three methods), the method that uses both multilocus genetic data and spatial coordinates (i.e. TESS) was used to divide the data set into populations for the following analyses (see description of the populations in the population structure result section).

As the inclusion of closely related individuals could impact population structure analyses, the Queller and Goodnight (Queller & Goodnight 1989) relatedness coefficient (r) was calculated using KINGROUP v.2 (Konovalov *et al.* 2004) within each population identified by TESS. TESS was then re-run by removing one individual from each pair of individuals showing a relatedness coefficient superior or equal to 0.45 as in Rosel *et al.* (2009).

Sex-biased dispersal was tested in FSTAT 2.9.3 by comparing sex-specific assignment indices, relatedness, F_{ST} and F_{IS} values using 10 000 permutations (Goudet *et al.* 2002). The test was performed on the whole data set using the populations identified by TESS and at the different levels of the hierarchical structure. Only adults were included in the test (biopsy samples were only

collected from adults, and for stranded animals, we kept only individuals with a minimum total length of 250 cm, i.e. an arbitrary threshold for which we considered that individuals were physically mature, $N = 292$ individuals).

Nuclear genetic differentiation and diversity

To characterize the level of genetic differentiation among the clusters identified by TESS, pairwise F_{ST} were estimated between populations using ARLEQUIN 3.5.1.3 (Michalakis & Excoffier 1996). The level of significance was assessed using 10 000 permutations. The analyses were also performed with the data set excluding closely related individuals. For each identified population, mean number of alleles (NA) and allele richness (AR) were calculated in FSTAT 2.9.3. (Goudet 1995). Inbreeding coefficient (F_{IS}), observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated in ARLEQUIN. Convert (Glaubitz 2004) was used to identify private alleles. Diversity indices were also calculated per locus. Mean AR and *Ho* were compared between pairs of populations using a Wilcoxon paired-sample test.

Mitochondrial DNA differentiation and diversity

A haplotype network was constructed to determine genealogical relationships using median-joining and maximum parsimony algorithms implemented in NETWORK 4.6.0.0 (Bandelt *et al.* 1999). Sequences were clustered according to the populations identified by TESS. Number of haplotypes (NH), number of polymorphic sites (S), haplotypic diversity (h) and nucleotide diversity (π) were determined for each population in ARLEQUIN. JMODELTEST 2.1.3 was used to determine the most accurate model of substitution using the Bayesian Information Criterion (BIC; Guindon & Gascuel 2003). Pairwise genetic differentiation was estimated between populations in ARLEQUIN using F_{ST} and Φ_{ST}. For Φ_{ST}, the Tamura and Nei (1993) model of substitution was chosen as it is the closest model to the HKY + I model, selected by JMODELTEST. Significance levels were tested using 10 000 permutations.

Sequences from this study were placed in the phylogeographical context of the North Atlantic basin. Haplotypes from the NWA and additional sequences from the Azores and Madeira were obtained from GenBank (Appendix S5, Supporting Information). A haplotype network was constructed as described above using a 324-bp consensus length for unique haplotypes.

Recent migration rates

Recent and asymmetric migration rates (within the last few generations) among populations identified by TESS were estimated using the Bayesian method implemented in BayesAss (Wilson & Rannala 2003) on microsatellite data (see Appendix S6, Supporting Information).

Effective population sizes

We used two methods for estimating contemporary effective population sizes for each population identified by TESS: a method that uses linkage disequilibrium in LDNe (Waples & Do 2008) and an approximate Bayesian computation method implemented in ONeSAMP (Tallmon *et al.* 2008). In LDNe, alleles frequencies <0.02 (P_{crit}) were excluded from the analyses to avoid bias caused by rare alleles but still get a high precision (Waples & Do 2010). In ONeSAMP, N_e priors were set from 2 to 500 and from 2 to 10 000 for the expected small and large populations, respectively. Influences of priors on the estimates were tested for the two coastal populations, using priors from 4 to 1000 and from 2 to 200. Our data set included multiple cohorts and age classes, which will bias N_e estimates downwards. For instance, a 10–15% downward bias in N_e estimates was observed in a study using mature bottlenose dolphins and a P_{crit} of 0.02 in LDNe (R. Waples, personal communication). We therefore applied a bias correction of 15% to our results for both LDNe and ONeSAMP ($N_e c$).

Results

Microsatellite validity

The genotyping error rate was 0.0097 (i.e. 10 incorrect genotypes/1025 genotypes reprocessed). The error rate for stranded individuals, which were fresh to moderately decomposed (0.013, i.e. seven incorrect genotypes/525 genotypes reprocessed), was twice as large as the error rate for live individuals (0.006, i.e. three incorrect genotypes/500 genotypes reprocessed). Significant departure from HWE was detected for the majority of the loci when considering the whole data set as a single population. However, this was the result of Wahlund effects as no significant departure was found when dividing the data set into the populations identified by TESS, except for loci MK9 and EV37 in one population each (Appendix S7, Supporting Information). As deviation was significant in only one population and results with and without these two loci were essentially the same (number of clusters and individual assignments), only results including MK9 and EV37 are reported. Linkage disequilibrium was significant for 0.50% of the pairwise comparisons, and when significant, it was not detected across all populations and was therefore considered negligible.

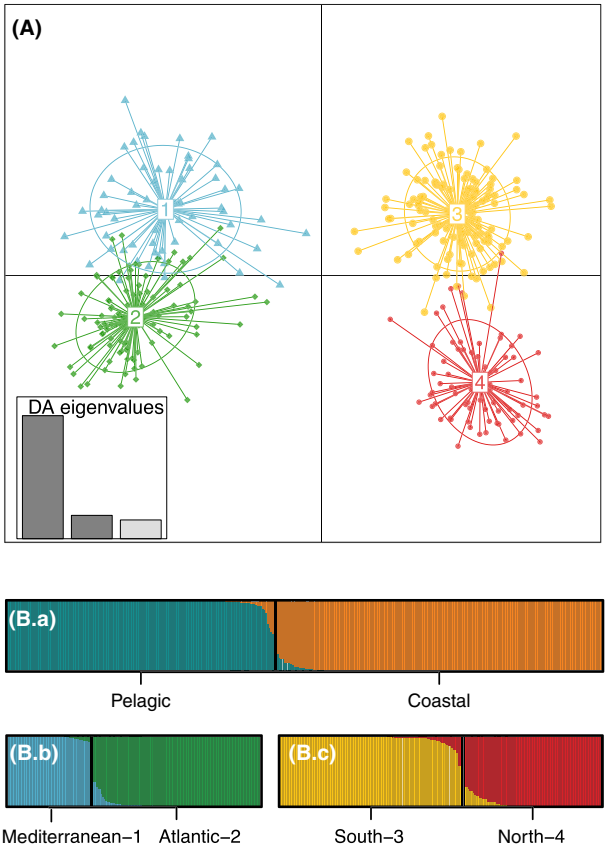

Fig. 3 (A) DAPC scatterplot showing the first two principal components for $K = 4$ (Mediterranean = 1, Atlantic = 2, South = 3, North = 4). (B) Bayesian assignment probabilities of individual bottlenose dolphins inferred using STRUCTURE. Each vertical column corresponds to one individual, with the colours representing the membership proportion to each of the two clusters. Dolphins were sorted using their maximum assignment probability. The black vertical lines delimit the inferred populations. (a) Barplot for the highest level of genetic structuring between pelagic and coastal dolphins. Barplots for the second level of genetic structuring between (b) Mediterranean and Atlantic pelagic dolphins, and (c) South and North coastal dolphins.

Drift prediction model

The drift prediction model indicated that individuals were likely to have died in coastal waters in the North Sea and the English Channel and from coastal to the outer shelf-edge waters in the Bay of Biscay (Appendix S1, Supporting Information).

Population structure

Four populations and a pattern of hierarchical structure were identified using DAPC (Fig. 3A). The first component separated two clusters that were further divided into two clusters by the second component (BIC plot in Appendix S8, Supporting Information). The most likely number of clusters identified using STRUCTURE and the Evanno method was two (Fig. 3Ba, Evanno plots in Appendix S9a, Supporting Information), using both the correlated and uncorrelated allele frequency models. The majority of individuals (98%) were strongly assigned to one of the clusters (assignment probability $Q > 0.80$). As the DAPC indicated a hierarchical structure, STRUCTURE was re-run inside each of the two clusters. A further division was found within each of the two clusters (Fig. 3Bb,Bc, Evanno plots in Appendix S9b,c, Supporting Information) with strong assignments for most individuals (96%). Finally, TESS detected four populations (Fig. 4, DIC plot in Appendix S10, Supporting Information), with 93% of individuals strongly assigned ($Q > 0.80$). Assignments were highly consistent among the methods with 93.5% of the individuals assigned to the same cluster across the three methods. Moreover, comparisons of TESS barplot ($K = 4$) and STRUCTURE barplot for $K = 4$ also indicated almost identical results for individual assignments (data not shown). Therefore, we considered that the population structure signal was strong and not linked to analytical artefacts.

The first population identified by TESS ($N = 119$) was composed of individuals that were biopsy-sampled or that stranded in the English Channel (France), three resident individuals that stranded in the Bay of Biscay (France) and stranded animals in South Galicia (Spain). The second cluster ($N = 77$) was composed of individuals biopsy-sampled or stranded in Ireland, England or Scotland (including 10 previously photo-identified resident dolphins for the latter). These two clusters grouped together in the first level of differentiation identified by STRUCTURE and DAPC. These individuals were biopsy-sampled in shallow and coastal waters (<20 m deep) or stranded in areas near resident populations [i.e. English Channel, Cardigan Bay (Wales, UK), Moray Firth (Scotland), South Galicia Rias (Spain)] and included dolphins previously photo-identified. Moreover, for these populations, the most likely area of death of individuals for which it was possible to apply the drift prediction model indicated that they came only from coastal and shallow waters. These two populations were therefore composed by coastal dolphins and named 'Coastal South' (English Channel, Arcachon estuary and South Galicia resident groups) and 'Coastal North' (UK and Ireland resident or mobile coastal groups) populations. Individuals biopsy-sampled in pelagic waters of the NEA (including the Azores archipelago) and stranded animals along the west coasts of Europe formed a third population ($N = 107$). According to the drift prediction model, individuals were likely to have died from coastal waters to the shelf-edge. The last population ($N = 52$) was composed of

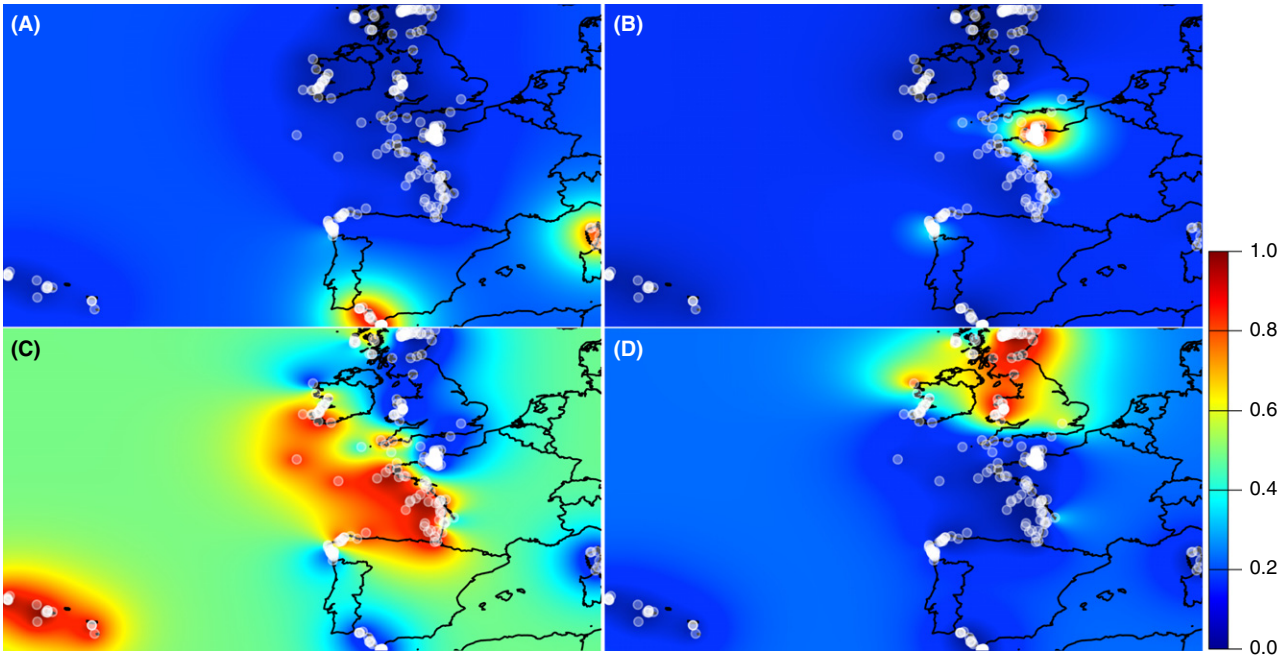

Fig. 4 Map of individual assignment probabilities per population identified by TESS. The colour scale bar indicates the assignment probabilities, (A) Pelagic Mediterranean, (B) Coastal South, (C) Pelagic Atlantic, (D) Coastal North.

individuals biopsy-sampled in the Gulf of Cadiz and the deep waters of the Gibraltar Strait and by individuals stranded in Corsica. These two populations grouped together in the upper level of structure revealed by STRUCTURE and the DAPC. As the biopsied dolphins in this group were sampled in deep waters (>200 m) of the Azores, the NEA and the Gibraltar Strait, these two populations were composed of pelagic individuals and named 'Pelagic Atlantic' and 'Pelagic Mediterranean' populations.

The removal of one individual per pair of closely related individuals (25, 21 and one individuals were removed from the Coastal South, Coastal North and Pelagic Mediterranean populations, respectively) did not change the inferred population structure.

Gender was determined for 370 individuals (153 females and 217 males). No significant sex-biased dispersal was found for any of the tested indices (all $P > 0.05$) either among the four populations or between each of two main groups (coastal and pelagic). We had reasonable numbers of males and females in each group for the 292 adults included in the sex-biased dispersal test (Coastal North = 23 females + 26 males, Coastal South = 39 females + 70 males, Pelagic Atlantic = 32 females + 56 males and Pelagic Mediterranean = 20 females and 26 males).

A total of 55 mitochondrial DNA (mtDNA) haplotypes were identified in the NEA data set (including 53 haplotypes for individuals that were also genotyped for microsatellites, see Appendix S11, Supporting Information for the table of polymorphic sites). The median-joining network (Fig. 5) indicated that the majority of individuals in the coastal group shared haplotypes forming a lineage separated by 12 base pairs (bp) from the lineage including most haplotypes found in the pelagic group. Only two haplotypes were shared between the coastal and the pelagic group. Some haplotypes within the pelagic group were highly divergent, with 49 bp separating the two most distant haplotypes.

When using only 324-bp sequences to include haplotypes from other studies, the number of haplotypes was reduced from 6 to 4 for NEA coastal dolphins and from 49 to 38 for NEA pelagic dolphins (Fig. 6). Haplotypes of the NWA were classified as coastal or pelagic following designation used in previous studies (listed in Appendix S5, Supporting Information and P. Rosel, personal communication). Coastal haplotypes from the NWA formed a completely separate lineage. Haplotypes from NEA and NWA pelagic individuals, from the Azores and Madeira and from NEA coastal individuals were clustered together in the network. Eighteen haplotypes were shared between NWA pelagic and NEA pelagic, NEA coastal or Azores and Madeira dolphins.

Genetic differentiation and genetic diversity in the NEA

All nuclear F_{ST}, mtDNA F_{ST} and Φ_{ST} pairwise comparisons were significant, with the highest level of differentiation found when comparing pelagic and coastal

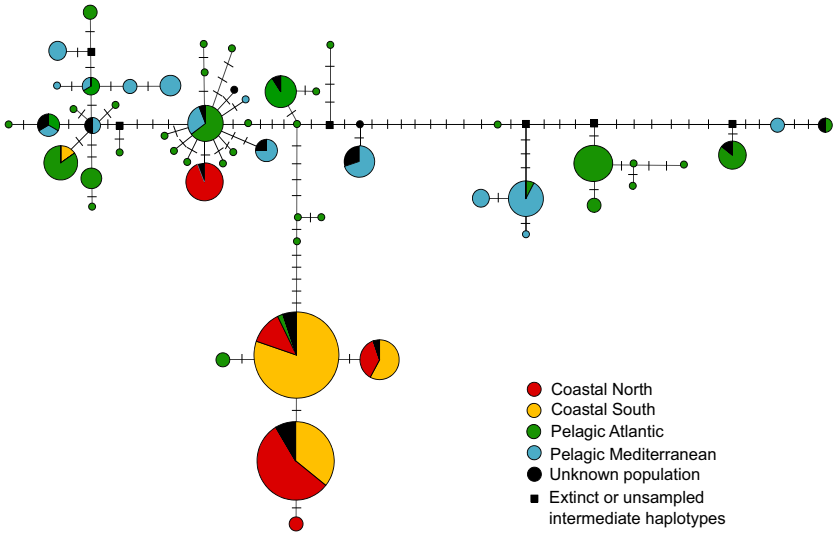

Fig. 5 Median-joining network of mtDNA control region haplotypes found in bottlenose dolphins from the North-East Atlantic. Each circle represents a unique haplotype coloured in proportion to the number of individuals from the populations inferred by TESS that share the haplotype (individuals for which the population could not be inferred by microsatellite data are shaded in black). Size of circles is proportional to haplotype frequencies. Black squares indicate either extinct or unsampled intermediate haplotypes. Black dashes indicate mutation steps between haplotypes.

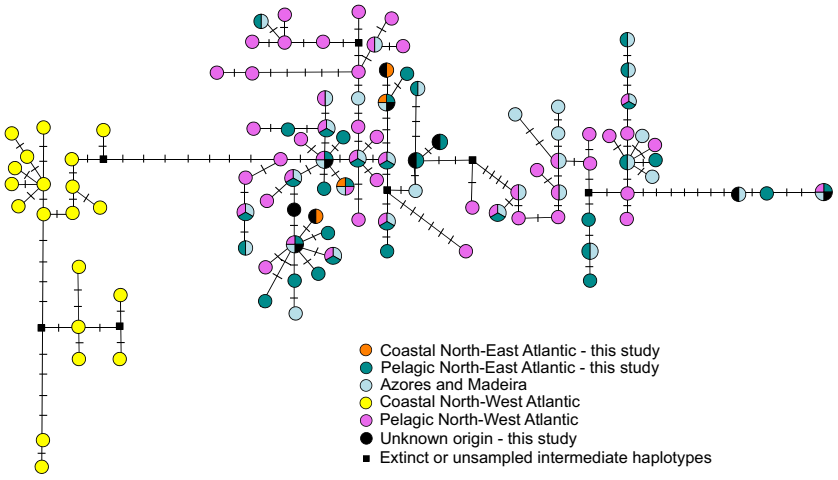

Fig. 6 Median-joining network of mtDNA control region haplotypes found in bottlenose dolphins from the North Atlantic. Each circle represents a unique haplotype coloured according to the population where it was found. The haplotype frequencies were not taken into account. The two pelagic and coastal populations of this study were grouped. Black squares indicate either extinct or unsampled intermediate haplotypes. Black dashes indicate intermediate mutation steps between haplotypes.

Table 1 Pairwise microsatellite F_{ST} between populations

Population	Coastal South	Coastal North	Pelagic Atlantic	Pelagic Mediterranean
Coastal South (N = 119)	—	0.057**	0.133**	0.118**
Coastal North (N = 77)		—	0.149**	0.157**
Pelagic Atlantic (N = 107)			—	0.043**
Pelagic Mediterranean (N = 52)				—

**$P < 0.01$ after sequential Bonferroni correction.

Table 2 Pairwise mitochondrial F_{ST} (above diagonal) and Φ_{ST} (below diagonal) between populations

Population	Coastal South	Coastal North	Pelagic Atlantic	Pelagic Mediterranean
Coastal South (N = 115)	—	0.252**	0.279**	0.326**
Coastal North (N = 76)	0.233**	—	0.195**	0.221**
Pelagic Atlantic (N = 101)	0.541**	0.349**	—	0.071**
Pelagic Mediterranean (N = 51)	0.671**	0.445**	0.056**	—

**$P < 0.01$ after sequential Bonferroni correction.

Table 3 Mitochondrial and nuclear diversities for each population inferred by TESS

	Mitochondrial					Microsatellites						
Populations	*N*	No hapl.	S	*h*	π	*N*	F_{IS}	*P*	*Ho*	*He*	NA	AR	PA
Coastal South	115	4	12	0.499 (0.044)	0.001 (0.001)	119	0.012	0.240	0.582 (0.180)	0.596 (0.172)	6.3 (2.8)	5.8 (2.6)	2
Coastal North	76	5	13	0.667 (0.042)	0.006 (0.003)	77	0.062	0.002	0.486 (0.180)	0.541 (0.191)	5.8 (2.4)	5.3 (2.2)	2
Pelagic Atlantic	101	38	41	0.929 (0.013)	0.014 (0.007)	107	0.008	0.236	0.734 (0.131)	0.770 (0.131)	9.8 (3.9)	9.0 (3.3)	48
Pelagic Mediterranean	51	15	28	0.902 (0.022)	0.013 (0.007)	52	0.018	0.154	0.700 (0.158)	0.726 (0.140)	7.8 (3.4)	7.8 (3.4)	8
Overall*	369	55	46	0.883 (0.011)	0.012 (0.006)	355	0.103	0.000	0.631 (0.139)	0.715 (0.142)	10.8 (5.2)	8.7 (3.7)	—

N, number of individuals; No hapl., number of haplotypes; S, number of polymorphic sites; *h*, haplotypic diversity; π, nucleotide diversity; F_{IS}, inbreeding coefficient; *P*, F_{IS} *P*-value; *Ho*, observed heterozygosity; *He*, expected heterozygosity; NA, mean number of alleles; AR, mean allele richness; PA, total number of private alleles; SD in parenthesis when appropriate.

*26 individuals that were not included in microsatellites analyses (due to amplification issues), and thus were not assigned to any population, were included in the overall values of mtDNA diversities. 12 individuals were successfully amplified for microsatellite markers, but not for mtDNA.

populations (Tables 1 and 2). Comparisons of the two coastal populations also had a high mtDNA F_{ST} value. As identical results were obtained when excluding closely related dolphins, they were kept in the analyses.

Mitochondrial genetic diversity was higher in pelagic populations than in coastal populations (Table 3). Despite similar sample sizes, the number of haplotypes in the coastal populations was considerably lower than in pelagic populations, with the majority of coastal individuals sharing two haplotypes and with no evidence of most common pelagic haplotypes (see Appendix S12, Supporting Information for haplotype frequencies by population).

Nuclear genetic diversity [allele richness (AR) and observed heterozygosity (*Ho*)] was significantly lower in coastal than in pelagic clusters (Wilcoxon test, $P < 0.01$) (Table 3, Appendix S7, Supporting Information for values per loci per populations). All pairwise comparisons were significant except for the AR, which was not significantly different between the two coastal clusters. Lower numbers of private alleles were identified in coastal populations than in pelagic populations (Table 3). A significant heterozygote deficiency was detected in the Coastal North population (Table 3), which was likely due to the inclusion of closely related individuals because F_{IS} was nonsignificant when they were removed ($F_{IS} = 0.029$, $P = 0.119$).

Recent migration rates

Estimates were highly consistent between runs; therefore, results for a randomly chosen run was selected (Table 4). Estimates of recent migrations rates were low among all clusters: 1.1% per generation at most, and with 95% confidence intervals that included 0 (Table 4).

Effective population sizes

The two methods produced approximately similar contemporary effective size ($N_e c$) estimates, with considerably lower estimates for coastal populations than for pelagic populations (Table 5). Using different priors for coastal populations in ONeSAMP, N_ec estimates varied only slightly. Despite months of computation, pelagic population $N_e c$ estimates using ONeSAMP never converged.

Discussion

Hierarchical structure

Bottlenose dolphins were hierarchically structured in the NEA. The strongest level of genetic differentiation was found between coastal and pelagic dolphins with both microsatellite and mtDNA markers. The NEA

Table 4 Mean (and 95% CI) recent migration rates inferred using BayesAss. The migration rate is the proportion of individuals in a population that immigrated from a source population per generation

From/To	Coastal South	Coastal North	Pelagic Atlantic	Pelagic Mediterranean
Coastal South	0.990 (0.979–1.000)	0.004 (0.000–0.012)	0.003 (0.000–0.008)	0.003 (0.000–0.009)
Coastal North	0.008 (0.000–0.021)	0.9837 (0.967–1.000)	0.004 (0.000–0.012)	0.004 (0.000–0.012)
Pelagic Atlantic	0.004 (0.000–0.010)	0.003 (0.000–0.009)	0.983 (0.956–1.000)	0.011 (0.000–0.026)
Pelagic Mediterranean	0.010 (0.000–0.026)	0.009 (0.000–0.024)	0.009 (0.000–0.024)	0.973 (0.947–0.999)

The diagonal values represent the proportion of nonimmigrants in a population.

Table 5 Contemporary effective population sizes corrected for overlapping generations ($N_e c$) estimated using LDNe and ONeSAMP

	LDNe	ONeSAMP
Coastal South	64 (56–74)	77 (62–108)
Coastal North	32 (28–37)	46 (36–62)
Pelagic Atlantic	7748 (1333–infinite)	Endless run
Pelagic Mediterranean	231 (168–360)	Endless run

haplotype network indicated two separate mitochondrial lineages with no complete lineage sorting between coastal and pelagic dolphins. Shared haplotypes indicated possible migration, incomplete lineage sorting or introgression. As in the NWA (Natoli *et al.* 2004), genetic diversities were higher in pelagic than in coastal populations. Significant genetic structure was found within each of the two groups. Migration rates between populations were low (about 1% per generation or less). In the coastal group, individuals sampled in the UK and Ireland (Coastal North) formed one population. Eight dolphins were reported moving between east and west Scotland and between Scotland and Ireland coastal groups through photo-identification (Robinson *et al.* 2012), which suggests that these wide-ranging individuals may maintain genetic connectivity between resident groups. This population was differentiated from neighbouring English Channel dolphins and more distant Galician individuals. However, several resident coastal groups [e.g. Shannon estuary (Ireland), Iroise Sea (France)] were not sampled. Moreover, the Shannon population is genetically isolated from other inshore dolphins in Ireland (Mirimin *et al.* 2011). Thus, more structuring is expected in coastal waters. In the pelagic group, individuals from the NEA were separated from individuals sampled in the Gulf of Cadiz, Gibraltar Strait and Mediterranean Sea. Individuals sampled in the Azores clustered with 88 individuals from the rest of the NEA, which can be surprising given the large distance between the Azores and the shelf-edge. Deep waters (>200 m) are found very close to shore for this archipelago, indicating that bottlenose dolphins inhabit oceanic environments. Photo-identification work indicated that resident individuals represented <5% of individuals found in the Azores, the majority of the individuals being transients or migrants (Silva *et al.* 2008). This could explain the lack of structure found in Quérouil *et al.* (2007) and our study, which contrasted with other oceanic archipelagos where genetic structure was found, like in Hawaii, where shallow water areas are larger and high site fidelity has been reported (Martien *et al.* 2011). Individuals of the Mediterranean Sea were considered as coastal in previous studies (Natoli *et al.* 2004, 2005), which contrasted with their high genetic diversity and with our results indicating that they were pelagic. Some coastal groups are resident but movements were reported between Corsica and France (Gnone *et al.* 2011), indicating that individuals crossed pelagic waters. The pelagic habitat use was confirmed by aerial surveys conducted during winter where bottlenose dolphins were mainly sighted in deep-water (>200 m) areas (SAMM, 2011/2012, E. Pettex, personal communication). We could, however, not exclude further population structuring within this area as we had a limited sample size and only samples from stranded individual for Corsica. Biopsy sampling of coastal and pelagic groups is therefore needed to assess Mediterranean Sea bottlenose dolphin population structure.

To our knowledge, this is the first time that the structure and connectivity between and within pelagic and coastal bottlenose dolphin populations was investigated in the NEA. Three clustering methods relying on different assumptions produced extremely consistent results. We therefore concluded that the genetic signal is strong and inferences reliable. We emphasize that using different methods is particularly important when working on highly mobile animals for which geographical barriers are not obvious. It is still rarely done in marine mammal studies. In our case, the landscape genetic method was efficient at detecting and geographically delineating four populations. However, marine mammal studies using a landscape genetics approach are still scarce (but see Fontaine *et al.* 2007; Möller *et al.* 2011). Our study shed light on global patterns of population structure of

bottlenose dolphins in the NEA. However, finer-scale population structure could exist within the identified populations, as Bayesian clustering methods have been shown to be inefficient at detecting structure when differentiation levels are below F_{ST} of 0.02 (Latch *et al.* 2006; Chen *et al.* 2007). This work could therefore be the basis for more localized studies inferring finer-scale population structure.

Although sampling stranded animals is a cost-effective method, we acknowledge that not all animals dying at sea are likely to strand (see review in Peltier *et al.* 2012), which confers uncertainty about the representativeness of these samples. The use of the most likely area of death (Peltier *et al.* 2012) for part of the stranded individuals shed light on their origin, which was consistent with the genetic results separating coastal and pelagic bottlenose dolphins. Unfortunately, meteorological data were not available for the whole area, making it impossible to apply the model for the complete data set. In addition, the most likely area of death does not necessarily correspond to living areas in particular if sick or weakened animals move to another area to die (e.g. closer to shore). Despite these caveats, the likely position of death was more indicative of the individual living area than stranding position. Moreover, Peltier *et al.'s* (2012) drift experiments with tagged individuals indicated a high precision of the model: 27.1 ± 24.5 km (mean distance between the observed stranding positions of the tagged animals and the positions predicted by the model). It is therefore a promising tool for the use of stranded dolphins in genetic studies, which has recently been questioned (Bilgmann *et al.* 2011).

Possible drivers of population structure

A complex interaction between historic environmental processes and contemporary ecological and behavioural factors is likely to drive social cetacean population structure (Möller 2011; Amaral *et al.* 2012a,b).

For bottlenose dolphins in the NEA, given the topology of the haplotype network, a single founding event of the coastal populations from the pelagic population could be a possible evolutionary scenario. This hypothesis is supported by the low genetic diversities and small effective population sizes of coastal populations. Founder events often involve few individuals, which leads to a loss of genetic diversity due to genetic drift. A similar scenario is suggested for NWA bottlenose dolphins (Hoelzel *et al.* 1998b; Natoli *et al.* 2004). When placing our samples in the Atlantic basin context, the NEA coastal haplotypes were more closely related to the NWA pelagic haplotypes than to the NWA coastal haplotypes. The pelagic population is possibly undifferentiated in the North Atlantic (Quérouil *et al.* 2007) although this needs to be confirmed using a larger sampling size and nuclear markers. Founder events might therefore have occurred independently from this wide-ranging pelagic population when suitable coastal habitats were released during interglacial periods (Natoli *et al.* 2004) on the two sides of the Atlantic basin, and more recently in the NEA than in the NWA. These hypotheses should be tested using coalescent approaches. Nevertheless, our work indicated that evolutionary history of bottlenose dolphins may differ among oceanic regions.

Genetically identified coastal bottlenose dolphins were only biopsy-sampled in shallow waters, whereas genetically identified pelagic individuals were sampled in deep waters. This supports a habitat-driven population structure in bottlenose dolphins. Although sex-biased dispersal methods are known to have low power (Goudet *et al.* 2002) and thus caution should be taken when interpreting the results, we showed that both males and females were philopatric as found in several other bottlenose dolphin populations (see review in Möller 2011). This situation contrasted with the mammalian mating system where females tend to be philopatric as their reproductive success is mainly limited by food resources, while males tend to disperse as their reproductive success is constrained by access to mates (Emlen & Oring 1977; Greenwood 1980). Familiarity with natal habitat, in particular resource specializations, together with social structure and culturally or vertically transmitted behaviour could possibly contribute towards philopatry for both sexes (Sellas *et al.* 2005; Sargeant & Mann 2009; Möller 2011; Cantor & Whitehead 2013). These processes could lead to assortative mating and maintain divergence at a large scale between the pelagic and coastal groups, and at a finer scale, within the two groups. Natal habitat preference through diet specializations was suggested as an important mechanism underlying cryptic population structure in terrestrial carnivores (Sacks *et al.* 2005; Pilot *et al.* 2012). Moreover, socio-ecological factors also drove genetic divergence between killer whale populations specialized on distinct prey (Hoelzel *et al.* 1998a; Foote *et al.* 2011). In our study, we had no direct evidence for different diets among populations and between pelagic and coastal dolphins. However, localized stomach content (Scotland and Bay of Biscay) and stable isotope (Galicia) studies suggested that coastal populations were feeding on estuarine species, while demersal or demerso-pelagic fishes mainly found on the shelf-edge (e.g. hake or blue whiting) were the main prey of presumably pelagic bottlenose dolphins (Santos *et al.* 2001; Spitz *et al.* 2006; Fernandez *et al.* 2011). Further diet studies are needed to document the niche specialization

of the two groups and investigate the hypotheses described above.

Effective population size estimates: small coastal vs. large pelagic populations

Effective population sizes were much larger for pelagic than for coastal populations, which was consistent with their genetic diversities. As pelagic populations were likely to be very large, N_e estimates for these populations were not reliable (Tallmon *et al.* 2010). In addition, our sample size for the Pelagic Mediterranean population was relatively low for these approaches. For coastal populations, we had a sufficient number of samples ($N = 77$ and 119) and high precision (25 microsatellites) to get reliable N_e estimates for small populations ($N < 500$) (Tallmon *et al.* 2010). However, our sampling scheme was not ideal. Two assumptions of both the linkage disequilibrium and approximate Bayesian computation methods were likely to be violated: closed populations and discrete generations. For the 'no immigration' assumption, the bias could be considered negligible as migration rates were very low and at least for LDNe, migration rates below 5–10% should have little effects on N_e estimates (Waples & England 2011). The 'discrete generations' assumption was clearly violated. First, bottlenose dolphins live up to 57 years and are sexually mature between 5 and 14 years (Wells & Scott 1999). Second, our data, collected across a 22-year time period, included multiple cohorts and generations. N_e estimates obtained using samples with overlapping generations are likely to be biased downward (Waples 2010). Nevertheless, a study comparing different N_e estimate methods for a brown bear population showed that the N_e estimate obtained in ONeSAMP on multiple cohorts was similar to the harmonic mean of N_e estimates obtained from single cohorts using another method, the Estimator by Parentage Assignment (Skrbinsek *et al.* 2012). Robinson and Moyer (2013) found that N_e estimates are closer to the per-generation N_e when only mature adults are sampled, which resulted to a downward bias of <15%. If it is not possible to sample only mature adults, Robinson and Moyer (2013) suggested that as many age classes as possible should be included in the analyses. As our data set contained multiple age classes and generations, results were likely to be biased downward. The downward bias depends also on the species' life history. We corrected our estimates for a 15% downward bias (N_ec) as a 10–15% downward bias was observed in a study using LDNe where mature adult bottlenose dolphins of different ages were sampled in Florida (R. Waples, personal communication). Last but not least, N_e estimated using LDNe related to the effective number of breeders N_b (Waples 2005). Further empirical research is needed on the relationships between N_b and N_e, which could be particularly complex when generations overlap (Waples 2010). Nevertheless, the order of magnitude of the bias should be similar across our data set. Our N_ec estimates are on par with abundance estimates obtained from surveys in areas inhabited by each of the four populations. The NEA pelagic population abundance estimate from Scotland to Spain was tens of thousands of individuals (Hammond *et al.* 2009, 2013). In the Mediterranean Sea, abundance was estimated to several thousands of individuals (Forcada *et al.* 2004; Gnone *et al.* 2011). According to mark–recapture studies, resident coastal population sizes were likely to be around 600–800 individuals for each of the two populations (M. Louis, unpublished data for the Normandy resident group, López 2003; Pesante *et al.* 2008; see review for Ireland in Mirimin *et al.* 2011; Cheney *et al.* 2012). For these two coastal populations, the ratio between effective population sizes and census sizes may be around 5–10% based on our N_ec estimates and abundances from mark–recapture studies, which is in the lower end of the range of values found in other species (Palstra & Ruzzante 2008).

Management implications

Coastal populations were isolated and their effective population size was small in comparison with pelagic populations. Estimated N_ec (range: ~30–80) was close to the value of $N_e = 50$ under which Mace and Lande (1991) proposed that a population is in a critical state. Low effective population sizes might lead to a low adaptive potential to environmental changes (Hare *et al.* 2011). Ecological adaptation to specific habitats is likely to drive coastal populations' structure (this study, Natoli *et al.* 2005; Rosel *et al.* 2009), which raises concerns about potential impacts from the currently increasing at-sea human activities. Habitat degradation in terms of organic contaminants and noise pollution from boat traffic and constructions (e.g. Pirotta *et al.* 2013) could strongly affect locally adapted coastal populations. In addition, in East England, a genetically differentiated population became extinct and the estuary was never repopulated (Nichols *et al.* 2007). Several Special Areas of Conservation have been created throughout Europe for the management of bottlenose dolphins; however, some important areas for the species still lack conservation measures. Given the vulnerability of small and isolated populations that live within increasingly disturbed environments, we recommend extending the habitat protection of the species in Europe. Moreover, ecotypes should be distinguished in management plans of the species.

Ecotype delineation and future directions

Our results showing weaker separation between the pelagic and coastal haplotypes in the NEA found using 324-bp in comparison with 682-bp sequences highlighted the importance of using long fragments of the mitochondrial control region to investigate ecotype delineation in bottlenose dolphins. We therefore recommend the use of long mitochondrial fragments to investigate recent and/or fine-scale genetic structure in delphinids displaying sequence variability levels similar to bottlenose dolphins.

We employed an original approach to define ecotypes, considering Lowry's (2012) definition as groups of ecologically distinct populations. In most studies, ecotypes were first described through diet, morphology or spatial distribution and then linked to genetic differentiation (e.g. Hoelzel *et al.* 1998b; Segura *et al.* 2006; Musiani *et al.* 2007). The latter approach sometimes led to the definition of ecotypes that were subsequently found not to be demographically and genetically isolated units (e.g. caribous Serrouya *et al.* 2012). For cryptic and mobile species for which we have only hints on ecology, genetic data could be an interesting first step in ecotype delineation. Previous distribution and diet studies gave us first clues on the ecological differentiation of coastal and pelagic bottlenose dolphins. Further investigations on diet specializations using stable isotopes and cranial skull features associated with feeding analyses (Perrin *et al.* 2011) may be required to further refine ecotype designations for bottlenose dolphins in the NEA.

Acknowledgements

We thank Vanessa Becquet and Eric Pante (LIENSs), Eric Taylor and two anonymous reviewers for their constructive comments on the manuscript. We also thank everyone involved in data collection or collaboration: Joanne O'Brien and Conor Ryan (GMIT, IWDG), Nigel Monaghan and Ruth Carden (National Museum of Ireland), Barry McGovern (SAC Inverness) and Julie Béesau, Gill Murray-Dickson and Paul Thompson (University of Aberdeen), GECC and Réseau National Echouages volunteers, Fabien Demaret, Ghislain Doremus, Vincent Ridoux and Olivier Van Canneyt (Pelagis), Sami Hassani (Océanopolis), Angela Llavona, Ruth Fernandez (CEMMA) and Paula Mendez-Fernandez (CEMMA and LIENSs), Philippe Verborgh and Ruth Esteban (CIRCE), Joan Giménez (EBD-CSIC). All samples were taken under the relevant permits. We thank Patricia Rosel for the information on the NWA haplotypes and Emeline Pettex for the information on bottlenose dolphin sightings of SAMM aerial surveys. We also thank the Molecular Core Facility at the University of La Rochelle. UK samples were collected under the aegis of the UK Cetacean Strandings Investigation Programme, which is funded by Defra and the Devolved Administrations of Scotland and Wales. Data from offshore Irish waters were collected on the Cetaceans on the Frontier cruise thanks to National Marine Research Vessels Ship-Time Grant Aid Programme 2010 funded under the Science Technology and Innovation Programme of National Development Plan 2007–2013. Biopsy samples in Ireland were carried out under licence from the National Parks and Wildlife Service Nos. C104/2011 and DER/Dolphin2012-10. Samples from Galicia were obtained with the support of Dirección Xeral de Conservación da Natureza-Xunta de Galicia, cofinanced with European Regional Development Funds (ERDF/FEDER). Southern Spain samples were collected thanks to LIFE 'Conservación de Cetáceos y tortugas de Murcia y Andalucía' (LIFE 02 NAT/E/8610). MAS was supported by an FCT postdoctoral grant (SFRH/BPD/29841/2006). Data collection in the Azores was funded by projects TRACE (PTDC/MAR/74071/2006) and MAPCET (M2.1.2/F/012/2011). Funding for sample collection in France and analyses was provided by Fondation Total, Agence de l'Eau Seine-Normandie, Fonds de Dotation pour la Biodiversité, Agence des Aires Marines Protégées, Association Nationale de la Recherche et de la Technologie, Direction Régionale de l'Environnement, de l'Aménagement et du Logement, Ministère de l'Ecologie, du Développement Durable et de l'Energie and Conseil Général de la Manche.

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M.L., A.V., C.G. and B.S.B. conceptualized the work and the analyses. M.L. and T.L. performed laboratory work. M.L., A.V., H.P. and B.S.B. analysed data. M.L., E.A., S.B., A.B., P.C., W.D., R.D., R.dS., F.G., P.G., R.P. and M.A.S. collected samples. M.L. wrote the paper. All authors approved the final manuscript.

Data accessibility

DNA sequences GenBank accession nos: KF650783–KF650837.

Newly developed microsatellites GenBank accession nos: KF887998–KF888002.

Microsatellite genotypes, DNA sequence alignment and analysis input files (TESS, DAPC, STRUCTURE and Arlequin): Dryad: doi:10.5061/dryad.57rr4.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Map of stranding locations (left) for individuals for which we applied a drift prediction model and map of their most likely area of death (right).

Appendix S2a PCR and genotyping conditions for each microsatellite locus.

Appendix S2b New microsatellite discovery method.

Appendix S3 PCR conditions for the amplification of a portion (682-bp) of the mitochondrial control region.

Appendix S4 DAPC settings.

Appendix S5 List of haplotypes obtained from GENBANK and used for the North Atlantic basin mtDNA haplotype network including information on accession nos (GENBANK), sampling locations (Origin with NWA = North-West Atlantic) and the articles where these sequences were reported and/or analysed.

Appendix S6 BayesAss settings.

Appendix S7 Test for Hardy–Weinberg equilibrium (HWE) deviation of each locus in each population and in the whole data set (*P*-values that are significant after sequential Bonferroni correction are highlighted in boldface).

Appendix S8 Selection of the optimal number of clusters for the DAPC analysis using the lowest BIC (Bayesian Information Criterion).

Appendix S9 Evanno plots of the STRUCTURE analyses separating (a) the coastal and pelagic groups, (b) the two coastal populations and (c) the two pelagic populations.

Appendix S10 Mean Deviance Information Criterion (and SD) values using the 10 replicate TESS runs for each *K* from 2 to 10.

Appendix S11 Polymorphic nucleotide sites defining the 55 mitochondrial control region haplotypes for bottlenose dolphins in the North-East Atlantic.

Appendix S12 Haplotype frequencies by group and population.