REPORT OF

ASCOBANS/HELCOM SMALL CETACEAN POPULATION STRUCTURE WORKSHOP

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At UN Campus, Hermann-Ehlers-Str. 10,
53113 Bonn, Germany

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CONTENTS

1. PREFACE

Over a 2.5-day period, twenty-four specialists in marine mammal genetics and ecology met at the UNEP Campus in Bonn, Germany, to review small cetacean population structure within the ASCOBANS Agreement Area. The target species were small cetaceans in general, but special attention was paid to harbour porpoise, bottlenose dolphin, white-beaked dolphin, Atlantic white-sided dolphin, and short-beaked common dolphin. This report summarises materials provided in the presentations made at the workshop, incorporating also the discussions and conclusions reached in relation to reviewing a) the nature of the various methods used to identify population structure, including their strengths and limitations; and b) the evidence for population structure amongst the five target species. The issue of population structure for harbour porpoise within the Baltic region was addressed separately, and a research proposal formulated to answer specific information gaps.

Since funding and other practicalities limited the number of participants that could attend, a further twelve specialists were invited to contribute material and/or participate in the review of this report, so as to ensure as comprehensive and balanced a coverage as possible. Sections 2-7 form the presentations made at the workshop, with authors given the opportunity to edit or update their contributions. Section 8 provides reviews of the five small cetacean species for which management units were to be proposed. Of the listed authors of each review, the first author was responsible for compiling the review, with input from the others. Where there was disagreement among co-authors, alternative views have been expressed. The introduction to section 8 was written by the first editor. Section 9 presents the report of the Baltic harbour porpoise workshop, and was written by the second editor.

The editors would like to thank everyone who participated in the workshops and the report arising, as well as to the ASCOBANS Secretariat, particularly Heidrun Frisch, and Tine Lindberg, Marco Barbieri and Rob Hepworth, for hosting the meeting. Funds were provided through ASCOBANS and the Swedish government on behalf of HELCOM, to whom we are very grateful.

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2. INTRODUCTION

Background

2.1 Workshop 1 – Small Cetacean Population Structure in the ASCOBANS Area

A pre-requisite to effective conservation management of small cetaceans is an understanding of how best to define populations in a biologically meaningful manner. To achieve this, there are substantive challenges to overcome since physical boundaries rarely exist, and a variety of approaches have been used that have different implications. Furthermore, there are both methodological and analytical issues that need addressing.

This workshop proposed to draw together persons with appropriate expertise for a review of small cetacean population structure throughout the entire ASCOBANS Agreement Area. The target species were small cetaceans in general but with particular attention to harbour porpoise, bottlenose dolphin, white-beaked dolphin, Atlantic white-sided dolphin, and short-beaked common dolphin.

2.2 Workshop 2 – Genetics and Population Structure of the Harbour Porpoise in the Baltic Sea

Arising from the Jastarnia Plan has been a management need to identify and agree upon appropriate population units for harbour porpoises in the Baltic Sea. To date, there have been a number of independent studies using samples obtained from different locations and time periods, and using different methodological approaches. These require synthesizing and evaluating. To achieve this, a separate workshop on genetics and population structure of the harbour porpoise in the Baltic Sea was organised.

3. MAIN OBJECTIVES

3.1 Common Objectives for both workshops

Both workshops should provide a forum for informal presentation and discussion of issues of interest by those involved and for a means of promoting and facilitating cooperation across borders.

Agreement should also be reached on the best way ahead, which could be one of two alternatives:

a) Establishment of a network that will gather and maybe re-analyse all available results to give the best overview of the population structure.
b) Application for a research project where new samples and other data are collected over the next few years to provide a more solid basis for understanding the population structure of the species in the region.

Specifically, the following aims should be achieved:

3.2 Workshop on small cetacean population structure

1) Establishment of a definition of population units of interest to management.
2) Identification of the strengths and limitations of different approaches used in discriminating between populations; these would include:
    a) genetic techniques (e.g. microsatellite, mtDNA, MHC studies)
    b) other approaches (e.g. metrical and non-metrical skeletal variation, contaminant and parasite burdens, fatty acid signatures, diet, variation in life history parameters, results of telemetry studies, etc)
3) Establishment of an agreed set of criteria for investigating population structure
4) Review of
    a) sampling protocols (sample sizes, spatial and temporal intervals between sampling points, etc)
    b) methodologies for sample collection
    c) standardisation of laboratory techniques
5) Review of statistical techniques for identifying population units (e.g. hypothesis testing versus clustering/other approaches)

3.3. Workshop on genetics and population structure of the harbour porpoise in the Baltic Sea

1) Review of status of knowledge on population structure of harbour porpoises in the Baltic Sea (including Kattegat and Belt Sea, as recommended by the Jastarnia Plan):
    a) review genetic evidence for separate Baltic porpoise populations
    b) review other lines of evidence for separate Baltic porpoise populations (morphometric skeletal variation, contaminant and parasite burdens, results of telemetry studies).
2) Agreement upon biologically meaningful boundaries for conservation management of the harbour porpoise in the Baltic Sea.
3) Identification of the characteristics of identified populations within the Baltic Sea in terms of
    a) genetic variability
    b) population history
    c) movement patterns and seasonality, including any gender differences.
4) Identification of gaps in our knowledge of evidence for distinct populations, and recommendation of research programs to address any such gaps.
5) Agreement upon best methods and standards (genetic and ecological) to allow for more comparable and informative results in the future.

4. METHODS FOR DISCRIMINATING POPULATIONS

4.1 Genetic*s*

4.1.1 *Markers*

a) mtDNA & Microsatellites: *Liselotte Andersen*
Microsatellites are sequences of di, tri, tetra or penta nucleotides repeated tandemly, randomly distributed in the nuclear DNA. They mostly occur in the non-coding regions but can be found in the coding region or coupled to genes. On each side of the repeated unit there is a flanking region, which makes it possible to develop microsatellites that are locus specific. They can also be found in mtDNA. Microsatellites are perfect when the repeated sequence consists of (AT) or other nucleotide combinations a certain number of times. They are imperfect when the repeat unit consists of different combinations of the pair of nucleotides and compound when the repeat unit is disrupted with a number of nucleotides within the unit $[(AC)_5TGC(TG)_3]$. This has implications for the mutation-model that the microsatellite is presumed to follow. They are highly polymorphic and can have several alleles per locus (up to 30 or more). The high variability is due to a fairly high mutation rate, which is around $3 \times 10^{-3(-4)}$ (Weber and Wong, 1992). The mutation models which microsatellites are thought to follow depending on their repeat unit, are the Infinite Alleles Model (IAM), where new alleles arise randomly; the Stepwise Mutation Model (SMM), where two alleles that differ by one repeat are more closely related than alleles differing by several repeats; and the K Alleles Model (KAM), where alleles mutate into any one "K" allele randomly. The high mutation rates make homoplasy a more common phenomenon, indicating that two alleles with identical lengths are not identical by origin.

As indicated, microsatellites can be both neutral and selective whilst the mode and rate of evolution may vary greatly amongst loci and taxa. The inheritance of microsatellites depends on the location. If autosomal, it is Mendelian inherited and co-dominant, where it is possible to distinguish heterozygotes from homozygotes. If sex-specific, it is either maternally or paternally inherited, but, if it is located in mtDNA, it is maternally inherited (Sunnucks, 2000; Selkoe and Toonen, 2006).

A typical animal cell has 100-1000 mitochondria with 5-10 copies of DNA (Wink, 2006). Mt DNA is composed of 16S rRNA, 12sRNA, tRNA, Cytochrome B, COI subunits I-III, NADH subunits I-VII, ATP synthase, Subunits a, b, and D-loop (non-coding region). The D-loop has the highest substitution rate, with a mutation rate of $\sim 5 \times 10^{-5}$ base substitutions / generation. It is maternally inherited; generally there is no recombination; and there is individual homoplasy (one haplotype / individual). It is thought to be neutral, although there is increasing evidence that this might not be the case, since a recent meta-population study analysing the D-loop diversity in several taxa did not find a correlation with population size and diversity (Bazin *et al*., 2006).

The use of microsatellites and D-loop variation in population/conservation genetics

	Microsatellites	D-loop
Individual level:		
Parentage analysis	X	
Relatedness	X	
Sub- and population level:		
Bottlenecks/founder events	X	X (Maternal)
Effective population size	X	X
Gene flow	X	X
Phylogeography	X	X
Species identification:		
Hybridization	X	X (combination)

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Sunnocks, P (2000) Efficient genetic markers for population biology. *Trends Ecol. Evol.*, 15: 199-203.

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b) MHC variation: *Rus Hoelzel*

The Major Histocompatibility Complex (MHC) is a multigene family, which codes for cell surface glycoproteins that bind peptides of processed foreign antigens, and present them to T-lymphocytes. MHC class I and class II loci have been shown to be highly polymorphic in primate, rodent, avian and bovine species. Furthermore, polymorphism in MHC genes is mainly restricted to the sites that specify the amino acids of the Peptide Binding Region (PBR), the region that is responsible for peptide collection and presentation. Two of the main reasons that MHC polymorphism has been attributed to frequency and/or overdominant selection are: i) the high number of non-synonymous (*dn*) relative to synonymous (*ds*) substitution rates in the PBR; and ii) trans-species polymorphism. Some suggest that MHC alleles may experience periods of neutral evolution, during which genetic drift and mutation may be more prominent in maintaining MHC polymorphism than selection. However, selection occurs at least when populations change habitats and environmental conditions, and thus new pathogens. Direct evidence for selection has come from the mapping of

allelic substitutions onto the inferred structural model of the MHC molecule. At the population level, comparisons may suggest balancing or directional selection or drift, but for cetaceans and other species groups investigated, selection seems a dominant force. Therefore, it can be useful as an indicator of local adaptation, but it is not likely to be useful as a neutral marker for investigations of isolation by distance.

c) SNPs: *Per Palsböll*

Single nucleotide polymorphisms (SNPs) are single base pair substitutions among DNA sequences and in essence are what is typically detected when a DNA sequence is surveyed at a non-repeat locus among individuals in populations. It is the most common genetic marker in the mammalian genome. The mode of inheritance depends upon the locus: autosomal SNPs are inherited in a Mendelian manner in a diploid, sexually reproducing species, whereas a mitochondrial SNP would be maternally inherited. The basic assumption regarding SNPs is the absence of recurrent mutation resulting in bi-allelic markers, for instance, and A (adenine) or G (guanine) as the target SNP. Typically, a single SNP per locus is surveyed and, often, by methods that are aimed at detecting the two possible nucleotides (here A or G).

A number of different survey methods have been developed, where the main advantage is the automated and unambiguous detection of the SNP genotype across many loci with little effort. Compared to single tandem repeats (STRs, i.e., microsatellite loci) SNPs require less hands-on work in terms of data generation and data extraction. The bi-allelic nature (and presumably low mutation rate) of SNPs yields them less suitable to kinship / paternity studies, but well suited for estimating population-level parameters, such as gene flow and effective population size. The fewer alleles per SNP locus compared with STRs implies that many more SNP loci need to be analysed to achieve an equal statistical power in terms of rejecting homogeneity (panmixia). However, as each locus is subject to less variance and more loci are genotyped, the estimates of population genetic parameters will be subject to less uncertainty compared to STR loci. SNPs are still not common in non-model species, in part because SNPs need to be identified prior to the onset of a new study. There are two main approaches; random cloning and identification of SNPs in a population sample; or surveying known candidate genes (or introns) for SNPs. The former ensures a random selection from the genome of SNP loci but requires more initial work. Common for both approaches is that candidate SNPs need to be identified randomly among target populations (as opposed to only one among a suite of populations) in order to avoid ascertainment bias.

The current dominant assumption that SNPs follow an infinite site mutation model (no recurrent mutations) is not likely to be met, and since mass DNA sequencing techniques are becoming more efficient, it has been suggested to sequence 2-300 base pairs surrounding (and including) the target SNP, which will permit detection of recurrent mutations.

4.1.2 Analytical Methods

a) Assignment of individuals: *Per Palsböll*

Assuming panmixis in sampled putative populations, the likelihood of each individuals' genotype in each population may be estimated from single and multi-locus Mendelian inherited genotypes. This approach is implemented in a variety of software packages: the most commonly employed are STRUCTURE and GENE CLASS. The approach is increasing in popularity and often hailed as a way to obtain a "real time" estimate of migration / gene flow. The ability to assign individuals to a specific population sample is positively correlated with the degree of genetic divergence. At low levels of genetic divergence, the migration rate (and thus the chance of sampling immigrants) is high, but the statistical rigour in assigning individuals is low. By contrast, a high degree of statistical certainty in assigning individuals is achieved when populations are genetically distinct from each other (i.e. low migration rates) but the probability of sampling an immigrant is then low. This limits the utility of individual assignment methods in management and conservation, and "real-time" estimates of migration rates from these methods require high levels of sampling both in terms of individuals and genetic markers to achieve a reasonable likelihood of sampling immigrants and individual assignments with a sufficient level of precision.

Very few programs translate assignment proportions into relevant and comparable population genetic parameter estimates of gene flow, such as N_m or F_{ST}, making it difficult to translate assignment proportions into meaningful measures of population connectivity/structure. BAYESASS extends the approach to identify first and second order offspring from immigrants and translates assignments into *m*. However, recent work has shown this approach to be biased at high levels of gene flow. Perhaps the best use of individual assignment methods (provided sufficient sampling) is in recently diverged populations (i.e., habitat/population fragmentation). In such assignments, proportions can be compared to those expected given the observed degree of population genetic divergence (e.g., F_{ST}) and if significantly lower would point to a recent reduction in gene flow. Alternatively, the temporal/spatial distribution of close kin (identified by the degree of consanguinity estimated from genetic markers, such as STRs) will provide insight into the level of connectivity among putative populations. This latter kind of "assignment" (in this case to individuals rather than populations) has the added advantage that it does not hinge upon the level of genetic divergence among putative sub-populations, but requires that a large enough fraction has been sampled that both members of dyads of close kin have been sampled, and is thus not feasible in larger populations. As is the case of assignment of individuals to populations, then the relationship between the spatial distribution of close kin and more traditional population genetic or demographic parameters is unknown – but could likely be approximated by simulations.

b) F_{ST}, R_{ST}, and their application to quantifying population structure in marine mammals: *Simon Goodman*

Wright's *F* statistics have played a central role in our understanding of the genetics of population structure since they were first conceived in the 1930s. Conceptually, they are based on the probability that alleles selected at random from individuals, sub-populations or populations, are identical by descent, and they measure the increase in inbreeding (or reduction in heterozygosity) relative to a randomly mating population arising from population structure. An alternative derivation can be obtained from considering the partitioning of the variance in allele frequency within and among populations (e.g. for Weir and Cockerham's (1984) estimator of F_{ST}), or the variance in allele size for Slatkin's R_{ST} statistic (a measure which is often applied to microsatellite data). These are measures of genetic differentiation that quantify the proportion of the total genetic variation that is distributed among populations, and take (in idealised circumstances) a value between 0 and 1, with 0 indicating complete panmixia, and 1 complete differentiation. Permutation tests are used to test if F_{ST} or R_{ST} estimates observed in real world data are significantly different from zero.

As an example, a brief review is given of the pattern of F_{ST} or R_{ST} estimates between porpoise samples from different regions in the ASCOBANS area. These are typically small (~0 to ~0.06) indicating relatively low levels of genetic differentiation.

F statistics have a number of fairly strict assumptions that are usually violated in real world datasets, and which can complicate their interpretation, whilst there are also additional confounding factors. Firstly, the calculation of these statistics requires prior assumptions to be made as to what constitutes a 'population', which can be problematic for highly mobile species in which there are no obvious geographic barriers to dispersal. Secondly, the value of the statistics for individual loci depends on the heterozygosity at that locus, which can confound the comparison of the magnitude of statistic values among different studies. Finally, the value of the statistics is sensitive to fluctuations in population size, which means it is often difficult to understand the biological meaning of F_{ST} or R_{ST} values without any knowledge of demographic history. Despite these limitations, F_{ST} and R_{ST} probably remain useful summary statistics for a first pass through datasets, but the most powerful hypothesis testing potential and inferences now come from recently developed analytical / simulation approaches such as Hey's *IM* (isolation with migration model).

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Weir, B.S. and Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, 38: 1358-1370.

c) Spatial Autocorrelation Methods: *Ada Natoli*

Spatial autocorrelation is an assessment of the correlation of a variable with reference to the spatial location of the variable. Spatial autocorrelation measures the level of interdependence between the variables, as well as the nature and strength of the interdependence. It may be classified as either positive or negative. Positive spatial autocorrelation has all similar values appearing together, while negative spatial autocorrelation has dissimilar values appearing in close association.

The computation implies the assignment of weights to the case, basically constructing weight matrices that represent the relationship between variables. Measures of the spatial autocorrelation are carried out using principally two global indices. The first is Moran's I. Computation of Moran's I, is achieved by division of the spatial co-variation by the total variation. Resultant values are in the range from approximately -1 to +1. Positive signage represents positive spatial autocorrelation, while the converse is true for negative signage, with a zero result representing no spatial autocorrelation.

Geary's C is the second index. Computation of Geary's C results in a value within the range of 0 to +2, with zero being a strong positive spatial autocorrelation, through to +2, which represents a strong negative spatial autocorrelation.

Spatial autocorrelation analysis has been applied to population genetic analysis. Analysis based on spatial statistics has proven highly powerful, even in cases where no structure is detected by F_{ST} statistics (Epperson and Li, 1996). It also performs well with small sample size. Spatial autocorrelation analysis can be applied to diploid multilocus genotype data and haplotypes.

A case study is briefly presented to illustrate how a spatial autocorrelation analysis was used to investigate population structure, and determine putative population boundaries. Bottlenose dolphin samples from the Kwa Zulu Natal coast (South Africa) were analysed for 9 microsatellites and 599bp of the mtDNA control region. This population is subject to a high rate of by-catch in shark nets, placed along the coast to protect bathers from shark attacks. Overall, the population showed very low genetic variability at both markers (microsat: Ho = 0.5, mtDNA: nucleotide diversity = 0.0039). The testing hypothesis was to determine whether there was more than one population inhabiting the areas.

Technicalities:

In the first analysis, performed with the programme Spatial Genetic Software (SGS, Degen *et al*., 2001), the Moran's Index Iq is calculated for a given distance class sq. For each allele or haplotype having higher frequency than some arbitrary threshold (X%) in all samples, Iq is calculated as follows:

$$I_q = \frac{n\sum_{i=1}^{n}\sum_{j\neq i}^{n} w_{ij}(a_i - \bar{a})(a_j - \bar{a})}{W\sum_{i=1}^{n}(a_i - \bar{a})^2} \qquad W = \sum_{i=1}^{n}\sum_{j\neq i}^{n} w_{ij}$$

n is the total number of samples and wij = 1 if the individuals i and j belong both to the spatial interval sq, otherwise wij = 0. For diploid data, ai is 1 if the i-th individual is homozygous for that allele, or 0.5 if heterozygous, or 0 if the individual has no copy of the allele. For haplotypes, ai is 1 if the i-th individual presents the haplotype, and 0 otherwise. The value *a* corresponds to the mean value of *ai* over all *n* individuals. Following Streiff *et al*. (1998), autocorrelation is calculated over all selected loci summing the numerator and denominator of the first equation over the total number of alleles. The expected values for the case of no autocorrelation are –1/(n-1) (Sokal and Wartenberg, 1983). Higher values indicate positive spatial autocorrelation, and smaller values indicate negative spatial autocorrelation.

A novel technique is proposed by Miller in the programme, Allele in Space (AIS, Miller, 2005). This technique is called Genetic Landscape Shape interpolation analysis, and can be used to obtain graphical representations of genetic distance patterns across landscapes. The three-dimensional surface plots generated by this procedure are referred to as ‘‘genetic landscape shapes.’’ AIS produces three-dimensional surface plots where X and Y coordinates correspond to geographical locations on the rectangular grid, and surface plot heights (Z) reflect genetic distances (for mathematical details, see Miller, 2005). It can be highly efficient to identify population discontinuities across a geographic range.

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d) Non-equilibrium approach using IM: *Rus Hoelzel*

IM is an isolation with migration model that explicitly incorporates parameters for time of population splitting, bidirectional gene flow after splitting, and population sizes, including the size of the ancestral population (Hey and Nielsen, 2004; Nielsen and Wakeley, 2001). The model uses a Bayesian framework that provides estimates for the posterior probability density of the model parameters, given the data. For the killer whale example, we used a uniform (i.e. uninformative) prior distribution. This means that the parameter estimates are essentially equivalent to maximum-likelihood estimates (Nielsen and Wakeley, 2001). We ran a number of linked simulations with varying levels of heating (35 to 80 chains, depending on the populations analysed) required in order to achieve adequate mixing (Hey and Nielsen, 2004). To obtain estimates of effective population sizes (N_e), migration rates, and the time of splitting, we included estimates of the mtDNA control region mutation rate (and scale rates for other loci based on the mtDNA data). With an estimate of the mutation rate, it is possible to obtain estimates of N_1, N_2, N_A (the effective size of populations 1 & 2, and the ancestral population, respectively) and t (time since isolation). Also, using estimates of $4N_1\mu$ and m_1/μ (where μ is the neutral mutation rate, and m_1 is the probability of migration per generation per gene copy), it is possible to obtain estimates of the effective number of migrants per generation into population 1 (i.e. $2N_1m_1 = 4N_1\mu / (2\ m_1/\mu)$), and similarly for $2N_2m_2$. To accommodate the uncertainty of substitution rates, we used two published rates: $1.5\mu\ 10^{-8}$ per base pair per year (Baker *et al*., 1993; Hoelzel *et al*., 1991); and $7.0\mu\ 10^{-8}$ per base per year (Harlin *et al*., 2003). For more detail and references, see Hoelzel *et al.* (2007).

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4.2 Skeletal Variation

4.2.1 Metrical Variation: *Anders Galatius and Carl Kinze:*

In recent years, geometric morphometrics have largely replaced traditional morphometrics based on length measurements, so rather than describe methods of the past, we will devote our time to what should be the present and is the future of morphometrics. Geometric morphometrics employ the capture of 2- or 3-dimensional coordinates from previously defined morphological landmarks from biological specimens to get an approximation of shape. Landmark-based geometric morphometrics are superior to traditional morphometrics since there is less redundancy in landmark positions relative to distance measurements that are often closely correlated with each other; and it facilitates graphical representation of morphological differences, in that original shapes are preserved throughout the analysis.

As an example, a 3-dimensional approach has been employed on three samples of harbour porpoise from the Danish North Sea, the inner Danish waters, and a sample taken in a drive catch in the northern Little Belt during the Second World War, the latter sample allegedly belonging to a seasonally migrating Baltic population. Multivariate statistics revealed highly significant differences among all three samples, corroborating genetic evidence for separate North Sea and inner Danish waters populations and suggesting the existence of a Baltic population, separate from these.

In conclusion, the geometric morphometric approach shows a lot of promise for application in this field, as it yielded much better separation of porpoises from inner Danish waters and the North Sea, than earlier analyses employing traditional morphometrics.

4.2.2 Non-metrical Variation: *Sinéad Murphy*

Non-metric characters, utilised in small cetacean studies, are described in Perrin *et al*., (1982, 1988), Kinze (1985), and Gao and Gaskin (1996). In a study on non metric morphometry of skulls of harbour porpoises from the western North Atlantic and eastern North Pacific, non-metric cranial characters were not found to be an efficient method for separating populations (Gao and Gaskin, 1996). Although differences could be detected, no really significant segregation developed in non-metric traits (Gao and Gaskin, 1996). By contrast, although Perrin *et al*. (1994) reported lower discriminating power in non-metric traits (82.5%) compared to metric (97.8%) characters, for separating populations of common dolphins *D. delphis* from the eastern Tropical Pacific, Black Sea, and western North Atlantic, it was reported that both were efficient. For non-metric characters, however there were difficulties concerning repeatability.

In conclusion, the limited studies undertaken using non metric cranial traits in small cetaceans, have suggested that, on its own, analysis of non metric cranial characters is not an efficient method for defining geographical variation, and should only be used in combination with analysis of metric characters.

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4.3 Stable Isotope Signatures: *Aleta Hohn*

Background

Stable isotopes are elements with unique atomic masses (same number of protons, but different numbers of neutrons) that do not undergo radioactive decay; and most elements have more than one stable isotope. The ratio of those isotopes for an element is the relative amount of the common to the rare isotope; generally the lighter isotope is more common in biological systems. Carbon isotope ratios ($\delta^{13}C$) of plant organic matter record environmental effects on photosynthesis; nitrogen isotope ratios ($\delta^{15}N$) record dietary and trophic-level information in animals and nitrogen fixation in plant–microbe symbioses; and hydrogen ($\delta^{2}H$) and oxygen isotope ratios ($\delta^{18}O$) record water-related dynamics in plants and animals. These recorders provide an 'isotopic signature', that is used to trace the movements of nutrients, compounds, particles, and organisms across landscapes and between components of the biosphere, and to reconstruct aspects of dietary, ecological and environmental histories. [Taken with minor modification from an excellent review by West *et al.*, 2006].

Summary of method

Isotopes are measured from samples run through a mass spectrometer. Depending on the samples, some preparation is required, for example, cleaning with de-ionised water, removal of lipids, and drying in an oven. Samples are weighed and an

appropriate amount (typically 0.4 to 0.6 mg for carbon / nitrogen sample, and 4 mg for sulphur) analysed for its stable isotopic composition using an elemental analyser (EA) coupled to the mass spectrometer. During the process, the samples are combusted to convert the organic samples into gases of suitable purity that can then be analysed by the mass spectrometer. Carbon is converted to CO_2, nitrogen to N_2, and sulphur to SO_2. Samples are run with accepted standards, and then the results from the samples compared to the standards. Isotopic ratios of samples (R_{sam}) are compared to the isotopic ratio of a standard for that element (R_{std}). R is the abundance ratio of the heavy isotope to that of the light isotope of the element, and differences in the ratios are expressed in 'delta' (δ) notation and are reported in per mil (‰): NE (‰) = (R_{sam} / R_{std} - 1) * 1000.

Discriminatory power, level of variance in estimating parameters
The reproducibility of isotopic measurements varies depending on the specific technique, instruments, and sample type used, but it is typically better than 0.2‰ for carbon and nitrogen, and 0.3‰ for the sulphur measurements.

Mode of acquisition and turn-over rate
The isotopic composition of a consumer reflects an integrated measure of the actual assimilated diet of an organism, although with a reproducible fractionation. Turnover rate depends on the tissue being sampled: skin turns over in weeks while teeth present a permanent record. Depending on the question, the appropriate tissue needs to be sampled.

Sampling issues
1. Generally only a few milligrams of sample are necessary for isotopic analysis. This small amount means that a biopsy sample of skin can be split between isotope analysis and genetic analysis. However, skin has a relatively fast turnover rate so identified isotope ratios will not provide a long-term record.
2. Hard tissues, such as teeth and bone, provide a longer-term record but often are available only from dead animals. An isotopic signature for an area should be developed from animals known to inhabit that area. Stranded animals may not be a good source for doing so unless they are known animals, such as through photo-ID.
3. Isotopes with the most potential for identifying habitat (rather than prey) differences are sulphur and oxygen. Sulphur can be extracted from many tissues, but oxygen is available only in mineralised tissue, such as bone and teeth. Also, sulphur and (organic) oxygen stable isotopes are not as frequently analysed as C and N, and thus it is more difficult to find facilities to process the samples.
4. Tissues used for stable isotope analyses should not be exposed to most chemicals, and thus archived samples stored in formalin or DMSO may not be usable. It is best to use frozen samples, or, for teeth or bone, dried samples

Information gaps and recommendations

1. One gap is more precise turnover rates of isotopes and tissues of particular cetacean species, especially for skin samples, because so many are (could be) available from biopsy studies. A related issue is whether turnover rates vary by species.
2. Interpretation of stable isotope results is constrained when not all dietary sources are known and when there is isotope overlap among food sources. Recently, mixing models models have been developed to try to quantify the relative contribution of various sources. The sensitivity of these models will be limited by the number of isotopes sampled, the number of prey species, and by variation in isotopic composition in prey species.
3. A potentially valuable, relatively new method is the use of compound-specific isotope analysis. It may provide much higher precision.

Reference

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4.4 Fatty Acids*:* *Mike Walton*

Background

Blubber is a store of lipid containing many different fatty acids (FA). Some of these are synthesised by the animal itself but others have to be derived from the diet, having been passed from phytoplankton, etc, upwards through the food chain. The relative composition (the % contribution by weight) of individual FA found in a tissue is known as the FA profile or signature. Although the blubber profile is influenced by the profiles of dietary species eaten, the relationship between them is quite complex. Because of practical problems, the use of the QFASA procedure (see note below) is likely to be very limited in cetaceans. However, differences in blubber FA profiles can be used as indicators of different feeding stocks.

Tissue

Blubber is usually collected by necropsy (which can provide a whole cross-section from a given body location) or dart biopsy (which provides a small portion of blubber attached to skin; the body site of sampling will vary). For those marine mammals that have been tested, the different areas of the backs and sides of the body have similar fatty acid profiles, so the use of dart biopsy is OK. However, there are differences in FA profiles across the depth of blubber and the degree of difference differs between species. Generally the inner areas are richer in PUFA (polyunsaturated FA) whereas the outer areas are richer in MUFA (monounsaturated FA). The inner areas are considered more metabolically active and show more similarity to the diet than outer areas.

Analysis
Lipid is extracted from blubber using organic solvents, and the fatty acids are converted to methyl (or occasionally butyl) esters and analysed by gas chromatography. The resultant FA profiles are compared by multivariate statistical techniques.

Practical considerations
The samples to be compared must be from similar cross-sectional areas of the blubber i.e. do not compare biopsy samples (outer blubber) with full depth necropsy samples. Within a stock, FA profiles can change over time if diet changes. Therefore when comparing stocks it is best if they are sampled at about the same time. Otherwise when comparing between stocks, one must check that the results are not compromised by possible changes over time.

Fatty acids (especially PUFA) are susceptible to attack by atmospheric oxygen, which would affect the FA profiles. Samples should be frozen as soon as possible after collection, or if impracticable, immersed in organic solvent containing an anti-oxidant. There are potential problems with using necropsy samples, since the time period between death and sampling is often unknown. Studies at Aberdeen with porpoise blubber have shown that FA profiles were fairly stable at room temperature for at least a couple of weeks, but often one will not know how much degradation has occurred.

A few examples
1) Grey seals (using whole blubber depth biopsied from captured animals) at North Rona and the Isle of May in Scotland show different FA profiles in each of the 12 years that they have been studied. Within each stock FA profiles, the variations between years have also been assessed.
2) Minke whales (hunted animals) in the North Atlantic could be assessed to different stocks. The same stock structure was found when using either whole cross-section or inner layer or outer layer blubber.
3) Killer whale (blubber biopsies from wild animals) could be differentiated into transient, resident and offshore types from their FA profiles.
4) Porpoises (using inner layer from necropsied animals) – differences were seen between those from the German Baltic and the German North Sea.

A note about QFASA (quantitative fatty acid signature analysis)
Iverson *et al.* (2004) proposed the QFASA method to identify and quantify what prey species had been eaten based on blubber FA profiles. It works by determining a "best fit" of prey profiles to match that of the blubber, once allowance has been made for metabolic effects. The method requires a set of FA profiles for potential prey species; a set of calibration coefficients, which are probably species dependent; and the software to perform the analyses. The calibration coefficients are derived from

controlled feeding experiments over many months, something that is difficult to achieve for many cetacean species. One should also bear in mind that they may also vary between species and within a species, between diets. Iverson *et al.* (2004) recommended that either inner layer blubber or whole depth blubber be used. Thus it is very unlikely that dart biopsy samples will be suitable for QFASA purposes.

Reference

Iverson, S., Field, C., Bowen, W.D., and Blanchard, W. (2004) Quantitative fatty acids signature analysis: a new method of estimating predator diets. *Ecological Monographs*, 74: 211-235.

4.5 Parasite Loads: *Kristina Lehnert*

When two or more population groups are separated geographically in different environments, their respective parasite faunas will exhibit differences. Under this assumption parasites have been used as biological indicators for stocks of fishes, small odontocetes and baleen whales. Although many studies have shown the usefulness of parasites as tags of stock levels and migrations of commercially important fish species, the application of these methods to marine mammals is usually limited by logistic factors: it is difficult to obtain large sample sizes, and sampling is often opportunistic rather than systematic. Researchers often have to rely on unpredictable, occasional strandings or by-catches of marine mammals, which may result in low sample sizes and/or severe sampling biases. For instance, it may be difficult to obtain age-stratified data to study variation with host age, or small sample sizes may miss heavily infected hosts. In addition, studies based on stranded animals might be unreliable because they are usually diseased and may not be representative of the whole population. Nevertheless, various studies have provided information on the population identity and local migrations of several marine mammal species. The technique consists of the comparison of infections with one or more parasite species between host groups, which are arranged according to ecological (migratory studies) or behavioural (social studies) criteria. These studies show the usefulness and also the limitations of parasitological surveys to provide information as stock indicators. The methodology requires that suitable parasite species have to be chosen as potential tags following certain criteria:

1) parasite species should be easy to locate and identify;
2) they must show different prevalences and/or intensities throughout the area studied;
3) knowledge on life cycle, direct lifecycle parasites are better suited;
4) life span must be long enough to cover the study period

The parameters to be measured have to be selected. Presence/absence data seem to deliver the most useful information because parasite species' occurrence tends to be more stable over time than their prevalence and abundance. Since the prey species are probably the intermediate hosts of cetacean helminths, variable dietary compositions within species of whales will manifest themselves in their respective parasite faunas. Therefore, knowledge of host dietary composition is important in studying cetacean

helminth zoogeography. The random nature of the sampling of marine mammals (strandings, occasional by-catches) makes it often impossible to achieve simultaneous samples from different localities. Additionally, seasonality can strongly affect the prevalence and abundance of helminth species. Therefore, differences observed between host animals may simply be the result of seasonal fluctuations. Despite this, long-term collections have revealed interesting patterns in spatial distributions of the host population. Differences in age structure and sex ratio between hosts may account for some differences as host-related and temporal factors are known to influence the structure of helminth communities in mammals. Parasitological surveys can give additional support to theories based on evidence from genetic or pollutant load techniques. They have the additional advantage of being relatively low priced and easy to implement.

4.6 Contaminant Loads: *Florence Caurant*

Background

Different types of contaminants are present in the marine environment: (i) POPs or Persistent Organic Compounds, and (ii) trace elements including heavy metals such as cadmium, lead or mercury. The biogeochemical cycles of these two families of contaminants exhibit different processes due to quite different physico-chemical properties. POPs result from chemical synthesis, and their presence in the environment is due to pollution, whereas heavy metals are naturally present in the earth crust and thus in the ocean. Thus their concentrations in the environment depend both upon geochemical characteristicsand also anthropogenic activities that induce increased concentrations compared to background levels. Nevertheless, besides the assessment of the toxicological risk, trace element concentrations in marine mammals can constitute new approaches in defining population structure of marine mammals. The oceans are not temporally and geographically uniform and the variability of the biological or physical characteristics induce the distribution of marine species such as abundance and distribution of a predator's prey. Because diet is the main source of trace elements in marine mammals, these chemicals can be used as ecological proxies by providing signatures allowing the retracing of aspects of long-term diet and any segregations or migrations of predator populations. These tracers provide information covering periods from days to years according to the turnover occurring in the tissue studied. As an example, Born *et al.* (2003), showed the existence of population substructure in North Atlantic minke whales (*Balaenoptera acutorostrata*) through the analysis of mercury, selenium and cadmium. The groups were consistent with those defined genetically by Andersen *et al.* (2003).

Summary of method

Trace elements are measured from samples run through furnace or flame Absorption Atomic Spectrometry (AAS) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS). When collected, samples must be stored in plastic bags to avoid any contamination. Less than 500 mg of powder of freeze-dried tissue is sufficient to carry out analysis of several trace elements after an acid digestion. Acid-digestion of

the samples is not necessarily required for mercury, when an Advanced Mercury Analyser spectrophotometer, ALTEC AMA 254 can be used. During sample processing, equipment has to be cleaned in acid solution to prevent from any contamination. Quality control of the analysis has to be assessed by using a standard such as dogfish liver (DOLT) from the National Research Council Canada. Detection limits differ according to the trace element and the tissue, but can be about 2 $ng.g^{-1}$ of dried tissue.

Mode of acquisition and turnover rate

Trace elements have generally no affinity for lipids (except mercury in the methylated form), and very few publications have demonstrated the relevance of skin samples for determining population segregation through trace element analysis (Kunito *et al*., 2002). This is why biopsy samples are not the most appropriate way for such studies. This implies that one should work on by-catch or stranded animals that can be sampled for liver, kidney or hard tissues such as teeth and bone. Cadmium, which has been shown to be a good candidate to discriminate diet or structure in populations (Bustamante *et al*., 1998; Lahaye *et al*., 2005), will provide a mid-term record when analysed in liver (a few months), and a long-term record in kidney (10 to 15 years). In the same way, mercury in liver or trace elements in bones and teeth will provide a long-term record. However, in all cases, an interpretation of trace element concentrations is constrained by all the biotic factors (such as specific metabolism, sex, age, etc), as well as toxico-kinetics and toxico-dynamics of these elements that are well known only in humans and small laboratory mammals. However, the joint use of fatty acids, stable isotopes, and trace elements increases the power for discriminating populations.

References

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

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Kunito, T., Watanabe, I., Yasunaga, G., Fujise, Y., and Tanabe, S. Using trace elements in skin to discriminate the populations of minke whales in southern hemisphere. *Marine Environmental Research*, 53: 175-197.

Lahaye, V., Bustamante, P., Spitz, J., Dabin, W., Das, K., Pierce, G.J., and Caurant, F. (2005). Long-term dietary segregation of common dolphins (*Delphinus delphis*) in the Bay of Biscay determined using cadmium as an ecological tracer. *Marine Ecology Progress Series*, 305: 275-285.

4.7 Diet: *Jerome Spitz and Vincent Ridoux*

Background

Species of small cetaceans have developed different foraging strategies according to their group structure, their size or their physiology, for example. Furthermore, most of these species are widely distributed in the ASCOBANS area, able to cover large distances, and to exploit different habitats during a lifetime, a year, a season, or even, a day. Thus, some variations in the diet of one small cetacean species have often been described in relation to time or location of sampling. Nevertheless, diet could be a further indicator used to define some population units because the prey community found in stomach contents may be considered as a descriptor of feeding stocks.

Methods and sampling

Stomachs of stranded or by-caught cetaceans can be collected during necropsy and then stored deep-frozen (-20°C). Sample analysis aims at describing the diet in terms of prey species occurrence, relative abundance, calculated mass and size distribution, following a general procedure, which is now standard for marine top predators.

The case of common and striped dolphins in and off the Bay of Biscay

The Bay of Biscay, which may be divided into two major habitats: the neritic area from the coast to the slope (depth <200m), and the oceanic area over the slope, supports two abundant small delphinid species: the common dolphin and the striped dolphin.

The results of striped dolphin stomach contents analysis have revealed that the individuals of this species clearly move between oceanic and neritic habitats to forage (Ringelstein *et al.*, 2006; Spitz *et al.*, 2006). By contrast, the study of the stomach contents of common dolphins indicates that this species has a distinct diet between the oceanic and the neritic habitats of the Bay of Biscay (Pusineri *et al.*, 2007; Meynier *et al.*, 2008). This complete absence of overlap in prey composition suggests that the groups of common dolphins forage in only one of the two main habitats rather than regularly switching back and forth. Hence, the study of stomach contents suggests that the common dolphins that forage in oceanic areas are isolated from those that forage in neritic areas. This hypothesis is strengthened by the distinct rates of accumulation of cadmium between common dolphins from the two habitats, referred to above.

Conclusion on discriminatory power

When different areas of management can be defined by different prey communities, stomach contents analysis of a small cetacean species may reveal the existence of different feeding stocks. Thus, diet coupled with feeding tracers like metallic contaminants, fatty acids or stable isotopes, can give pertinent information for the definition of populations units of interest for management.

References

Ringelstein, J., Pusineri, C., Hassani, S., Meynier, L., Nicolas, R., and Ridoux, V. (2006). The food and feeding ecology of the striped dolphin, *Stenella coerulaeoalba*, in the oceanic Northeast Atlantic. *Journal of the Marine Biological Association of the UK*, 86: 909-918.

Spitz, J., Richard, E., Meynier, L., Pusineri, C., and Ridoux, V. (2006). Dietary plasticity of the oceanic striped dolphin, *Stenella coeruleoalba*, in the neritic Bay of Biscay. *Journal of Sea Research*, 55: 309-320.

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Meynier, L., Pusineri, C., Spitz, J., Santos, M.B., Pierce, G.J., and Ridoux, V. (2008) Diet and feeding ecology of the common dolphin, *Delphinus delphis*, in the Bay of Biscay: importance of fat fish. *Marine Ecology Progress Series*, 354: 267-276.

4.8 Life History Variation: *Christina Lockyer*

Parameters to consider:

1) Age (longevity, recruitment, mortality & survival)
2) Reproduction (age & length/weight at sex. maturity, seasonal mating/parturition, ovulation/pregnancy rates & reproductive interval, gestation, foetal sex ratio, post-natal sex ratio, neonatal size, length of lactation (and weaning of calf)
3) Growth (growth rates/formulae, asymptotic length/weight, age at physical maturity, foetal growth, size/age at weaning

Important points to consider when trying to discriminate between stocks / populations using life history parameters:

They can vary within populations over time according to:

- Environmental conditions, e.g. variable food resource, climate
- Population status, e.g. overall population size relative to carrying capacity, sex ratio, age distribution
- Health factors, e.g. body fat condition, parasites, pollutant load, disease

Other factors to consider when interpreting results:

- Possible changes in geographic distribution over time – is this the same population? Migration?
- Individual variations in social species that form pods – familial trends, e.g. pilot whales
- Variations seasonally in sexual and age class segregation
- Variations seasonally in body weight and condition

- Variations between years in body condition affecting many growth parameters and reproduction
- Response of population to exploitation pressures – whether direct or indirect (by-catch)
- Actual natural hybridization between different species and even genera, e.g. fin-blue whale, Dall's & harbour porpoise
- Sample bias, e.g. if using strandings. Most problematic for parameters like pregnancy rate

Conclusion on the usefulness of life history parameters for discriminating between populations:

Most Useful:

- Timing of reproductive events – mating, birthing seasons; different seasons effectively isolate populations thus preventing mixing of genes
- Length at sexual maturity – usually fairly stable, regardless of feeding conditions, unlike weight

Least Useful:

- Age-related parameters – unless compared populations are in the same time period; these may provide more information about the local environmental conditions affecting growth rates
- Weight-related parameters – these are very variable, both seasonally and between years, and they depend on food resource, and general health
- Gestation period – usually fixed for a species, and difficult to determine accurately

4.9 Telemetry: *Jonas Teilmann*

In contrast to genetics and morphology, movements and distribution of live animals monitored by satellite telemetry provide the most accurate and recent picture of how animals from different areas are mixing. This is very useful when determining population and management unit boundaries. However, conclusions from telemetry studies are often limited by samples size and duration of the tracks.

In general terms, telemetry covers transmitters or receivers attached to an animal in order to be able to locate it. These electronic devices are used for many purposes like short-range detailed tracking or following long-range movements. For short-range tracking, underwater acoustic transmitters or VHF transmitters are often used, while tracking animals for more than a few kilometres, Argos satellite transmitters, potentially Fast-loc GPS receivers, and in special cases, geo-location by light are more appropriate. When studying the range of populations or management units, we need to know where the animals are at any time. For these studies, the Argos satellite transmitter is the first choice when considering size, coverage, cost and reliability in

receiving data. Attaching a transmitter to the dorsal fin has proven successful in following small cetaceans for more than a year. The limiting factors on resolution of individual tracks are the surface behaviour of the species, the battery capacity/duty cycle, the position error, and the satellite coverage.

Tracking small cetaceans for population studies require that the age, gender, individual and seasonal variations are covered. In some species that live in family groups or display a systematic general movement pattern, only few animals may be tracked in an area to know how the whereabouts of the group (e.g. killer whales) or the whole population (e.g. some bottlenose dolphins, gray whales or humpback whales). Other species may show individual opportunistic behaviour, moving around alone or in small groups to optimise feeding success (e.g. harbour porpoises). In such species, more individuals must be tracked to be able to determine population boundaries.

Telemetry alone may be used to derive management units, but to determine boundaries of a population that is not completely isolated from other areas used by the same species, genetic studies may be required. Telemetry and genetics can supplement each other if population boundaries have been stable over hundreds or thousands of years. However, the dramatic environmental changes in recent centuries may have changed the ecology of a species in a way that is not shown by genetic studies. Therefore, comprehensive telemetry may stand alone, indicating the present picture of what animals, stocks, populations or management units are doing, although conclusions will be much stronger if supported by other evidence such as from genetics and morphology.

Future recommendations for the use of telemetry to determine populations and management units would be to:

1) Develop methods to catch animals randomly in the distribution range of a species.
2) Develop tags that can transmit for longer time, e.g. over two breeding seasons.

4.10 Photo-Identification: *Peter Evans*

In the last three decades, photo-ID has become a standard tool in cetacean monitoring (see, for example, Hammond *et al.*, 1990), being used for the estimation of abundance, home range size and use, movements, aspects of life history, and for the better understanding of social structure. It relies upon natural markings (such as nicks in the dorsal fin or tail flukes, pigmentation patterns or chevrons) being unique for a particular individual, and that individual being recognisable over time.

As with telemetry, it has the advantage of providing current information on movements of individuals that can contribute to identification of separate management units rather than having to rely upon the effects of evolutionary history. Photo-ID has the potential to sample a wider range of individuals within and between

populations than with telemetry since it does not rely upon physical attachment of any device. On the other hand, it is much more difficult to accurately track individuals with photo-ID since one tends to obtain only snapshots of their range and this is affected greatly by the number of sightings obtained which may mean a lot of survey effort.

Only some species are amenable to photo-ID. Amongst small cetaceans, probably the best example is the bottlenose dolphin, and this is the species most commonly used for photo-ID in European seas. In a population of more than 200 individuals of this species occupying Cardigan Bay in West Wales, around 40% have recognizable markings (Pesante and Evans, 2008). However, in other species, like the white-beaked dolphin, white-sided and short-beaked common dolphin, the proportion of individuals in the population that is recognisable would be much lower, whilst for some species like the harbour porpoise, it would be unrealistic to use photo-ID at all for distinguishing populations.

There are a number of considerations with photo-ID that need careful attention. First, everything depends upon an individual being re-sighted and recognised. If the marking is subtle, this may not occur. Furthermore, even distinct markings may change over time, so there is greatest confidence if an individual has been re-sighted on a regular basis such that any changes can be tracked. There is also the possibility that a marking is duplicated (at least to the human eye) in more than one individual, and care is therefore required, particularly with apparent re-sightings over a wide geographical area. In those cases, the use of combinations of markings provides greater confidence in avoiding false positives.

References

Hammond, P.S., Mizroch, S.A. and Donovan, G.P. (editors) (1990) *Individual Recognition of Cetaceans.* Reports of the International Whaling Commission (Special Issue 12).

Pesante, G. and Evans, P.G.H. (2008) *Sea Watch Foundation Welsh Bottlenose Dolphin Photo-Identification Catalogue*. CCW Marine Monitoring Report No. 66, i-xii, 1-204.

4.11 Statistical Considerations: *Simon Goodman*

This workshop attempts to synthesise the present state of knowledge and potential gaps in our understanding of population structure for small cetaceans in the ASCOBANS Area. During the workshop, information has been considered from a wide range of data types and analytical approaches, which will obviously all have different statistical considerations and limitations.. Of primary interest is whether the present sampling of the distribution of species is adequate to give a realistic picture of contemporary population structure, or whether there may be systematic biases in some cases, given that studies rely almost exclusively on opportunistic sampling from strandings or by-catch, due to geographic or temporal gaps in sample availability. In

this context, power analysis or simulations can be useful. Another important point is how well different genetic quantifications of population structure and dispersal rates cope with violations of their underlying assumptions, especially with regard to continuously distributed populations.

4.12 Integrating Different Lines of Evidence: *Christina Lockyer*

Here I consider two main approaches for population definitions: biological and management. These definitions may result in an overlap and essentially describe the same population; however, one is defined according to strict biological characteristics, and the other usually according to human need criteria. The decision to choose one or the other depends upon the eventual conservation goal. This contribution focuses on how to begin comparing putative populations by integrating data that are important:

- Distributional data – defines geographical barriers and boundaries between putative populations / stocks, i.e. allopatric, parapatric, or sympatric;
- Population Response data – defines population demography and life history, and other biological parameters including behaviour, e.g. breeding season;
- Phenotypic data – morphological expression of genotype e.g. colour pattern, skeletal shape and form, meristics;
- Genotypic data – genetic data derived from isozyme, molecular cytogenetic, and DNA analyses of different types.

The putative populations are then categorised into four phylogeographic types by using geographic localisation as a proxy for gene flow. The categories are:

- Category I – generally allopatric populations with presence of actual geographic barriers to mixing so that these populations are effectively isolated. Discontinuous genetic divergence is implied, if not known.
- Category II – may be sympatric or parapatric with weak geographic partitioning, but great genetic divergence. Might be expected to be managed together despite separate origins.
- Category III – allopatric or parapatric with no clear geographic separation, but continuous genetic divergence.
- Category IV – often sympatric with continuous genetic divergence, and no barriers to movements or genetic mixing; most “nebulous” of all categories in management decisions.

All these data are then combined using a technique described by Dizon *et al.* (1992), so integrating all known information in a manner that allows both a comparison of putative populations, and also provides a guide as to how distant or close these populations are. This latter information may be the most useful when having to consider management issues where the need to manage strictly by population or geographic locality depends on how critical and urgent the conservation issue is. Several examples using harbour porpoise data are presented and explained.

Reference

Dizon, A., Lockyer, C., Perrin, W.F., DeMaster, D.P. and Sisson, J. (1992) Rethinking the stock concept: a phylogeographic approach. *Conservation Biology*, 6: 24-36.

4.13 Justification for Re-evaluation of the Concept of Management Units:

Per Palsböll

The identification of management units (MUs) is central to the management of natural populations and is crucial for monitoring the effects of human activity upon species abundance. Here, we propose that the identification of MUs from population genetic data should be based upon the amount of genetic divergence at which populations become demographically independent instead of the current criterion that focuses on rejecting panmixia.

MU status should only be assigned when the observed estimate of genetic divergence is significantly greater than a predefined threshold value. We emphasize the need for a demographic interpretation of estimates of genetic divergence given that it is often the dispersal rate of individuals that is the parameter of immediate interest to conservationists rather than the historical amount of gene flow.

Management units or units of conservation are typically defined as demographic entities between which birth and growth rates are uncorrelated (Palsbøll *et al*., 2007). However, to date, genetic analyses have been utilised towards this issue aimed at rejecting homogeneity in allele or mtDNA haplotype frequencies among putative management units (Moritz, 1994). With sufficient data (i.e., samples and genetic markers analyzed in each sample), homogeneity will be rejected even if the putative management units are demographically highly correlated (e.g., if migration rates are well above 10% per generation) (Palsbøll *et al*., 2007). Hence, the concept of simply rejecting homogeneity as a basis for defining units for conservation/management has become outdated, given that the statistical power of genetic assessments has now increased tremendously.

Palsbøll, Bérubé and Allendorf (2007) have argued that the initial step in defining management units is to determine at what rate of connectivity putative management units become sufficiently isolated from each other that vital demographic rates, such as birth and mortality rates, become uncorrelated. Consequently, any measure of genetic divergence needs to be translated into the equivalent demographic migration rate to determine if the genetic estimate of connectivity is significantly above or below the pre-determined threshold rate. In such cases, a decision may be made as to whether the units under study should be monitored as a single or multiple units of management/conservation. If, by contrast, the observed genetic estimate of connectivity does not differ statistically from the pre-defined threshold rate then the status of the target populations is unresolved and will require either (1) additional data, and/or (2) calculation of the relative likelihood of the two possible outcomes (one or more management units) (Palsbøll *et al*., 2007).

The translation of genetic estimates of divergence into a measure of demographic connectivity is not simple, especially as most population genetic estimators of connectivity have an evolutionary basis and thus rest upon assumptions about population sizes, gene flow and mutation rates over similar time scales, and would warrant further investigation (Palsbøll *et al*, 2007).

References

Moritz, C (1994) Defining Evolutionarily-Significant-Units For Conservation. *Trends in Ecology & Evolution*, 9(10): 373-375.

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5. CRITERIA FOR INVESTIGATING POPULATION STRUCTURE, AND THE STRENGTHS & LIMITATIONS OF DIFFERENT METHODOLOGICAL APPROACHES

In the previous section, a number of different methodological approaches for investigating population structure were reviewed by specialists in each. Those various approaches may provide different types of information: genetic markers may be largely neutral or to a greater or lesser extent under the influence of selection; they may be subject to Mendelian inheritance or be maternally inherited. Some are of greater cost than others. Genetic markers and skeletal variation tend to provide information about population structure at longer time scales than approaches like dietary studies using stomach contents or fatty acid profiles, and telemetry or photo-ID in studies of individual animals. On the other hand, there are analytical procedures for genetic markers that can be used which will to an extent address this.

Table 2 summarises the features of each approach. For each genetic marker (Table 2a), the mode of inheritance is shown, whether it is under selection or effectively neutral, the time scale to which the marker applies, likely sample sizes needed, overall relative cost, whether it is possible to obtain samples from living animals, how well it is at discriminating populations, and the level of variability in estimating parameters. For other non-genetic approaches (Table 2b), mode of acquisition, relative importance of evolutionary versus environmental influences, relevant time scale, relative cost, sample sizes needed, discriminatory power, and level of variability in the measurements are all considered. In a number of these cases, it is impossible to be prescriptive because it depends very much upon specific circumstances.

Frequently, a combination of approaches has been used in the assessment of population structure. It is important, however, to recognise the different information they convey, as well as the limitations that each may have. There is no single panacea for discriminating populations, and a suite of methods is generally the preferred option. Most are subject to the influences of both evolutionary history and local adaptation. For management purposes, differences in demography (birth & death rates, movements & ranging patterns) are likely to be more useful than the identification of differences in gene frequency that reflect historical levels of gene flow. On the other hand, the morphological and genetic changes that may have taken place over a longer time scale may be the best evidence of barriers to gene flow that persist to the present time. Finally, it is worth noting that the fact that no significant genetic differences have been found in a population sampled across a wide area such as the North Atlantic does not mean that there is no sub-structuring within the population, and from a management perspective, it may be prudent to split rather than lump management units on the basis of partial evidence such as spatial gaps in range, or lack of interchange of individual animals where reasonable sample sizes exist. These considerations have influenced our decisions over proposed management units.

Table 2. Methodological Approaches for the Study of Cetacean Population Structure

a) Genetic

MARKERS	Mode of Inheritance	Selection vs Neutral	Time scale of measurement	Size of sample needed	Overall Cost	Live sampling possible	Discriminatory Power	Level of variance in parameter estimation
mtDNA	Maternal	Both (commonly interpreted as neutral but selection can be important)	Can resolve contemporary and historical processes with appropriate analysis	Depends on question and variation within species	Moderate	Yes (skin swabs are fine)	Acts as single locus, so can have limitations in answering some questions because of this	Depends on parameters, sample size, and type of analysis
Microsatellites	Mendelian	Both (commonly interpreted as neutral but selection can be important)	Can resolve contemporary and historical processes with appropriate analysis	Depends on question and variation within species	Relatively Low	Yes (skin swabs can be problematic)	Power increases with number of loci, variation, and sample sizes of individuals	Depends on parameters, sample size, and type of analysis
MHC	Mendelian	Both (commonly interpreted as neutral but selection can be important)	Can resolve contemporary and historical processes with appropriate analysis	Depends on question and variation within species (Peptide binding regions for class I and/or class II loci)	Moderate	Yes (skin swabs are possible)	Depends on the extent and nature of selection (and drift)	Depends on parameters, sample size, and type of analysis
SNPs	Mendelian	Both (as depends on target loci)	Can resolve contemporary and historical processes with appropriate analysis (but depends on numbers of loci, and loci selected)	Depends on question and variation within species	Moderate (if locus markers available)	Yes (skin swabs are possible)	Need more loci relative to microsatellites to get equivalent resolution, but genotyping large numbers of loci easier	Depends on parameters, sample size, and type of analysis

b) Other Methods

METHOD	Mode of Acquisition	Evolutionary vs Ecological Influence	Applicable Time scale	Size of sample needed	Overall Cost (if samples available)	Live sampling possible	Discriminatory Power	Level of Variance in Measurement
Metrical skeletal	Genetic and Environmental	Both	Unknown, but probably several generations	Moderate to Low	Low	No	High	Variable
Non metrical	Genetic and Environmental	Both	Unknown, but probably several generations	Moderate to Low	Low	No	Low to Moderate	Unclear
Stable isotopes	Diet and water	Ecological	Weeks to a Lifetime	Low	Medium	Yes (but skin swabs problematic)	High	Low
Fatty acids	Diet	Ecological	Days to a Lifetime	Low	High	Yes	Moderate	Moderate
Parasites	Diet and Behaviour	Both	Days to a Lifetime	Low to Moderate	Low	No (with a few exceptions)	High	Low
Contaminants	Diet	Ecological	Days to a Lifetime	Low to Moderate	Medium to High	Mostly No	Variable	Low
Diet	Prey availability and energetic needs	Ecological	Days	Low to Moderate	Low	No	Variable	Moderate
Life History	Genetic and Environmental (including population carrying capacity)	Both	Generation(s)	Moderate to High	Low to Medium	No (unless live capture)	Variable	Moderate
Telemetry	Movements and Distribution	Both	Weeks to a Lifetime	Low	High	Yes (but capture may be necessary)	High	Low
Photo ID	Movements and Distribution	Both	Months to a Lifetime	Moderate	Low	Yes	Moderate	Variable

6. SAMPLING CONSIDERATIONS

The original objectives of this Population Structure workshop included a more detailed review of sampling protocols (sample sizes, spatial and temporal intervals between sampling points, etc.), methodologies for sample collection, and standardisation of laboratory techniques. However, it was decided that much of that was beyond the scope of this particular workshop, given the time available and the fact that these issues are very complex. It would require a wider representation of laboratories around Europe as well as some considerable time devoted to consideration of the different procedures required depending upon the specific approach, cetacean species, region, and circumstance.

Instead, it was felt that the most constructive measure would be to summarise where possible what data are already held by different research groups within the ASCOBANS region, together with their format, by developing meta-databases for the major small cetacean species (harbour porpoise, bottlenose dolphin, short-beaked common dolphin, white-beaked and Atlantic white-sided dolphin) that were the focus of attention in this workshop. These meta-databases are available as Excel spreadsheets and though certainly incomplete, they can at least form the basis for future development of sampling protocols.

We did, however, attempt to provide a rough power analysis with some guidelines in relation to recommended sample sizes and costs, and these are presented below:

Genetic analyses: *Rus Hoelzel*
As with other approaches, sampling considerations need to be on a case-by-case basis. The sample size will depend on the power needed, and for this one may use some type of power analysis (e.g. the simulation approach implemented in POWSIM - see Ryman and Palm, 2006). A first approximation is 30-50 samples per putative population, although this depends upon effective population size (Ne). Much more samples will be necessary if Ne is large, so it is better to use a power analysis whenever one can. The cost varies depending on the type of study. For microsatellite analyses, ten microsatellites multiplexed may be possible for 20-25 Euros per sample, while adding mtDNA would increase this overall to about 35 Euros per sample. This does not include the labour costs, however.

Reference
Ryman, N. and Palm, S. 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes*, 6: 600-602.

Skeletal variation: *Anders Galatius*
Useful studies can be done with around 20 specimens from each sample compared. If differences are large and one limit oneself to few variables, as a rule of thumb, less than the minimum sample size, this can be done. The more variables that one includes and the smaller inter-population differences there are, the larger the sample size that will be needed.

As for costs, equipment prices from 100 Euros up to 100,000 Euros depending on what one needs, from calipers to high/end 3D scanners. At least 3,500 Euros are needed for quality 3D analysis equipment. If one has museum samples available, then

sampling costs are more or less equivalent to just travel expenses. Otherwise, if one has to build up collections of skeletal material, this will be rather labour intensive.

Parasites: *Kristina Lehnert*
For a parasitological analysis to discrimante between host populations, one would need to investigate c. 30 animals per region. The overall costs including storage and delivery of carcasses, necropsy and all the standard examinations (histology, bacteriology plus parasitological work), as well as labour for investigations of parasite prevalence, intensity, species identification, etc, amount to c. 2,000 Euros per sample.

Contaminants: *Florence Caurant*
Trace elements: Sample sizes of 30-50 per putative population are recommended. Because of the influence of age on trace element concentrations, the age distribution of individuals has to be comparable between sampled populations. Several trace elements can be run together (when ICPMS is used), and analysis can be conducted for 10-20 Euros per sample.

Organic compounds: Males are preferred to females, for which pregnancies and lactation constitute a way of POPs elimination. Organochlorines are numerous, but for PCBs, the cost per sample is 100-150 Euros.

Stable Isotopes: *Rus Hoelzel*
Sample sizes of 30-50 per putative population are recommended initially. Costs (excluding labour) depend upon the stable isotope being examined. For isotopes of carbon and nitrogen (which can be run together), analyses can be conducted for 11.5-17.5 Euros per sample; for isotopes of hydrogen and oxygen (also run together), the costs per sample are 16-18 Euros per sample; whilst analyses of isotopes of sulphur cost c. 35 Euros per sample.

Telemetry: *Jonas Teilmann*
The tagging of 20-30 animals from each area for at least a few months will provide a good knowledge of population structure. However, it will depend on species: in some species, tagging a few animals will tell the whole story while in others one will need a lot of tracks to understand population movements and distribution. The cost per tag depends mostly on the logistic cost in tagging the animals. Otherwise, the cost of instruments, obtaining the data and analysing them, will be in the order of 3,000 Euros per animal, i.e. 90,000 Euros for a sample size of thirty animals.

Photo-ID: *Peter Evans*
The number of identifiable individuals required to have a reasonable chance of understanding population structure will depend largely upon the estimated population size and proportion exhibiting unique markings. This will vary between species and areas. For a population size of c. 200 individuals with 40% recognisably marked, this amounts to a potential maximum number available for re-sighting of 80 individuals. This value will be lower if a smaller proportion can be identified, and in such cases, it may be impossible to resolve populations from movements. In the absence of a full power analysis, as a rule of thumb, ideally one should have c. 20-30 re-sightings of at least one-quarter of the putative population available for re-sighting. In the above example, that would represent 20 individuals. On the other hand, it may be that a few

re-sightings in the range of another putative population may occur with smaller numbers of sightings, and thus indicate mixing.

Besides labour, the cost of photo-ID comprises mainly camera equipment and that of boat charter/usage. For a digital SLR camera equipped with telephoto zoom lens capable of shooting at 5 frames per second or more, the current cost is c. 2,000 Euros. Following image taking, the time involved in sorting & editing images, and then looking for matches can be considerable, although semi-automatic matching software exists that can speed up the latter process. In our experience, with a catalogue of 300 individuals, these tasks require c. 3 months work per year.

Life history variation: *Christina Lockyer*
In some cases, samples of a minimum of 30 may be sufficient to provide a good ball-park idea of whether distinct populations exist. However, some life history parameters will require stratification of samples by sex and maturity, or even age, so that overall sample sizes may exceed 100–150, or even more, to be meaningful, e.g. age at sexual maturity, growth parameters, etc. Clearly, for comparisons between putative populations, the mean life history values should have acceptably low SDs to distinguish between them.

Approximate costs (in USD) are given below (although these will vary from country to country):

Age determination of toothed small cetaceans:
1) Capital equipment investment - cutting machine, tools and diamond saw blades - ca 1,500 USD;
2) Cryostat or freezing microtome - costs variable from 100,000 USD (expensive cryostat) to 5,000 USD (for simplest portable machine).
3) Lab reagents and chemicals, plus glassware for basic batch to do up to 200 hundred teeth - 800 USD
4) Labour - in total about an hour per tooth; could do up to 50 teeth in a working week - depends on method and tooth size and species. Note this does not include the extraction and pre-preparation of the teeth.

Reproduction:
Ovaries -
Examination of corpora and follicles:
1) Can be sliced (1mm) by hand or with a slicing machine - this latter will increase capital expenditure. Labour - ca 30 min per animal (2 ovaries) - depends on age of female; could take an hour plus, if photos required, etc.
2) If histological examination required, then capital investment could be very costly if using automatic Shandon (for example) tissue processor and microtome.
2a.) Equipment - up to 80,000 USD for all items; maybe 20,000 USD for simplest sledge microtome if process tissues by hand.
2b) Consumables - solvents, chemicals and reagents, glassware - approx. 1,500 USD per 200 samples.
2c.) Labour - intensive if by hand - up to 2 days per specimen.
Testes -
Histological examination required for accurate diagnosis of maturity status.
Similar equipment and labour costs to ovary examination 2).

7. SPECIES CASE STUDIES

The following are abstracts concerning studies of population structure for the five target species, which were provided specifically for this workshop.

7.1 Harbour Porpoise

7.1.1 Population structure of the harbour porpoise, *Phocoena phocoena*, in eastern and central North Atlantic in 2001

Liselotte W. Andersen, Daniel E. Ruzzante, Michael Walton, Per Berggren, Arne Bjørge, and Christina Lockyer

Based on variation at 12 microsatellite loci, the population structure of 807 harbour porpoises, *Phocoena phocoena*, collected from Ireland, England, Scotland, Shetland, Netherlands, Danish North Sea, Skagerrak, Kattegat, Belts Swedish Baltic, Norwegian Westcoast and West Greenland were analysed. No structure within the different regions was observed. This was illustrated focusing on the samples from the Danish North Sea, Skagerrak, Kattegat-Belts and Swedish Baltic Sea where neither significant multilocus tests for allele frequency differences nor significant F_{ST} estimates were observed between Danish North Sea – Skagerrak and Kattegat-Belts and the Swedish Baltic samples. Only between Skagerrak and the pooled IDW (Inner Danish Waters) sample (Kattegat-Belts and Swedish Baltic Sea) were significantly different allele frequency differences across the 12 loci observed. After pooling samples within regions, multilocus tests for allele frequency differences, population structure estimates (F_{ST}) and genetic distance measures (D_{LR} and D_C) all indicated six genetically differentiated populations/sub-populations. Harbour porpoises from West Greenland, the Norwegian Westcoast, Ireland, the British North Sea, the Danish North Sea and the inland waters of Denmark (IDW) were all genetically distinguishable from each other. A sample of harbour porpoises collected off the Dutch coast (mainly during winter) was genetically heterogeneous and likely comprised a mixture of individuals of diverse origin. Mixed stock analysis indicated that most of the individuals in this sample (~77%) were likely migrants from the British and Danish North Sea.

7.1.2 Morphometric and genetic differentiation of the Harbour Porpoise (*Phocoena phocoena*) around the North Sea and adjacent seas

Carlos De Luna, Oliver Thatcher, Simon Goodman, Liselotte Andersen, Krystal Tolley and A. Rus Hoelzel

The harbour porpoise *Phocoena phocoena* is a small odontocete inhabiting cold and temperate waters in the Northern Hemisphere, and we studied putative populations that inhabit a relatively small geographic area in the North Sea and adjacent seas. To assess differentiation among putative populations, we measured 16 traits on 462 skulls and DNA variation at 12 microsatellite DNA loci. Discriminant function analysis (DFA) was performed for the assignment of individuals by skull morphometry. One discriminant function (DF1) was significant ($p<0.001$). DF1

reflected the length and width of the oral cavity; DF2, while not significant, reflected the size of the ocular orbit.

Three populations were successfully classified: British (BRIT), Danish (DK) and Norwegian (NOR). The discrimination between NOR from both BRIT and DK was by far the strongest based on the morphological characters (and especially DF1). For molecular genetic analyses, both assignment tests (implemented in the software STRUCTURE) and F_{ST} (values for nine microsatellite loci ranged between 0.04 and 0.05) showed weak but significant differentiation between the three putative populations. Previous studies have shown a difference in the choice of prey for porpoises inhabiting these different areas. BRIT and DK porpoises forage in relatively shallow waters preying mainly on benthic species; whereas NOR porpoises prey mainly on mesopelagic and pelagic fish. Here we suggest that the differentiation observed may be explained as a result of resource specialization and adaptation to local habitat.

7.1.3 Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters

Michael Fontaine

Understanding the role of seascape in shaping genetic and demographic population structure is highly challenging for marine pelagic species such as cetaceans for which there is generally little evidence of what could effectively restrict their dispersal. In the present work, we applied a combination of recent individual-based landscape genetic approaches to investigate the population genetic structure of a highly mobile extensive range cetacean, the harbour porpoise, in the eastern North Atlantic, with regards to oceanographic characteristics that could constrain its dispersal.

Analyses of 10 microsatellite loci for 752 individuals revealed that most of the sampled range in the eastern North Atlantic behaves as a 'continuous' population that widely extends over thousands of kilometres with significant isolation by distance (IBD). However, strong barriers to gene flow were detected in the south-eastern part of the range. These barriers coincided with profound changes in environmental characteristics and isolated, on a relatively small spatial scale, porpoises from Iberian waters and on a larger scale, porpoises from the Black Sea. The presence of these barriers to gene flow that coincide with profound changes in oceanographic features, together with the spatial variation in IBD strength, provide for the first time strong evidence that physical processes have a major impact on the demographic and genetic structure of a cetacean. This genetic pattern further suggests habitat-related fragmentation of the porpoise range that is likely to intensify with predicted surface-ocean warming.

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7.1.4 A genetically separate Baltic population? Some statistical issues

Anna Palmé

There is a strong conservation concern for the harbour porpoises *Phocoena phocoena* of the Baltic Sea that rests on the assumption that these porpoises represent a genetically distinct population that is reproductively separated from adjacent populations to the west. We argue that current scientific support for this claim is weak, and that additional analyses are needed to identify the most appropriate management approach for the Baltic porpoises.

We reviewed current knowledge on the population genetic structure of harbour porpoise in Swedish and adjacent waters. The work was aimed at providing management guidelines and was initiated by the Swedish Environmental Protection Agency (Palmé *et al.*, 2004, 2008a). The basic question regarding management is whether Baltic harbour porpoises should represent a separate management unit, or if they should be managed jointly with porpoises further west.

We re-analyzed the data of Wang and Berggren (1997) focusing on the mtDNA haplotype frequency difference between the Swedish Baltic and the Kattegat-Skagerrak areas. Wang and Berggren report a significant P-value of P=0.035 for this difference. In contrast, our recalculations did not support the notion of statistical significance (Palmé *et al*., 2008a,b). Thus, if delineation of management units were to be based on the existence of statistically significant gene frequency differences, then in our view there is no support for treating the Baltic segment as a separate unit. We note, however, that the evidence supporting such a notion is quite weak, considering that data refer exclusively to mtDNA and that the test is associated with a low statistical power (Ryman *et al*., 2006).

The reason for our contrasting results (significance vs. non-significance) appears to be as follows. In standard statistical hypothesis testing, the probability of interest is that of obtaining (under the null hypothesis) an outcome “as bad as or worse than” the one observed. Most basic statistics textbooks (e.g. Sokal and Rohlf 1995) recommend this approach, which is reflected in the non-significant P-values we presented (Palmé *et al*., 2008a). Wang and Berggren (1997), however, only reported the probability of an outcome “worse than” the one observed (ignoring “as bad as”), thereby making it “easier” to obtain significance.

Palsbøll *et al.* (2007) suggested an alternative way of delineating management units on the basis of the rate of migration (m). Following Palsbøll *et al.* (2007), we considered an exchange of migrants of 10% or more ($m \geq 0.10$) to imply a degree of demographic connectivity justifying the two population segments to be managed jointly as a single unit. By contrast, a migration rate of less than 10% ($m < 0.10$) could justify separate management units.

The true degree of migration between porpoises in the Baltic and waters further west, is unknown. However, assuming an effective population size of 200 in the Baltic

(Hiby and Lovell 1996; Berggren 2003), an even sex ratio, and migration-drift equilibrium, a migration rate of 10% or more would correspond to an $F_{ST} \leq 0.05$ for mtDNA. Conversely, a higher degree of isolation (i.e. $m<0.10$) would correspond to a larger F_{ST} under the present assumptions ($F_{ST}>0.05$).

The haplotype frequency point estimates are very similar for the Baltic and the Kattegat-Skagerrak, yielding an $F_{ST}=0.007$, as calculated from the data given in Wang and Berggren (1997; Table 1). We used a random number simulation approach to address the question whether the observed estimate of $F_{ST}=0.007$ was more likely to be obtained under either of the two contrasting hypotheses on migration rates ($m \geq 0.10$ corresponding to $F_{ST} \leq 0.05$ vs. $m<0.10$ corresponding to $F_{ST}>0.05$). We found that the observed $F_{ST}=0.007$ was about equally likely under both hypotheses. Thus, we consider the results of Wang and Berggren (1997) inconclusive with respect to the basic question whether the Baltic segment should be managed as a separate unit or not. In other words, their data are insufficient to resolve the management unit status of the Baltic porpoises, if delineation of such units is to be based on the level of migration.

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7.1.5 The Baltic Harbour Porpoise

Per Berggren

Wang and Berggren (1997) investigated the population structure of harbour porpoises in the Baltic region using mitochondrial DNA analysis (nine restriction enzymes) of samples from 65 by-caught animals retrieved from the Baltic Sea (n=27), the Kattegat-Skagerrak Sea (n=25), and the west coast of Norway (n=13). The stated objective in the study was to investigate whether porpoises from these areas belonged to the same or separate genetic entities. Analysis of heterogeneity in the frequency distribution of haplotypes among the three sampling areas revealed significant differences, which supported the recognition of three different subpopulations.

The results in the paper by Wang and Berggren (1997) have been criticised in a recent publication by Palmé *et al.* (2008); more specifically, Palmé *et al.* (2008) questioned the p-value presented by Wang and Berggren (1997) that indicated significant differences between the Baltic and the Kattegat-Skagerrak sampling areas (P=0.035). Berggren and Wang (2008) then responded to the critique outlined below. Neither we (Wang and Berggren, 1997) nor Palmé *et al.* (2008) presented the two P-values from the Monte Carlo χ^2 simulation analysis in *REAP v. 4.1* (McElroy *et al.*, 1992): the probability of exceeding the original χ^2 by chance, and the probability that includes ties. We (Wang & Berggren, 1997) believed that the correct P-value to report was the former. In retrospect, both P-values should have been presented and discussed. Regardless, the results and conclusion are unaffected.

Our dataset was re-analysed independently by Dr Patricia Rosel (National Marine Fisheries Service, USA) using *REAP v. 4.1* with the following results: the calculated χ^2 value was 8.3 (mean χ^2 = 6.1, range 3.9–10.2); P = 0.034 ± 0.0018 (341 replicates without ties) and 0.079 ± 0.0028 (449 replicates with ties). The slight differences between these values and those in Palmé *et al.* (2008) and Wang and Berggren (1997) are probably due to the algorithm's randomisation procedure.

The many tied χ^2 values are due to the small sample size relative to the population's genetic diversity, and reflect low analytical power for detecting differences. Both P-values represent potential correct values derived from the analyses; with the actual probability in the range 0.034–0.079, but further resolution requires a larger sample size. Nevertheless, even the higher P-value (0.079) suggests structure. Given low analytical power, this P-value may even be stronger evidence of differentiation than the significant P-value that excludes ties. Furthermore, we argue that any P-value <0.1 is grounds for prudent conservative management (i.e. in this case recognising Baltic porpoises as genetically distinct). Finally, the claim by Palme *et al.* (2008) of no evidence for genetic distinctness ignored differences in other characters (e.g. craniometry; Börjesson and Berggren, 1997) and ecological markers (e.g. organochlorine contaminants (Berggren *et al.* 1999). Furthermore, satellite telemetry movement data of harbour porpoises tagged in inner Danish Waters (the Danish Belt Seas) show limited movement of tagged animals into the Baltic (Teilmann *et al*., 2008). Only one juvenile male porpoise of the 37 tagged animals moved into the Baltic proper for a short time period during spring in year 2000. This information indicates limited movement of migrants into the Baltic Sea from neighbouring areas.

Misunderstanding statistical results in analyses of population structure can jeopardise biodiversity conservation. Consideration of the errors in hypothesis testing is crucial, i.e. of Type I (rejecting a true null hypothesis) and Type II errors (failing to reject a false null hypothesis). In tests for population structure, the null hypothesis is usually that there are no differences between provisional populations, and the threshold for rejection is α . 0.05. Thus, there is a 1/20 chance of a Type I error; the probability of a Type II error depends on both α and sample size; increasing either reduces the Type II error risk and improves the power to detect population differences. With the Baltic harbour porpoise population being very small (Berggren *et al.*, 2004), the consequence of a Type II error increases the likelihood of extirpation (a non-reversible outcome), and conflicts with the precautionary principle of conservation.

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7.2 Bottlenose Dolphin

7.2.1 Bottlenose Dolphins from the Western North Atlantic

Aleta Hohn and Larry J. Hansen

Summary of findings
Using genetics, stable isotope ratios, photo-identification, and telemetry, the single stock of bottlenose dolphins along the Atlantic coast of the U.S. was redefined as seven management units several years ago. Since then, additional results from telemetry and photo-identification indicate a more complicated structure on the coast, as well as within the estuaries. Photo-identification has proven useful not only for individual identification and recapture, but also for defining characteristics of groups, such as *Xenobalanus* load, that contribute to distinguishing possible management units. Structure seems to occur as a function of many factors, including latitude, distance from shore or depth, localized habitats (estuaries or areas with estuarine systems), and behaviour (e.g., migratory or non-migratory) in the absence of physical barriers to movements or mixing.

Implications for using particular method(s)
Genetic results are not yet sufficient to assign an individual animal to a management unit. The other methods complement the genetics, sometimes providing initial evidence of differentiation that help determine where genetic sampling should be conducted. Photo-identification effort has been limited to a few discrete sites, with the "footprint" of each site being small relative to the area inhabited by the likely metapopulation. Results can be overly narrow and miss mid- to large-scale movements. Stable isotopes analyzed from skin samples reflect only the recent history of movements/habitat for an animal while samples from crushed, homogenized teeth represent the average over a lifetime, obscuring possible migratory patterns or movements between estuaries and the coast. Telemetry has possibly the greatest potential for defining limits of subunits if a sufficient number of tags can be deployed, which is difficult to achieve for many small cetaceans. More recently, it seems that even low-tech methods, such as using group characteristics, may prove useful.

Metapopulation considerations
We cannot yet define the limits of our metapopulation(s), and do not yet know the number and limits of subunits included in any metapopulation(s). In the coastal environment, we have identified separate management units even though there are no fragmented "landscapes" or barriers along the coast as are often considered necessary for metapopulations. Individual estuaries could be considered fragmented habitat and it does appear that many of the estuaries have resident bottlenose dolphins that may be considered management units.

Boundary recommendations for management purposes
We think it is important to fully explore and understand the complex, underlying structure of the population. However, for management purposes, boundaries may have to be practical rather than based on biological parameters. For example, off the coast of North Carolina, we have three sympatric management units during the

winter. Yet, because we cannot assign an individual animal to a stock, the three management units are managed as one "Winter Mixed Management Unit" with a PBR calculated from the estimated winter abundance of the mixed units. Thus, if an animal becomes entangled, that mortality is applied toward the Winter Mixed Management Unit. This approach may not be conservative because if the mortality came from the smallest of the management units, the implications may be different than if it came from the largest.

Recommendations
- focus on science then overlay management
- use multiple techniques where possible because they are complementary
- develop capability for simultaneous analysis of results from various methodologies (genetics, isotopes, telemetry, morphology, *Xenobalanus* load, etc).

7.2.2 Bottlenose dolphin genetic variation in UK waters

Valentina Islas

Previous work by Parsons *et al* (2002) on the population structure of bottlenose dolphins in UK coastal waters found low levels of genetic diversity along the East Coast of Scotland, and in Welsh populations. They also found less evidence of genetic differentiation between the populations of the East Coast of Scotland and those in Wales than between the neighbouring coastal populations of East and West Scotland. Since the sample size of this initial study was limited (29 individuals), these surprising findings need to be confirmed with larger samples. In addition to a larger number of samples provided by the stranding networks in the country, we have biopsy samples from the East Coast of Scotland as part of our project that looks at the patterns of association and relatedness of the dolphins in this population. The total number of samples collected from biopsies and strandings is the following: East Coast (n=69), West Coast (n=19), Wales (n=15), and England (n=7). We are using 15 microsatellite *loci* and a 500 bp segment of the mitochondrial control region.

Preliminary mtDNA results confirm the findings of Parsons *et al*. with significant population structure between the East Coast of Scotland and Wales, and a higher separation between the West and East Coasts of Scotland.

We also found a lower genetic diversity in the East Scottish population, in both nucleotide and gene diversities. These results confirm that the low level of genetic diversity previously found for the mitochondrial control region of the East Coast population of bottlenose dolphins is unlikely to be due to small sample size, and suggests that further analysis of samples and additional genetic markers will determine the population genetic structure.

Reference

Parsons, K.M., Noble, L.R., Reid, R.J., and Thompson, P.M. (2002) Mitochondrial genetic diversity and population structuring of UK bottlenose dolphins (*Tursiops truncatus*): is the NE Scotland population demographically and geographically isolated? *Biological Conservation*, 1038: 175-182.

7.2.3 Genetic variation of bottlenose dolphins in Eastern North Atlantic

Ada Natoli

The ASCOBANS area includes the northernmost habitat for the bottlenose dolphin. In my research, twenty samples were analysed from Scotland and 35 samples from the Eastern North Atlantic (6 from south of England, 18 from Spain, and 11 from Portugal) in the context of a broader study that included samples from the Mediterranean and Black Sea.

The target of the study was to assess the population structure across this geographic range and to identify the possible mechanisms that could drive such structure.

A total of 145 samples were analysed using 9 microsatellite loci (bi-parentally inherited) and 630 bp for the mtDNA control region (maternally inherited).

Strong population structure was found across the whole range reflecting the different habitat characteristics observed in the different areas, using both a Bayesian base method (Structure) and a classic F_{ST} statistics. Also, both markers gave consistent results showing that both males and females have a similar dispersal pattern and no sex-biased dispersal was detected. Asymmetrical migration (Migrate) rate based on the mtDNA data showed some directional migration from the populations inhabiting the peripheral habitat areas, including Scotland.

Within the ASCOBANS area, marked genetic differentiation was detected between the Scottish samples and the samples from the rest of the Eastern North Atlantic (F_{ST}=0.068, $p<0.001$). No significant genetic differentiation was detected among the samples from different areas of the Eastern North Atlantic (Portugal, Spain, and South of England). However, the data set is limited, as it does not cover areas like Ireland, France and the Baltic coast. Also, it does not analyse any local resident populations, often observed in the area (e.g. Shannon Estuary, Cardigan Bay, Cornish waters). Considering the pattern of population structure observed for this species, it would be advisable to extend the analysis, assessing the identity of the local populations as well as including samples from the areas not covered by previous studies.

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7.2.4 Historical genetic variability in bottlenose dolphin populations in UK waters

Courtney Nichols, Jerry Herman, Oscar E. Gaggiotti, Keith M. Dobney, Kim Parsons, and A. Rus Hoelzel

A number of dolphin species, though highly mobile, show genetic structure among parapatric, and sometimes, sympatric populations. However, little is known about the temporal patterns of population structure for these species. Here we apply Bayesian inference and data from ancient DNA to assess the structure and dynamics of bottlenose dolphin (*Tursiops truncatus*) populations in the coastal waters of the UK. We show that regional population structure in UK waters is consistent with earlier studies suggesting local habitat dependence for this species in the Mediterranean Sea and North Atlantic. One genetically differentiated UK population went extinct at least 100 years ago and has not been replaced. The data indicate that this was a local extinction, and not a case of historical range shift or contraction. One possible interpretation is a declining meta-population and conservation need for this species in the UK.

Reference

Nichols, C., Herman, J., Gaggiotti, O.E., Dobney, K.M., Parsons, K., and Hoelzel, A.R. (2007) Genetic isolation of a now extinct population of bottlenose dolphins (*Tursiops truncatus*). *Proc. Royal Soc*. B, 247: 1611-1616.

7.3 White-beaked & Atlantic White-sided Dolphins

7.3.1 Status and distribution of white-beaked and Atlantic white-sided dolphins in the Eastern North Atlantic

Peter Evans

White-beaked dolphin

Population distribution Restricted to northern North Atlantic, from South-west and Central East Greenland, Svalbard and Barents Sea, south to about Cape Cod (USA) and the Bay of Biscay (Reeves *et al*., 1999a). It occurs over much of the Northern European continental shelf; it is common in British and Irish waters, most abundantly in the Central and northern North Sea across to West Scotland and Ireland; occasional off S Ireland, in the Irish Sea, Western Channel, and northern Bay of Biscay (Evans, 1980, 1990, 1992; Baptist, 1987; Northridge *et al*., 1995, 1997; Williams *et al*., 1999; Hammond *et al*., 2002; Evans *et al*., 2003; Reid *et al*., 2003). It has a similar distribution to the Atlantic white-sided dolphin, though less pelagic, generally more abundant on the continental shelf.

A general decline (numbers sighted per unit effort) observed in NW Scotland since the early 1980s, may reflect distributional change (Evans, 1992; Evans *et al*., 2003; MacLeod *et al*., 2005). Strandings have significantly increased in the southern North

Sea since the 1960s, and it now regularly occurs in the Southern Bight (Bakker and Smeenk, 1987; Kinze *et al.,* 1997). Other important concentrations occur off northern Norway (Øien, 1996).

Seasonal occurrence The species is recorded throughout the year in British waters, but largest numbers are seen in late summer, July-September. It may move offshore in winter, although poorer coverage may explain lower numbers seen during this period in near-shore waters (Evans, 1980, 1992; Northridge *et al.,* 1995, 1997; Evans *et al*., 2003).

Habitat Preferences It is found in cool and sub-arctic waters, usually over the continental shelf in waters 50-100 m deep. From the Sea Watch database, 75% of sightings in NW European waters occurred at SSTs of 11-13^{o} C (total range including outliers 3-17^{o} C) (Anderwald, 2002).

Population Abundance SCANS II, July 2005, covering European continental shelf seas from SW Norway, south to Atlantic Portugal), gave an estimate of 22,700 (CV = 0.42) (P.S. Hammond, *pers. comm.*).

Atlantic white-sided dolphin

Population distribution Restricted to northern N Atlantic, mainly in offshore waters, from SW Greenland, Iceland and western Barents Sea south to Virginia (USA) and the Bay of Biscay. It is less common than white-beaked dolphin on the European continental shelf (Evans, 1992; Øien, 1996; Northridge *et al.,* 1997; Hammond *et al*., 2002; Evans *et al*., 2003; Reid *et al*., 2003).

Off the British Isles, it is concentrated around the Hebrides, Northern Isles and northern North Sea, but extends south along the Atlantic seaboard, mainly outside or near the continental shelf (c. 200 m depth), W and S of Ireland, and in the Bay of Biscay; it is rare in the Irish Sea, the English Channel and southern North Sea. (Evans, 1980, 1992; Leopold and Couperus, 1995; Couperus, 1997; Kinze *et al.,* 1997; Northridge *et al.,* 1997; Reeves *et al.,* 1999b; Williams *et al*., 1999; Evans *et al.,* 2003; Reid *et al*., 2003).

Seasonal occurrence It is most commonly observed over the UK continental shelf July-September; apparently concentrated in deep waters off shelf edge between November-May (Evans 1980, 1992; Northridge *et al*., 1995, 1997; Leopold and Couperus, 1995; Evans *et al*., 2003; Reid *et al*., 2003).

Habitat The species is more pelagic than white-beaked dolphin, occurring mainly along edges or seaward of continental shelves, over depths of 100-300 m. It sometimes comes onto continental shelf, and may enter fjords and inlets with depths of less than 50 m. From the Sea Watch database, 75% of sightings in NW European seas recorded at SSTs of 7-13^{o} C (total range including outliers 6-17.5^{o} C) (Anderwald, 2002). In eastern United States, the species occupies waters of 1-13^{o} C in spring and autumn, but most occur in waters of c. 5-11^{o} C (Selzer and Payne, 1988).

Population abundance There are no comprehensive population estimates; an estimate of 5,867-18,528 *Lagenorhynchus* including an unknown proportion of white-sided

dolphins in North Sea and adjacent waters, was made during SCANS I in July 1994 (Hammond *et al*., 2002).

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7.3.2 Life history variation in white-beaked and Atlantic white-sided dolphins in the North Atlantic

Anders Galatus & Carl C. Kinze

White-beaked Dolphin

Population structure

Reflecting on a gross scale the distribution of shelf waters of the Northern North Atlantic, four principal centres of high density can be identified: 1. The Labrador Shelf including South-western Greenland. 2. Icelandic waters. 3. The waters around Scotland, including the northern North Sea. 4. The narrow shelf stretch along the Norwegian coast, extending north into the White Sea. The population structure is not known in any detail. So far, a comparison has only been conducted between areas 1 and 3 revealing significant differences in a suite of skull distance measurements (Mikkelsen and Lund, 1994). The size of these putative populations is not known, but abundance estimates are available from the Labrador coast; "at least several thousands" (Alling and Whitehead 1987) and the greater North Sea; 7,856 (95% CI 4,032-13,301) (Hammond *et al.*, 1995).

Life history

In an ongoing effort to establish hitherto poorly known biological parameters for this species, we have performed age estimates on a sample of 86 specimens stranded in Danish waters since 1980. Preliminary results reveal that females on average become physically mature at lengths of 251 cm, males at lengths of 271 cm, corresponding to mean ages of 15.6 yrs (95%CI: 9.8-23.1) and 11.4 yrs (95%CI: 7.7-18.1), respectively. Females attain sexual maturity at a mean age of 8.7 yrs (95%CI: 5.1-14.6), males at 11.6 yrs (95% CI: 8.2-16.1). The mean lengths at sexual maturity were found to be 240 cm and 270 cm in females and males, respectively. There is a marked seasonality in the testes size of mature males. During the mating season (July and August) the combined testes mass has increased six times from 500 g to nearly 3 kg. The gestation period lasts about 11 months. Our studies indicate a rather high annual ovulation rate of 0.7. Pregnant females were rare in the sample (2 of 23 sexually

mature specimens) indicating either longer periods of resting between parturitions, lower mortality, and/or segregation of breeding females.

7.3.3 Genetic variation in white-beaked and Atlantic white-sided dolphins in the North Atlantic

Eulalia Banguera-Hinestroza, Bob Reid, Arne Bjørge, Luca Mirimin and A. Rus Hoelzel

Two species in the genus *Lagenorhynchus* are found in the North Atlantic, each of which is subject to fisheries by-catch and of conservation concern. In this workshop we show results on the genetic diversity and population structure of white-sided dolphins (*Lagenorhynchus acutus*) and white-beaked dolphins (*Lagenorhynchus albirostris*) in the eastern North Atlantic.

We examined variation at 10 microsatellite loci for 83 samples of *L. acutus* and 15 microsatellite loci for 70 samples of *L. albirostris* from different geographical areas. A subset of tooth samples from museums were also analysed with 5 microsatellite loci for each species. We also tested the variation for each species in the control region for 166 samples of *L. acutus* and 122 samples of *L. albirostris* in four geographical areas, including a population from the Northwest Atlantic.

The overall mtDNA gene diversity was moderate in *L. acutus* (0.814 ± 0.026, π: 0.00714 ± 0.00053), and slightly lower in *L. albirostris* (0.719 ± 0.031; π: 0.0060 ± 0.00051). Two different approaches were used to assess population genetic structure in both species, the assignment test showed evidence for geographic structure in *L. albirostris* but not in *L. acutus*; however the Fixation index test (F_{ST}) showed a clear differentiation between some geographic populations for both species.

The more pelagic white-sided dolphin shows relatively little differentiation across the North Atlantic, while the more coastal white-beaked dolphin shows fine-scale population structure and relatively high F_{ST} values, although no differentiation within the North Sea. Mismatch distributions and diversity levels suggest the possibility of a bottleneck sometime in the past for both species, possible associated with expansion after the LGM.

7.4 Short-beaked Common Dolphin

7.4.1 Genetic variation in short-beaked common dolphins from the Atlantic, Mediterranean and Black Seas

Ada Natoli

Common dolphins are widely distributed around the world in both temperate and tropical waters. As for the bottlenose dolphin, the ASCOBANS area represents one of the northernmost habitat ranges for this species.

A total of 122 samples from the ASCOBANS area were analysed for 9 microsatellite loci and 369bp of the mtDNA control region (16 samples from Portugal, 39 from Galicia, 41 from the Celtic Sea, and 26 from Scotland). Samples from the Black Sea, Mediterranean Sea, South Eastern Atlantic and Western North Atlantic, were also included (Natoli *et al*., 2006, 2008).

The analyses were conducted on the microsatellite dataset using a Bayesian based method (Structure) and classic F_{ST} statistics. Structure failed to identify any genetic structure among these populations, indicating the most probable number of population K=1. However, F_{ST} was significant for some of the population comparisons (Scotland versus the Celtic population and the Galicia population) indicating the presence of some population structure. Low or not significant F_{ST} values were also observed between eastern Atlantic populations and the WNA population, suggesting gene flow among these regions, despite the large geographic distance.

Table 1: F_{ST} values between pairwise populations. The values in red are not significant (p>0.05) SEA = South Eastern Atlantic, WNA (Western North Atlantic), Alboran Sea = Western Mediterranean; Ionian Sea = Eastern Mediterranean

	BlackSea	Ionian	Alboran	Port	Galicia	Celtic	Scotland	SEA
BlackSea								
Ionian	0.09936							
Alboran	0.10162	0.05219						
Port	0.09554	0.05292	-0.00183					
Galicia	0.09665	0.04807	0.003	-0.00027				
Celtic	0.09854	0.04983	0.01075	0.00246	0.0047			
Scotland	0.13057	0.05984	0.01279	0.00705	0.01225	0.01134		
SEA	0.08251	0.04014	0.00779	0.00523	-0.00201	0.00115	0.01019	
WNA	0.11665	0.05233	0.01964	0.01364	0.01379	0.02285	0.01147	0.02137

The mtDNA analysis showed similar results with the Galicia, Celtic and Scottish population not showing any significant differentiation. The high number of shared haplotypes and the lack of any geographic clustering suggested a high level of gene flow among these populations. Interestingly, neutrality tests, based on Fu's Fs, showed high and significant values for the Portugal, Galicia and Celtic populations, suggesting possible population expansion. In conclusion, short beaked common dolphins show low differentiation over large geographic ranges and evidence of gene flow across oceans.

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7.4.2 Skeletal and life history variation of common dolphins from Eastern North Atlantic

Sinéad Murphy

- NECESSITY project (Mirimin *et al*., 2007) – A total of 192 samples collected from animals stranded on the coasts of Ireland, France, Portugal, and Scotland (mtDNA only), and from individuals by-caught in the Irish albacore tuna driftnet fishery (operated in the Celtic Sea) and in the UK (microsatellite only) bass pelagic trawl fishery (operates in the western English Channel) were processed. Results from an Analysis of Molecular Variance (AMOVA) indicated no significant genetic structure among all sampled areas (*i.e*. most genetic variability resided within not between samples). This lack of genetic structure was observed using both microsatellite and mtDNA control region markers for all estimators calculated. Similarly, results using the Bayesian approach (STRUCTURE) suggested that individuals from the sampled areas may belong to the same genetic stock. Furthermore, no significant genetic differentiation was detected when the sexes were analysed separately, suggesting similar patterns of dispersal for male and female common dolphins. Overall, Mirimin *et al*., (2007) found low (non significant) levels of genetic differentiation within common dolphins in the Northeast Atlantic.

- Cranial morphometric analysis undertaken by Murphy *et al*. (2006) did reveal some evidence of population differentiation within the eastern North Atlantic, with female *D. delphis* off Portugal showing segregation from more northerly sampled areas. The segregation of female Portuguese common dolphins from other areas was attributed to animals off Portugal possibly mixing with the Mediterranean Sea population, or with animals inhabiting waters further south of the sampled region. The inconsistent results from morphometric and recent genetic studies could suggest that variations in morphological features - caused by adaptation to different habitats - may occur more rapidly than in genetic markers at the population level (Mirimin *et al*., 2007).

- Murphy *et al*. (in prep.) analysed data from by-caught and stranded common dolphins from Irish, UK, Galician and Portuguese waters (maturity status determined for 417 females). The overall annual pregnancy rate for the Northeast Atlantic population was 25%, and a calving interval of approx. 4 years was determined. The average age and body length attained at sexual maturity using the SOFI method were 8.82 years and 187 cm, respectively. As the maximum age in the sample was 29 years, lifetime reproductive output is approx. 4-5 calves. Average length and weight at birth was calculated as 93 cm and 8,700 g. Conception dates estimated using foetal data ranged from 5th April – 2nd October (outer limits), although 40% of individuals were conceived in July, and the average day of conception was calculated as 19th July.

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7.4.3 Genetic variation in common dolphins from Eastern North Atlantic

Ana Rita Amaral

The existence of genetic population structure of common dolphins in the Northeast Atlantic region was investigated through the analysis of DNA sequences from two mitochondrial regions, the cytochrome *b* gene and the control region. Four putative populations from the Scottish, Northern Spain, West Portuguese and South Portuguese coasts were analysed. No significant genetic structure was detected between these populations, although when putative populations were separately analysed by sex, significant F_{ST} values were obtained for both females and males. This may be indicative of sex-biased dispersal, though further analyses with nuclear or Y chromosome markers will be essential in order to confirm this. Neutrality tests and the star-shape of the median-joining networks obtained suggested that these populations are in expansion.

In the phylogenetic analysis of the cytochrome *b* gene sequences, a highly differentiated group of individuals representative of the four putative populations was found (Clade X). When this group of individuals was compared with the *D. delphis* clade, a value of 1.59% was obtained, which is much higher than the one found to separate *D. delphis* from *D. capensis* (1.07%). High levels of differentiation between individuals of Clade X and *D. delphis* were also obtained in preliminary analyses of other molecular markers such as the mitochondrial cytochrome *c* oxidase I gene, the 7 intron of the β-fibrinogen gene and amplified fragment length polymorphisms. These individuals may constitute a divergent lineage within *D. delphis* or even be a different taxonomic entity. The clarification of the taxonomic status of the common dolphin in the NE Atlantic area is thus of extreme importance for the establishment of population boundaries. This will only be achieved by increasing sample size and by gathering all relevant molecular, morphological and ecological information.

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8 PROPOSED MANAGEMENT UNITS

8.1 Introduction

Understanding population structure is critical if conservation management is to be effective. The focus has generally been upon genetic markers (Hoelzel, 1991), from isozymes through to mtDNA sequences, nuclear microsatellite loci, and more recently, variation of the Major Histocompatibility Complex and Single Nucleotide Polymorphisms. However, the use of a suite of approaches has increasingly been advocated, with the distinction made between those that address evolutionary aspects of population separation (that may involve tens, hundreds or thousands of generations), and those that reflect contemporary structure (Evans, 1991; Dizon *et al*., 1992; Taylor and Dizon, 1999).

A variety of criteria have been proposed by which conservation biologists assign population distinctiveness while incorporating molecular genetic data (Crandall *et al*., 2000). Historically, this classification incorporated both ecological data and genetic variation to define 'evolutionary significant units' (ESU: Ryder, 1986; Waples, 1991). Some later ESU concepts were based more extensively on molecular phylogenies (Moritz, 1994, 1995). Crandall *et al*. (2000) then argued for a broader categorization of population distinctiveness that was based on concepts of both ecological and genetic exchangeability.

For differentiation that has been established over shorter time-scales, the identification of management units was proposed (MUs; Moritz 1994). This was based on evidence for significant deviation from the assumption of panmixia, even without reciprocal monophyly at mtDNA (but see Waples and Gagliotti, 2005). More recently, there has been an effort to define demographically independent populations whose population dynamics depend largely on local birth and death rates rather than upon immigration (Palsbøll *et al*., 2007). The implications of this when interpreting genetic data are that instead of focusing upon rejecting panmixia, one should assign MUs on the basis of the amount of genetic divergence at which populations become demographically independent. Thus emphasis is placed upon the contemporary dispersal rate of individuals rather than the historical amount of gene flow. However, it is important to recognize that dispersal rate estimates (based on physical or genetic tagging) and levels of contemporary gene flow may not be equivalent if immigrants are not contributing successfully to the local gene pool. Thus to define an appropriate threshold level of population genetic divergence at which populations should constitute separate MUs, it is proposed to establish the relationship between the demographic characteristics and population genetics dynamics of the target species, but for the present time linking biologically realistic demographic models with population genetic estimation remains challenging (Palsbøll *et al*., 2007; Schwartz *et al*., 2007).

In the past, the tendency has been to assume one large MU, and then to subdivide this once differences have been detected by various methods. However, a precautionary approach would be to start with a number of smaller MUs based upon preliminary evidence of differences, and then to pool these once one has data to show the differences are unlikely to be significant. We have tended to use this latter approach

(as does the International Whaling Commission Scientific Committee – see, for example, Martien and Taylor, 2003; IWC, 2004).

A first step in considering populations as demographically distinct has been whether or not they are spatially separated. Thus an isolated population of harbour porpoise in the Black Sea might be considered as likely to be distinct from one in the North Atlantic. However, in most cases no such obvious geographical separation exists, and it becomes very difficult to use such initial criteria. Furthermore, whether or not populations are spatially distinct does not mean they are demographically or genetically so.

In section 4, the strengths and limitations of the various methods for discriminating populations are detailed. In summary, the two most commonly used genetic markers are the mtDNA control region and microsatellite DNA loci. The former (as with cytochrome *b*) is haploid and maternally inherited and therefore is associated with a four-fold smaller effective population size compared to nuclear by-parentally inherited markers. However, the lower mutational rate of the cytochrome *b* gene tends to make this marker more suitable than the control region for phylogenetic and taxonomic studies. By contrast, the mtDNA control region and nuclear microsatellite loci (generally found in non-coding regions) show higher levels of polymorphism due to a relatively high mutation rate, and so they tend to be more sensitive in detecting fine-scale population structure. Single nucleotide polymorphisms (SNPs) form a new set of markers that offer much potential due to their higher genotyping efficiency, data quality, genome-wide coverage and analytical simplicity (Morin *et al*., 2004).

Most genetic analysis are based upon a demographically simple population model: random mating, constant population size, as well as constant migration rates among populations, equal reproductive success among individuals, non-overlapping generations, and equilibrium between drift and migration. Although this is too artificial for most natural populations, the impact of violating these assumptions can be assessed (and is often minor or not relevant to a particular analysis) and so working with this simplified model nevertheless provides useful information.

The relevant genetic analyses typically estimate parameters that result from the interaction between mutation, genetic drift and migration (though natural selection is also highly relevant for markers expressed phenotypically). The rate of change is influenced by the rate of mutation and migration, and by the effective population size (which influences the rate of genetic drift – higher in small populations). The fact that most natural populations will be out of mutation-drift-migration equilibrium means that there can be a considerable lag between demographic changes and their reflection in parameter values assessed using molecular markers. In other words, traditional genetic estimates of migration rates (e.g. based on F_{ST}) and effective population sizes (based on genetic diversity) represent evolutionary means, which may not reflect the current population parameter values.

Many of the analyses summarized here and presented in the literature use Wright's Inbreeding Coefficient (F_{ST}) (which assumes an infnite mutation model) as a measure of population structure / genetic divergence, or the R_{ST} statistic (which assumes a step-wise mutation model). The calculation of *F* statistics requires prior assumptions as to what constitutes a 'population', which can lead to arbitrary designations based, for

example, on political boundaries (though a signal for this can be detected by the 'Wahlund effect', where artificially pooled populations show a deviation from Hardy-Weinberg expectations). Furthermore, the value of the statistics for individual loci depends on sample size, the heterozygosity at a given locus (which is why multiple loci are typically used), and fluctuations in population size. The latter means it can be difficult to understand the biological meaning of F_{ST} or R_{ST} values without any knowledge of demographic history (although this can be assessed independently using molecular markers).

Another standard approach has been to ask "are the allele frequencies different?" which is the most common manner in which "stocks" have been defined from genetic data. Since the ability to detect a significant difference not only depends upon how genetically divergent two samples are but also on sample sizes, the number of genetic markers, and the specific markers used, then a comparison of A-B and A-C can be difficult, since different results may be due to non-biological aspects (i.e. sample size, etc). This incidentally also applies to all other kinds of analyses that simply go for assessing if samples are "different". This is why one needs to have a measure of *how* different the samples are, and thus ultimately a comparison of the degree of difference. This is demonstrated in the results from several of the genetic studies on harbour porpoise, where some F_{ST} values are as high within MUs as between them, suggesting that either (a) insufficient genetic data had been collected to obtain the necessary level of precision, or (b) that the MUs are incorrect.

Some recently developed methods address in part the problems described above. For example, assignment methods that assume equilibrium (e.g. Hardy Weinberg and linkage equilibrium) can partition populations without making any *a priori* assumption about population divisions by testing for deviations from equilibrium assumptions (e.g. as run in the program STRUCTURE; Pritchard *et al*., 2000). There are also methods to assess migration rates based on the coalescent, which interprets historical lineage structure inferred from extant genotypes (e.g. see Beerli and Felsenstein, 2001). This method is less dependent on sample size and can provide directional estimates of gene flow. And there are also non-equilibrium models, again based on the coalescent, that can estimate gene flow after some point of division (e.g. IM; Hey and Nielsen, 2004), though these often make strict assumptions that restrict gene flow to the pair of populations being assessed. Other applications allow the testing of different models including the incorporation of multiple populations (e.g. ABC; Excoffier *et al*., 2005). In general, the application of multiple markers and analytical methods helps with the, often tricky, task of interpreting data in the context of model assumptions.

When no differentiation can be found at genetic markers, this does not mean that the populations cannot be diverging in a significant way (due to some of the factors outlined above). For example, it could mean that divergence is very recent or obscured by population expansion. Thus a range of other "ecological" approaches may be helpful as complementary evidence in informing us where structure exists. Some of these are likely to be more useful than others. Differences in diet or in certain life history parameters such as gestation periods or ones that are age or weight related, are less useful than those which provide signals of longer term differentiation (measured in years or decades). Some will be adaptive and reflect local environmental conditions – metrical differences may be related to growth conditions, for example,

and such characters are often inter-correlated. For this reason, geometric morphometric approaches are preferable. Of other approaches, stable isotope signatures and levels of contaminants such as mercury in the liver, or cadmium in the kidney (which provides a long-term record measured in 10-15 years), are good candidates to discriminate diet or structure in populations. Differences in parasite loads, or the timing of reproductive events, or length at sexual maturity, all offer further indications of population differentiation, although it should be borne in mind for all of these approaches that similarities or differences may be coincidental, reflecting whether or not local environmental conditions happened to be similar or different. And we do not necessarily understand the underlying processes resulting in what we observe, so that it becomes difficult to model the expectations and how sensitive they are, or how exactly they relate to dispersal or migration rates.

A major limitation of many of the above approaches (both genetic and ecological) is that the location in which the animals were living is rarely known. Most result from biological samples obtained as strandings, whilst even if they derive from by-catches, their exact locations when alive may not be clear. And drift is not equivalent in different locations or areas. For example, in the North Sea, currents are predominantly from the north, in the Irish Sea from the south, and along Atlantic coasts from the west or south-west. Furthermore, researchers are usually confronted by small numbers of samples scattered over wide areas spanning long time periods, and they may analyse these by arbitrarily combining samples more on political than upon biological grounds. It would be more meaningful to use biological processes (which in turn may be influenced by physical processes such as features of bathymetry or ocean circulation – see maps in Appendix) in hypothesis testing for defining management units, although identifying appropriate ones remains a challenge since populations may be structured on the basis of parameters that we are unable to easily recognize. One approach of potential promise is to investigate how ecology may influence movement patterns and thus shape social and population structure. Where prey is sedentary, predictable, and persistent (as tends to occur with benthic or demersal fish and invertebrate species), forming localized areas of suitable foraging habitat for cetaceans, discrete local populations may arise. Where prey is pelagic and wide-ranging, population structure is much less likely to develop. If dietary specialization develops amongst individuals, there is potential for sympatric yet demographically (and ultimately genetically) distinct populations to occur.

More direct measures of dispersal can be obtained from techniques such as photo-ID of recognizable individuals, genetic tagging, or telemetry. However, these may not necessarily inform one about actual gene flow or even dispersal rates (unless this can be quantitatively assessed), and generally they involve limited numbers of individuals and/or relatively short time periods. Like all the other methods described, they are best used in combination to better inform one another. In general, the integration of both genetic and ecological markers is necessary to obtain the best possible indication of relevant stock structure. A major challenge that still needs fully addressing is how to integrate these rather different lines of evidence, and what time frame is most appropriate to consider here in the context of conservation management. For the time being, we consider a few generations (equivalent to low tens of years) as the appropriate time frame for defining a management unit, and we identify an MU as a group of individuals for which there are different lines of complementary evidence suggesting reduced exchange (migration/dispersal) rates. Ideally, one should set

quantitative parameters (e.g. maximum of ten percent migration per generation), but in most cases we do not have the information as yet to do this, nor has the theoretical framework for integration of different evidence bases been fully developed.

In the species accounts that follow, we have attempted to include wherever possible the sample sizes, sampling locations and summary of tests conducted (with F_{ST} values where appropriate), as well as drawing attention to possible weaknesses in inferences drawn. We have not tried to weight the different lines of evidence in any quantitative manner. It should be emphasized that the management units proposed remain both preliminary and precautionary, and doubtless will change with new information and new analyses.

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8.2 Harbour Porpoise *Phocoena phocoena*

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Introduction

The harbour porpoise is restricted to temperate and sub-arctic (mainly 11-14^{o} C) seas of the northern hemisphere. In the North Atlantic, the species occurs mainly from Central West Greenland and Novaya Zemlya in the north to North Carolina and Senegal in the south (Figure 1). A geographically distinct population exists in the Black Sea (although there is evdence that it has not always been isolated – see Rosel *et al*., 2003).

Figure 1. North Atlantic Distribution of Harbour Porpoise
(depicting those areas where the species is thought to regularly occur)

In European seas, it is common and widely distributed over the continental shelf (mainly at depths of 20-200 m) from the Barents Sea and Iceland south to the coasts of France and Spain, although in the 1970s it became scarce in the southernmost North Sea, English Channel, and Bay of Biscay. Nevertheless, it remains the most widely distributed and frequently observed cetacean in NW European shelf seas (Figure 3), and since the 1990s, has returned to the southernmost North Sea, English Channel and French Biscay coast (Rogan and Berrow, 1996; Hammond *et al*., 2002; Reid *et al*., 2003; Evans *et al*., 2003; Evans and Wang, 2003; Camphuysen, 2004; Kiszka *et al*., 2004, 2007; Evans *et al*., 2008; Hammond, 2008).

Although porpoises can be found in deep waters off the edge of the continental shelf (for example within the Faroe Bank Channel – see Pollock *et al*., 2000), they are comparatively rare in waters exceeding 200 metres. The species frequently uses tidal conditions for foraging (see e.g., Evans, 1997; Pierpoint, 2008; Marubini *et al*., 2009).

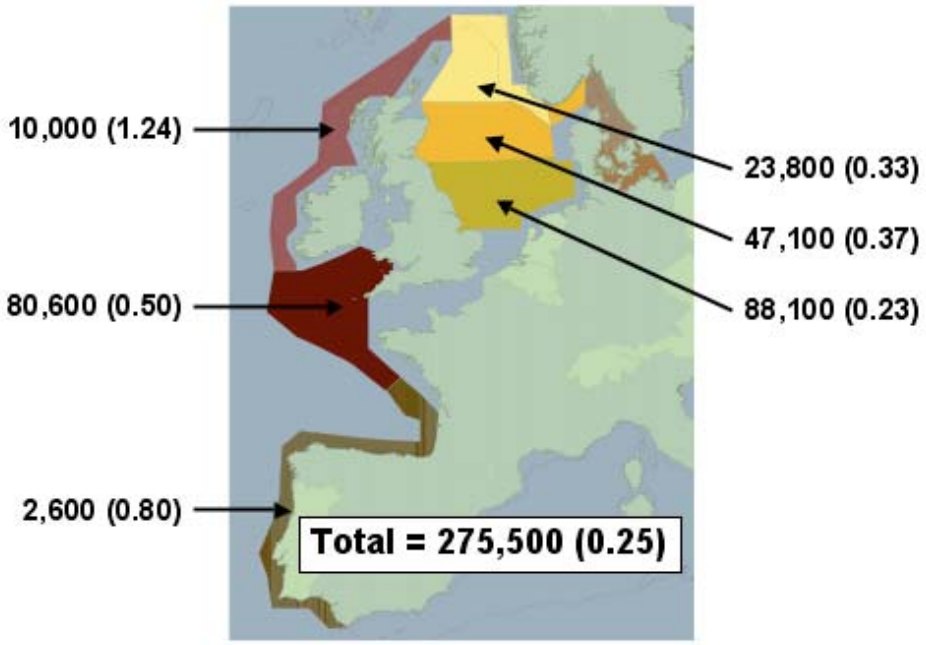

Figure 2a. Abundance Estimates (and CVs) for Harbour Porpoises from SCANS II Survey (shipboard), July 2005

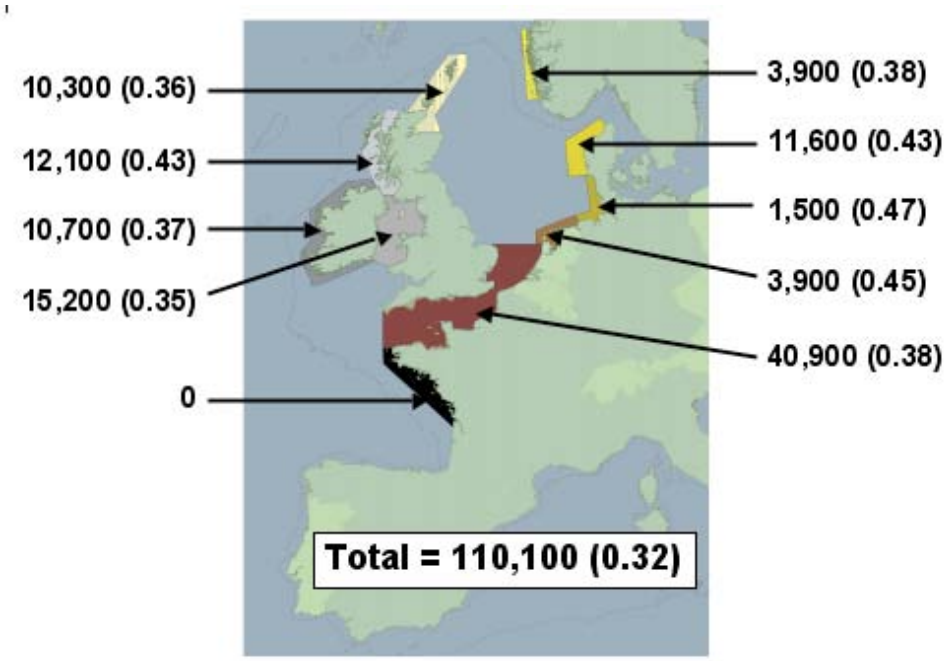

Figure 2b. Abundance Estimates (and CVs) for Harbour Porpoises from SCANS II Survey (aerial), July 2005

From line transect surveys in July 1994 (Hammond *et al.*, 2002), an overall population estimate of 341,000 porpoises (CV=0.14; 95% CI: 260,000-449,000) was made, with the following regional estimates: the North Sea (c. 250,000), Baltic region (36,600 in Kattegat/Skagerrak/Belt Seas/Western Baltic Sea), Channel (0), and Celtic Shelf (36,300). A repeat survey in July 2005 (SCANS II), covering a wider area (continental shelf seas from SW Norway, south to Atlantic Portugal), gave an

estimate of 385,600 (CV = 0.20) (Hammond, 2008), with regional estimates: North Sea (c. 231,000), Baltic (23,000 in Kattegat/Skagerrak/Belt Seas/Western Baltic Sea), Channel (40.900), and Celtic Shelf (58,400). Figures 2a & 2b depict the abundance estimates (and associated coefficients of variation) from the SCANS II shipboard and aerial surveys respectively. Comparing the two surveys, although the overall number estimated for the North Sea, Channel and Celtic Sea was comparable (341,000 in 1994, and 335,000 in 2005), numbers in the northern North Sea and Danish waters had declined from 239,000 to 120,000, whereas in the central and southern North Sea, Channel and Celtic Shelf, they had increased from 102,000 to 215,000. This is thought to represent a southwards range shift rather than actual changes in population size (Winship, 2009), at least for the month of July. It suggests some connection between northern and southern areas although whether or not these may cross the proposed Management Unit boundaries, as indicated later, is not clear.

In Norwegian waters, estimates of 11,000 porpoises (95% CI: 4,790-25,200) for the Barents Sea and Norwegian waters north of 66ºN, and 82,600 (95% CI: 52,100-131,000) for Southern Norway and the northern North Sea, were made during July 1989 (Bjørge and Øien, 1995).

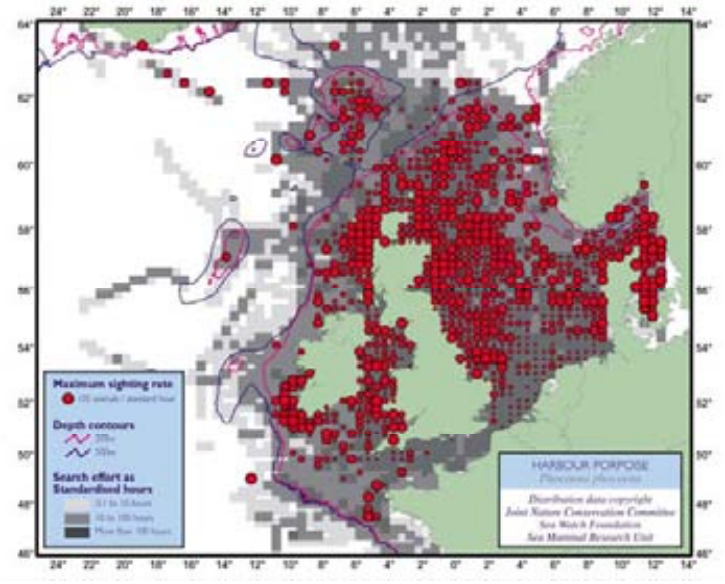

Figure 3. Sighting rates of Harbour Porpoise
[Records from 1979-98. Red circles are scaled in proportion to the number of animals observed per hour of observation. Sightings rates are standardised for observations made under different sea conditions but have not been corrected for differing efficiencies of the various people & vessels used to collect the data. The grey shaded cells indicate observation effort (from Reid *et al*., 2003)]

Skeletal & Tooth Ultrastructure Variation

Kinze (1985) in a study of metric and non-metric skull characters of porpoises from the northern North Sea, Baltic Sea and Dutch coast, found significant differences between Baltic and Dutch animals, but suggested a mixing of individuals between the Baltic and northern North Sea.

Following this, Yurick and Gaskin (1987) conducted a study of metric and non-metric skull characters of porpoises from the western and eastern North Atlantic, Black Sea and eastern Pacific, and found clear differences between the four regions. There was

also some indication of segregation in the North Sea into Dutch coastal, eastern North Sea and Baltic subpopulations, but sample sizes from each area were too small for a statistical analysis.

Kinze (1990a, b) then demonstrated non-metric differences between porpoise skulls collected from the Dutch and German North Sea; between the German and Danish North Sea; and between the Swedish Skagerrak and Inner Danish Waters (Danish Skagerrak and western Baltic). He proposed four separate population units: 1) Dutch coast, 2) German Wadden Sea, 3) northern North Sea through to the Belt region, and 4) Swedish Skagerrak. Following Kinze's (1985) suggestion that seasonal migrations may result in some mixing within Danish waters, Börjesson and Berggren (1997) examined porpoise skulls from the Baltic Sea and Kattegat-Skagerrak region and compared samples from different seasons. They found that females but not males could be separated between the regions, irrespective of season.

Huggenberger *et al*. (2002) used both metric and non-metric skull characters in a study of porpoises (n=242) from the German Bight, the central Baltic Sea (Arkona seas and eastern Sweden), and an area in between the two (Skagerrak, Kattegat, Belt Seas, Øresund and Western Baltic Sea). They were able to distinguish all three areas using a variety of statistical approaches.

In a study employing 3D geometric morphometrics on skulls, Galatius and Kinze (*in prep.*) found highly significant differences among three samples of porpoises from Danish waters, recent samples from the Danish North Sea (n=38) and Kattegat/Belt Seas (n=26), and a sample (n=15) of animals taken in drive catches in Gamborg Fjord (Belt Seas), during the Second World War. The differences between the Gamborg Fjord sample and the others were so significant that the authors concluded that these animals belonged to a different population, although it should be borne in mind that the spatial comparisons are confounded by a temporal difference in sampling. Møhl-Hansen (1956), who described the porpoises from the drive catch, believed that they were part of a seasonal migration from the central Baltic. The existence of such a migration has since been the object of scepticism (Huggenberger *et al*., 2002). However, it is possible that ice conditions in the Baltic proper during cold winters may have forced porpoises out of the central Baltic.

A study of tooth dentinal and cemental ultrastructure and Growth Layer Group (GLG) characteristics by Lockyer (1995) found variation in the incidence of particular mineralization characteristics with Danish and British porpoises being most similar, differing from Norwegian, eastern Canadian and Californian porpoises, all of which also differed from each other. In an expanded study, including additional areas (West Greenland, Iceland, Sweden, Poland, German Baltic, and sub-areas of the North Sea: British Isles, Netherlands and Germany), using eight different mineralization anomaly characteristics for comparison, Lockyer (1999) found that West Greenland was very distinct from the Bay of Fundy (Canada), with some further differentiation into three regions (particularly northern *vs* central/southern) within West Greenland. Although Iceland and West Greenland showed few differences, there were significant differences in the incidence of marker lines and GLG type. Samples from Iceland also differed significantly from those in the North Sea/Celtic Shelf region. Other significant differences were observed between 1) northern North Sea, 2) central North Sea, 3) southern North Sea, 4) Skagerrak, 5) Inner Danish waters and Kattegat, and 6)

Baltic Sea. In the latter three areas, the differences observed involved very different characters from the two noted in the North Sea.

More recently, De Luna (2005) measured 16 traits on 462 porpoise skulls from three different regions in the eastern North Atlantic (Norwegian, n=50: Barents Sea = 21, Norwegian Sea = 9, Norwegian North Sea = 20); Danish, n=93: Danish North Sea = 11, Skagerrak = 2, Kattegat = 41, Belts Seas = 38, Baltic Proper = 1); British, n=319: British North Sea = 113, Irish Sea-Wales-West England-West Scotland = 107, English Channel-Southern England = 13). Discriminant function analysis was performed for the assignment of individuals by skull morphometry, and four were found to be highly significant ($p<0.001$), three of them reflecting the length and width of the oral cavity, and the fourth reflecting the size of the orbit. Three populations were successfully classified: British, Danish, and Norwegian, with by far the strongest discrimination between Norway and the other two regions. He interpreted this as reflecting differences in foraging behaviour, British and Danish porpoises foraging in relatively shallow waters preying mainly on benthic species, whereas Norwegian porpoises prey mainly on mesopelagic and pelagic fish.

Together, these studies indicate separation between western and eastern North Atlantic porpoises, and differences between eastern Canada and western Greenland (with possible sub-structuring within West Greenland). Within the eastern North Atlantic, there is differentiation within the North Sea and within the Skagerrak to Baltic regions.

Recently, significant differences in skull morphology as well as greater body lengths (maximum 191 cm) and body length-to-age ratios have been observed between porpoises from Cornwall and other parts of England (Jepson, 2003). Some porpoises from West Wales were also similar to those from Cornwall.

Genetic Analyses

The first genetic studies conducted upon porpoises used isozyme markers, and revealed significant differences at two polymorphic loci between samples from eastern Canada (Gulf of St Lawrence, n=12) and the eastern North Atlantic (Dutch coast, n=40, and inner Danish waters, n=93), and between West Greenlandic (n=66) and both Canadian and Dutch samples (Andersen, 1993). She also found some evidence of sub-structuring within the European samples, with summer Dutch samples having significantly different gene frequencies to those from inner Danish waters. This study was later repeated using microsatellites, comparing the samples from West Greenland, North Sea and inner Danish waters (Andersen *et al*., 1995). The West Greenland sample was distinct from the other two regions, but those latter samples could not be differentiated. The North Sea sample had a significant excess of heterozygotes, interpreted as either the result of mixing of a number of sub-populations, or due to males occasionally coming into the North Sea from adjacent areas. Enlarging the number of loci examined (to five), and using a combination of isozyme and microsatellite analyses, Andersen *et al*. (1997) recognized three distinct populations: West Greenland, the North Sea, and inner Danish waters, but with some degree of gene flow apparent between areas. Females appeared to be more sedentary than males. Walton (1997) obtained a similar result, using single locus mtDNA to examine samples from around the British Isles (Ireland/Celtic Shelf, n=64; Irish Sea, n=56; West Scotland, n=18; northern North Sea including Northern Isles, n=105;

southern North Sea including the Dutch coast, n=73; and English Channel, n=11). However, he found significant differences between northern and southern North Sea porpoises and between northern North Sea and Celtic Shelf/Irish Sea animals. Since mtDNA is maternally inherited, he concluded that these differences reflected limited movement amongst females (bearing in mind that mtDNA tells nothing about male movements). Maternal philopatry is commonly claimed when a study finds a higher level of genetic divergence/lower degree of gene flow at mtDNA compared to nuclearDNA. On the other hand, such studies rarely make a statistical assessment of the point estimates of divergence/gene flow from the two kinds of markers so we do not necessarily know if the observed difference is statistically significant or just a chance difference.

Indication of some sub-structuring within the North Sea came also from the study by Tolley *et al.* (1999), where they analysed porpoise samples from the Barents Sea (n=20 females), northern North Sea west of Norway (n=16 females), and British northern North Sea (n=35 females), using a single locus mtDNA marker. They found differences between the Barents Sea and British North Sea but only when samples from Shetland had been excluded. They also found indications of philopatry in females.

Tiedemann *et al*. (1996), in a study using mtDNA markers, proposed that porpoises from the German Baltic (n=39) were distinct from those in the German sector of the North Sea (n=20), based upon the presence of a particular haplotype in all of the Baltic samples that was absent in the majority of the southern North Sea animals. They also found low nucleotide and haplotype diversity in the Baltic animals, and suggested population separation several thousand years ago with limited genetic exchange since then.

Wang and Berggren (1997) also compared porpoises from the Swedish Baltic (n=27), Kattegat-Skagerrak region (n=25), and the west coast of Norway (n=13), using a single locus mtDNA RFLP (nine restriction enzymes), and obtained similar results, with the three areas showing significant differences (P=0.035 between Baltic and Kattegat-Skagerrak sampling areas), and the former two having much lower nucleotide and haplotype diversities than those in the northern North Sea west of Norway. Palmé *et al*. (2008a, b) re-analysed the data of Wang and Berggren (1997) and concluded that the evidence equally supported one single management unit as two, the P-values being non-significant. They further attempted to delineate management units on the basis of rate of migration, using an exchange rate of 10% or more as the threshold to imply a degree of demographic connectivity justifying the two population segments to be managed jointly as a single unit, following Palsbøll *et al.* (2007). In this particular case, this was estimated to correspond to an F_{ST} of 0.05 or less for mtDNA, whereas F_{ST} for the Baltic and Kattegat-Skagerrak region was 0.007. A random number simulation indicated that the observed value of F_{ST} was equally likely under both hypotheses.

Berggren and Wang (2008) responded to this critique by having the dataset reanalyzed independently with resultant P values ranging between 0.034 and 0.079. They argued that the prudent conservation measure was to take as the null hypothesis that there are no differences between provisional populations, with the threshold for rejection being 0.05, and that when there is low analytical power for detecting

differences due to small sample size, any P-value <0.1 is grounds for prudent conservation management.

Tiedemann (unpubl. data) in a further analysis of 316 porpoises using mtDNA markers and 217 porpoises with 15 microsatellite loci also proposes a separate population in the Baltic Proper, based on samples from the Polish and eastern German Baltic coast while samples from the Swedish south coast had a closer relationship to the Kattegat and Belt Sea samples.

There have also been a number of genetic studies on a wider geographical scale, including samples from the western North Atlantic. Tolley *et al*. (2001) in a single locus mtDNA study of 370 porpoises from six locations, found that porpoises from West Greenland (n=50), Gulf of St Lawrence (n=40), Newfoundland (n=41), and Gulf of Maine (n=80) were more similar to those from Iceland (n=72) than with animals from the eastern North Atlantic (in this case, Norway, n=87), but recommended that Iceland be treated as a separate population. Rosel *et al*. (1999) using both mtDNA and microsatellite analyss of western North Atlantic porpoises, (Gulf of Maine, n=80; eastern Newfoundland, n=42; Gulf of St Lawrence, n=40; mid-Atlantic states, n=48; and West Greenland, n=50) found on the basis of haplotype frequencies, that animals from West Greenland were distinct from those in the Gulf of Maine and Newfoundland, whilst there was further sub-structuring between Gulf of Maine, Newfundland and Gulf of St Lawrence. They also found females to be more philopatric, and interpreted gene flow as occurring mainly by male dispersal.

Andersen *et al*. (2001) examined 807 porpoises in a study using 12 microsatellite loci, and distinguished six populations genetically: West Greenland (n=151 from three areas), west coast of Norway (n=49), Ireland (n=105), British (western) North Sea (n=131), Danish (eastern) North Sea (including Skagerrak) (n=151), and inner Danish waters (Kattegat, Belts seas, and Øresund) (n=169). F_{ST} values (significant at P=<0.05) varied from 0.003-0.004 (British vs Danish North Sea, Ireland vs Danish North Sea, Ireland vs British North Sea) to 0.010-0.014 (Inner Danish waters vs West Greenland, Inner Danish Waters vs Ireland, Inner Danish waters vs Norway). All comparisons were significant, although most F_{ST} values indicated only weak population structuring (the greatest being between Inner Danish waters and elsewhere). However, samples assigned to regions combined Atlantic Ireland with the Irish Sea and western English Channel, and Shetland with eastern Scotland and east England).

A sample of harbour porpoises (n=51) collected off the Dutch coast (mainly during winter) in particular was genetically heterogeneous, suggesting it comprised a mixture of individuals of diverse origin (Andersen *et al*., 2001). Mixed stock analysis in fact indicated that around 75% of the individuals in this sample were likely migrants from the British and Danish North Sea (although which sectors of either was not identified).

De Luna (2005) in a combined study of skeletal and DNA variation in porpoises from the British Isles (n=223: British North Sea = 113, Irish Sea-Wales-Western England-West Scotland = 107; English Channel-Southern England = 3), Denmark (n=93: Danish North Sea = 11, Skagerrak = 2, Kattegat = 41, Belts seas = 38, Baltic proper = 1) and Norway (n=47: Barents Sea = 21, Norwegian Sea = 6, Norwegian North Sea =

20), examined 12 microsatellite DNA loci, and found each of the three areas to be distinct (using both assignment tests, implemented in the software STRUCTURE, and F_{ST}). Values of F_{ST} based upon 8 of the 12 microsatellite loci (De Luna, unpubl. data) ranged from 0.04 for the comparison between the British Isles and Denmark, to 0.06 for the comparisons between Norway and Denmark, and Norway and Britain (in all three cases, P=<0.01, although the F_{ST} values indicate relatively weak substructuring). No differences were found *within* the three main areas, although sample sizes were small for a number of locations.

Fontaine *et al*. (2007a) analysed 10 microsatellite loci for 752 porpoises, and found that most of the sampled range in the eastern North Atlantic (sampling locations excluded the England & Wales, Inner Danish waters and Baltic) behaved as a 'continuous' population that extends over thousands of kilometres with significant isolation by distance, and local habitat-related variation in its strength. This was consistent with previous results in the North Sea, where significant, but generally weak differences have been observed at similar kinds of markers when comparing groups artificially defined. They did, however, find strong barriers to gene flow (at both microsatellite and mtDNA loci) in the south and eastern parts of the range (in Iberian waters, and the geographically near-isolated population in the Black Sea) (cf. Tolley and Rosel, 2006), coinciding with profound changes in environmental characteristics (i.e. depth, sea surface temperature and primary biomass). They concluded that physical processes and especially the factors determining food availability for the species, have a major impact on the demographic and population genetic structure of porpoises.

Fontaine *et al.* (2007a, in review.) has concluded that the pattern of population genetic structure suggests an ongoing habitat-related fragmentation of the range of the species, probably related to changes in features of its habitat and thus to climate change. Comparing genetic inferences with historical records on fisheries and paleoceanographic data, Fontaine *et al.* (in review) conclude that porpoise poulations have responded markedly to recent climate-induced reorganisation in NE Atlantic ecosystems, beginning with the retreat of porpoises from the Mediterranean Sea during its postglacial warming, followed by the isolation of Iberian porpoises from those inhabiting waters further north in tandem with the contemporaneous warming trend underway since the “Little Ice Age” period around three hundred years ago, and the retreat of cold water species from the Bay of Biscay. The Iberian population is thought to persist in those marginal areas due to their special upwelling conditions.

The genetic structure of 592 harbour porpoises from UK waters was analysed using nine microsatellite loci, and the results compared with analyses of toxicological profiles of a subset of these (Bull *et al*., 2008). An individual-based analytical approach was used that did not require *a priori* delimitation of groups. Bayesian model-based approaches (as implemented in STRUCTURE and GENELAND) used multilocus genotypes to partition individuals into random mating populations. The posterior density distribution of the number of clusters estimated from GENELAND indicated that the genetic data around UK consisted of a single cluster. However, the significant deficit in heterozygosity (P=<0.001) showed that porpoises do not breed randomly around UK waters. An alternative hypothesis, however, is that porpoises form a single continuous system, but under isolation by distance. This trend primarily occurs in juvenile animals.

Fatty Acid & Dietary Studies

Using fatty acid signatures in blubber and body fats to indicate different diets, Møller (1999) and Møller *et al*. (2003) found significant differences between porpoises sampled within areas in West Greenland, and between Greenland and Denmark.

There are many studies (for example, Rae, 1965, 1973; Desportes, 1985; Lick, 1991; Aarefjord *et al*., 1995; Berrow and Rogan, 1995; Berggren, 1996; Börjesson and Berggren, 1996; Malinga and Kuklik 1996; Martin, 1996; Benke *et al*., 1998, Szefer *et al*., 2002; Vikingsson *et al*., 2003; Borjesson *et al*., 2003; Lockyer *et al*., 2003; Lockyer and Andreason, 2004; Santos *et al*., 2004, 2005; Fontaine *et al*., 2007) that have shown differences in diet for porpoises between regions in the North Atlantic, North Sea and Baltic, but caution is required in interpreting these as reflecting population differentation. Besides sampling issues such as age-related, seasonal and annual changes in diet that can take place, they may simply reflect short-term prey availability rather than differences in diet of discrete populations.

Stable Isotope Studies

Das *et al*. (2003) conducted a stable isotope study (using $\delta^{13}C$ and $\delta^{15}N$) of porpoises from the southern North Sea (n=49, stranded), and compared these with samples from the German North Sea (n=11, stranded), German Baltic (n=8, by-caught), Denmark (n=15, by-caught), Norway (n=23 by-caught), and Iceland (n=11, by-caught). They found that geographic location significantly affected $\delta^{13}C$ and $\delta^{15}N$ measurements obtained. Porpoises from the German North Sea displayed significantly higher $\delta^{15}N$ values than porpoises from Belgian coasts, Denmark, German Baltic, Norway and Iceland. Porpoises from Belgian coasts were significantly enriched in ^{13}C compared to individuals from Denmark, German Baltic, Norway and Iceland.

Muscle $\delta^{13}C$ and $\delta^{15}N$ were compared between porpoises from Belgian and German North Sea coasts, for animals displaying a good, moderate and emaciated body condition, and stable isotope ratios remained similar between the three categories, indicating that any geographic differences were not due to differences in condition of the animals sampled.

These results suggest that porpoises from the German North Sea are feeding at a higher trophic level than individuals from other locations. Porpoises from the German Baltic, Danish and Belgian coasts displayed similar $\delta^{15}N$ values whereas $\delta^{13}C$ values varied widely between locations. Trophic positions were estimated according to the model described by Lesage *et al*. (2001), for porpoises from the southern North Sea, German Baltic and Norway coasts, for which $\delta^{15}N$ values in the particulate organic matter were available. A mean trophic position of 3.4 was calculated for porpoises from the Belgian part of the southern North Sea. Assuming a similar $\delta^{15}N$ value around $9^{o}/_{oo}$ for the German North Sea particulate organic matter, porpoises from this area occupy a trophic position of 3.7, i.e. somewhat higher than off the Belgian coast. By contrast, porpoises from Norwegian coasts display a lower trophic position of 3.2.

The depletion in $\delta^{13}C$ that was observed for individuals from Norway and Iceland was thought to be related to a more offshore feeding, as the continental shelf area is considerably reduced along these coasts compared with the southern North Sea. These results are enhanced by the high hepatic and renal cadmium concentrations observed

in porpoises from Norway and Iceland (Das *et al*., 2004), suggesting a significant contribution of oceanic cephalopods in their diet.

In a study of the feeding ecology and habitat use of 32 porpoises by-caught in four localities along the Scandinavian coast from the North Sea to the Barents Sea (Finnmark: n=3; Nordland, n=7; Southwest Norway, n=9, and Southern Norway, n=13), two stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and six trace elements (Zn, Cu, Fe, Se, total Hg and Cd) were investigated as ecological markers (Fontaine *et al*., 2007b). Among the trace elements analysed, only Cd with an oceanic specific food origin was found to be useful. Cd and the two stable isotopes were found to be highly correlated with each other as well as with local bathymetry and geographic location. Variation in the isotope ratios indicated a shift in feeding habits from pelagic prey species in deep northern waters (Finnmark and Nordland) to more coastal and/or demersal prey in the relatively shallow North Sea (SW Norway) and Skagerrak (Southern Norway) waters.

At present, it is difficult to see how to relate these differences to any quantitative measure of exchange rates for animals in different locations. For one thing, it is not clear how long it takes for differences in isotope ratios to appear. It would be advisable to have larger sample sizes and to be able to examine whether spatial differences were maintained over time periods measured at least in years. For the time being, we use them primarily as supplementary evidence for structure.

Parasite Loads

A number of studies of parasite loads have revealed differences between geographical areas (Balbuena *et al*., 1995). The incidence of helminths in the ear sinuses, stomach, lungs and liver of porpoises differed between Denmark and West Greenland, with a higher incidence of ear worms and stomach worms in West Greenlandic animals (Kinze, 1989). Lick (1991) reported the presence of the nematode parasite, *Pseudoterranova* in the North Sea but could not find it in porpoises from the Baltic Sea. Fernandez *et al*. (1993) found differences in gut parasite occurrence between animals from the Bay of Fundy (East Canada) and those from the British Isles. And in a study of porpoises from the Faroe Islands, two lungworm species were found that had previously been unrecorded elsewhere (Larsen, 1995). On the other hand, differences in numbers and incidence may occur annually, seasonally and in relation to food availability or preference, as Herreras *et al*. (1997) found when they examined the incidence of helminth parasites in porpoises from Danish waters and compared these with porpoises elsewhere. More recently, Lockyer and Kinze (2003) produced a comparison of parasite loads in porpoises fron the Danish North Sea and Inner Danish waters between three periods (1943-44, 1962-65, and 1985-90), finding no obvious changes with time, although the comparison was primarily in terms of species presence rather than incidence rates.

In addition to geographic differences in incidence of parasite infections, Danish and Baltic porpoises were found to have a much greater inflammatory response to parasites and certain bacterial infections than those from West Greenland, that generally were more healthy (Wunschmann *et al*., 2001). On the other hand, both populations contained antibodies to a morbillivirus (CMV), and Müller *et al*. (2000) found a high incidence of PMV-specific antibodies in porpoises from German North Sea and Baltic waters.

As with the application of stable isotope ratios, it is not clear how one may use geographic differences in incidence rates of specific parasites to assign animals to particular management units except where there is already spatial discontinuity in the ocurrence of the species.

Contaminant Loads

Organochlorines and heavy metals have also been used to discriminate between populations (Aguilar, 1987), and there are several studies that have shown geographic differences in contaminant loads. Clausen *et al*. (1974) reported very low levels of tDDT in porpoises from West Greenland compared with porpoises from other areas in the North Atlantic. Granby and Kinze (1991) also found low levels in West Greenlandic porpoises compared with Danish animals, and, similarly, Bruhn *et al*. (1999), analysing more recent samples, obtained significantly higher levels of certain chlorinated biphenyls and chlorinated pesticides in porpoises from the North Sea and Baltic Sea compared with West Greenland, as well as differences in PCB loads between the North Sea and Baltic Sea, while Berggren *et al*. (1999) found differences in PCB levels between the Swedish Baltic coast and the Swedish Kattegat/Skagerrak coast. Levels were generally higher in the Baltic, which could be explained either by differences in geographical background levels or the diet of the porpoises. However, analyses of stable isotopes, that might be used to determine differences in diet, did not show any significant results along the Swedish coastline (Angerbjörn *et al*., 2006).

Care is needed in interpreting geographical differences because contaminant levels in an area may change over time. Berggren *et al*. (1995, 1999), for example, observed that levels of DDTs and PCBs in porpoises from inner Danish waters had decreased between 1978-81 and 1988-91, and Koschinski (2002) in a review of levels of both organochlorines and heavy metals in the Baltic Sea and adjacent area showed that they had generally fallen between the mid-1970s and mid-1990s. Similar declines have been observed for a number of marine mammal species in previously heavily polluted areas in the Northern Hemisphere (Borrell and Reijnders, 1999).

Berggren *et al*. (1995) compared levels of DDTs, PCBs, non-ortho-PCBs and PCDD/Fs in porpoise blubber from the Baltic Sea, Kattegat-Skagerrak and western Norway, and found a significantly different contaminant pattern amongst Norwegian porpoises. Kleivane *et al.* (1995) also compared organochlorine loads in young male porpoises from the Danish Kattegat and Norwegian waters, and found that Danish animals had the higher loads. Elsewhere, samples from Scotland and Ireland had similar levels of organochlorines and PCBs, but these were lower than from Denmark or Norway (Smyth *et al*., 2000).

Heavy metal levels also have been shown to vary geographically, with both hepatic and renal cadmium being at much higher levels in West Greenland than in British, German, Danish and Polish waters, with lowest levels in the Baltic (Paludan-Møller *et al*., 1993; Szefer *et al*., 1995, 2002; Strand *et al*., 2005). This was interpreted as reflecting porpoises having a different diet in the two regions, with a higher diet of cephalopods (which are known to selectively concentrate cadmium) in Greenlandic waters. Levels of mercury and butyltin, on the other hand, were higher in Danish than West Greenland porpoises, although as with contaminant studies generally, there are potential confounding effects of age, sex, reproductive state, and nutritional status (though some of these were accounted for – see, for example, Strand *et al*., 2005).

In a study of trace metal levels, porpoises from the southern North Sea (n=49, stranded) were compared with those from the German North Sea (n=11, stranded), German Baltic (n=8, by-caught), Denmark (n=15, by-caught), Norway (n=23, by-caught), and Iceland (n=11, by-caught) (Das *et al*., 2004). They found that porpoises collected along the southern North Sea coast (Belgian and German sectors) generally had significantly higher zinc and mercury concentrations compared to samples collected from the German Baltic, Denmark, Norway and Iceland.

As described in the section on stable isotopes, a study of 32 porpoises by-caught in four localities along the Scandinavian coast from the North Sea to the Barents Sea (Finnmark: n=3; Nordland, n=2; Southwest Norway, n=9, and Southern Norway, n=13) investigated two six trace elements (Zn, Cu, Fe, Se, total Hg and Cd) as potential ecological markers (Fontaine *et al*., 2007b). In the same way as others have concluded, among the trace elements analysed, only Cd with an oceanic specific food origin was found to be useful. Cadmium was found to be highly correlated with the two stable isotopes analysed, as well as with local bathymetry and geographic location. A northwards trend in Cd-enrichment was observed, suggesting that Cd-contaminated prey (notably oceanic cephalopods) were more likely to be included in the diet of porpoises in deep northern waters.

In another study, five trace elements (Cd, Cu, Hg, Se, Zn) were measured in the kidneys and liver of 104 porpoises stranded between 1997-2003 along the coasts of France (n=24), Spain (Galicia) (n=3), Ireland (n=22), Scotland (n=36), and the Netherlands (n=19) (Lahaye *et al.*, 2007). Generally, relatively low concentrations of toxic elements were found. Elevated Cd levels in Scottish porpoises were related to their feeding preference, an apparent increase of cephalopods in their diet having been observed with latitude (Santos and Pierce, 2003; Santos *et al.*, 2004). Significant geographic differences were observed in hepatic Zn concentrations, with elevated levels in porpoises from the Netherlands which the authors thought may relate to their poor health status. Variation in metal concentrations within porpoises from the North Sea was thought to reflect a long-term segregation between animals from northern (Scotland) and southern areas (the Netherlands).

A study of radionuclide levels in porpoises from the Irish Sea found elevated levels of caesium-137 (Long *et al.*, 1996; Berrow *et al*., 1998), and concluded that this suggested the population might be resident. Other investigations have shown declining levels in porpoise tissues with distance from the British nuclear power plant at Sellafield (Cumbria) (Watson *et al.*, 1999), with levels also generally declining northwards from the North Sea along the Norwegian coast into the Barents Sea (Tolley and Heldal, 2002), indicating limited north-south movements.

Recently, 284 porpoises found to have stranded due to physical trauma (as opposed to infectious disease or other causes) on the coasts of Scotland, England and Wales were used in a principal components analysis of 25 chlorinated biphenyl congeners and 12 metals (Cr, Ma, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Hg & Pb) to provide a toxicological profile for an investigation of population structure (Bull *et al*., 2008). Hierarchical cluster aalysis of principal components allowed a blind test, not subject to comparison between pre-conceived (for example, geographic) groups, of porpoise population structure. The absolute values of loadings from the eight principal components showed that PCBs contributed most of the first two principal components, with metals

featuring more dominantly in subsequent components. Mercury was the most influential metal in the PCA. For both mercury and PCBs, they found significant isolation by distance. The hierarchical analysis revealed two major clusters of animals, although with no obvious spatial pattern. A range of ecological hypotheses (for example, differences in foraging strategy) is currently being investigated, as well as a comparison with population genetic data. As with all the above studies, the precise origins of the samples are rarely known and will be variably influenced by drift in different regions, whilst clustering of toxicological profiles may be coincidental rather than reflecting higher gene exchange.

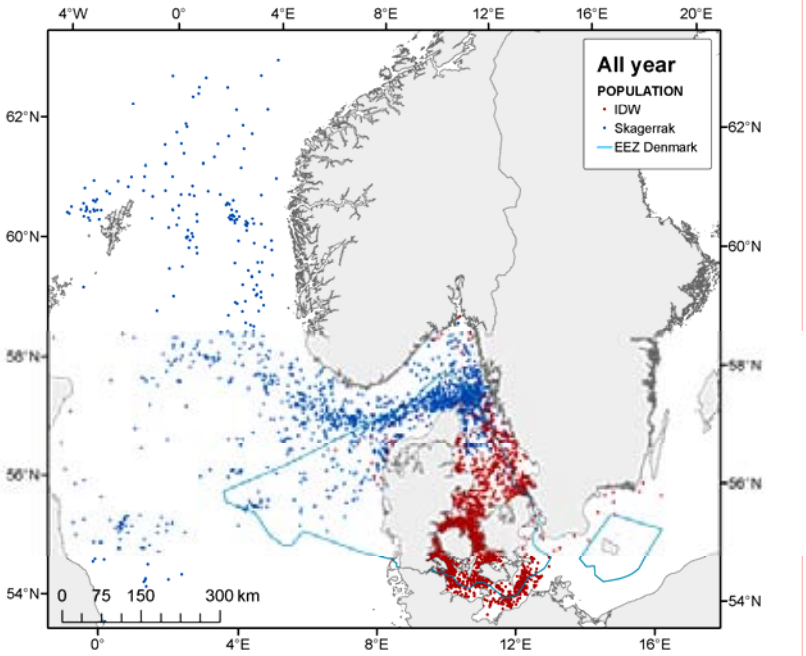

Figure 4. Locations (one per day) of 63 radio-tagged porpoises. Porpoises tagged in the IDW are red, and porpoises tagged in the northern tip of Jutland (Skagen) are blue
(N=63 porpoises, n=4287 locations)

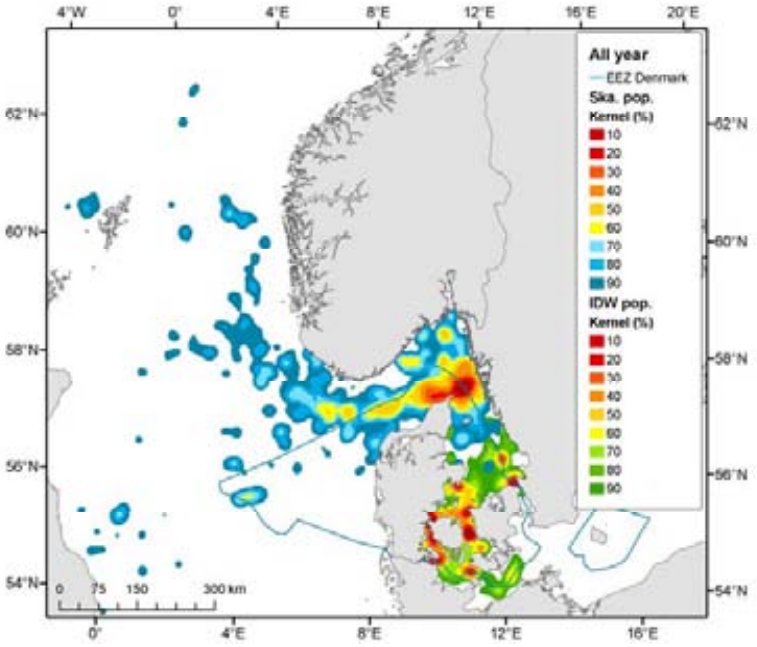

Figure 5. Kernel distribution all year showing the 10% to 90% volume contours (IDW population: N=37 porpoises, n=2765 locations; Skagerrak/North Sea population: N=26, n= 1522

Telemetry

In Danish waters, a satellite telemetry study has indicated that animals in the northern Kattegat, the Skagerrak and northern North Sea may consist of one continuum of porpoises while the inner Danish Waters from the northern Kattegat south through the Belt Seas to the western Baltic appear to consist of another group (Teilmann *et al.*, 2008; Sveegaard *et al.*, submitted; see also Figs 4-6). This was based upon 63 radio-tagged porpoises over a period of 11 years (1997-2007), after being incidentally caught in pound nets in Danish waters from Skagen to Gedser.

Thirty-seven animals (6 adult females, 5 adult males, 26 juveniles) were tagged in Inner Danish waters, and 26 (2 adult females, 5 adult males, 19 juveniles) in the Skagerrak. Satellite locations were received in every month of the year but with the highest numbers of transmitting tags between April and July. Average transmission time was four months, with a maximum of one year.

The satellite telemetry study showed that mature female porpoises (n=8) ranged on average about the same distances as mature males (n=10), although they were not recorded over in the western North Sea (Fig. 6). Porpoises tagged in Skagen moved seasonally from the northern Kattegat and Skagerrak northwards as far as the Shetland Islands, but did not range into the southern portion of the North Sea (Fig. 3). Those that were tagged in Inner Danish Waters were only rarely recorded outside this area.

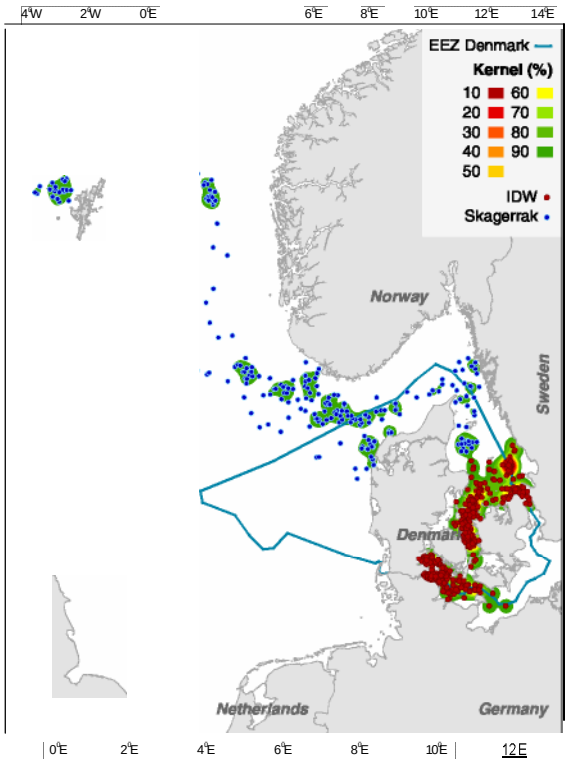

Figure 6. Kernel density estimates in 10% intervals based upon two mature female porpoises tagged in Skagen (in blue) and six mature female porpoises tagged in Inner Danish waters (in red)

Recommended Management Units

A number of attempts have been made in the past to distinguish North Atlantic porpoise populations or stocks for management purposes, not least because of significant anthropogenic pressures they face, particularly with respect to fisheries by-catch but also from changes in food resources in part as a result of over-fishing, as well as from pollution and disturbance.

Gaskin (1984) proposed 14 stocks or subpopulations for porpoises in the North Atlantic, and, later, IWC (1996) revised this to 13 (with one more in the Black Sea), lumping together as one unit, the English Channel, NW French, Spanish and Portuguese waters, including the Bay of Biscay. In March 1999, a joint IWC-ASCOBANS working group reviewed population structure evidence and recognised extra subdivisions within the North Sea, Kattegat and Skagerrak (IWC, 2000). Since then, there have been two important reviews of porpoise population structure by Andersen (2003) and Lockyer (2003).

The current ASCOBANS Population Structure Workshop recommends only minor changes to earlier divisions, and these mainly in the light of recent more comprehensive genetic studies and the combining of information from other approaches (e.g. telemetry) so as to derive Management Units that were not so heavily based upon genetics.

Table 1. Supporting Evidence for Proposed Management Units in Harbour Porpoise
[MU = Management Units; GoM = Gulf of Maine & Bay of Fundy; GoSL = Gulf of St Lawrence; NEW = Newfoundland; WGR = West Greenland; ICE = Iceland; FAR = Faroe Islands; NOR = Northwest/Centralwest Norway & Barents Sea; NENS = Northeastern North Sea & Skagerrak; SWNS = Southwestern North Sea & Eastern Channel; IDW = Inner Danish Waters; BAL = Baltic Sea; CES = Celtic Sea (plus South-west Ireland, Irish Sea & Western Channel); NWIS = North-west Ireland & West Scotland; BoB = Bay of Biscay (West France); IBNA (NW Spain, Portugal & NW Africa)] √ = evidence for differentiation; x = evidence for no differentiation)

MU	Isozymes	mtDNA	Microsat.	Skeletal	Tooth ultra-structure	Dietary	Contam.	Paras.	Telemetry
GoM		√	√		√			√	
GoSL	√	√	√						
NEW		√	√						
WGR	√	√	√		√	√	√	√	
ICE		√			√	√			
FAR								√	
NOR		√	√	√	√	√	√		√
NENS	√	√	√		√	√	√		√
SWNS	√		√	√		√	√	√	
IDW	√	√	√	√	√	√	√	√	√
BAL		√		√	√		√		√
CES		√	√	√	√	√	√		
NWIS		√							
BoB									
IBNA			√						

Table 1 lists the 15 Management Units proposed for the North Atlantic, along with the supporting evidence for those divisions. These include nine MUs within the ASCOBANS Agreement Area, and three others (NOR, ICE, WGR) adjacent to these, as well as three in North America. Figure 7 illustrates the geographical areas covered.

The main changes from earlier stock divisions are:

1) Division of the North Sea into two MUs along a median (at this stage arbitrary) line, running NNW-SSE;
2) Inclusion of the Shetland Islands, Skagerrak and northern Kattegat within the Northeastern North Sea MU;
3) Northern boundary shift of the Northeastern North Sea MU along the Norwegian coast;
4) Inner Danish Waters MU to include part of the Kattegat, all of the Danish Belt seas, and the Western Baltic;
5) The Baltic Sea proper to form a separate MU, with its western boundary being around the Darss/Gedser underwater ridge or Rügen;
6) The coasts of Portugal and NW Spain forming a separate MU (at this stage placed with NW Africa, but this needs to be verified). Recent studies suggest this is also an evolutionary significant unit and should be given priority for separate management.

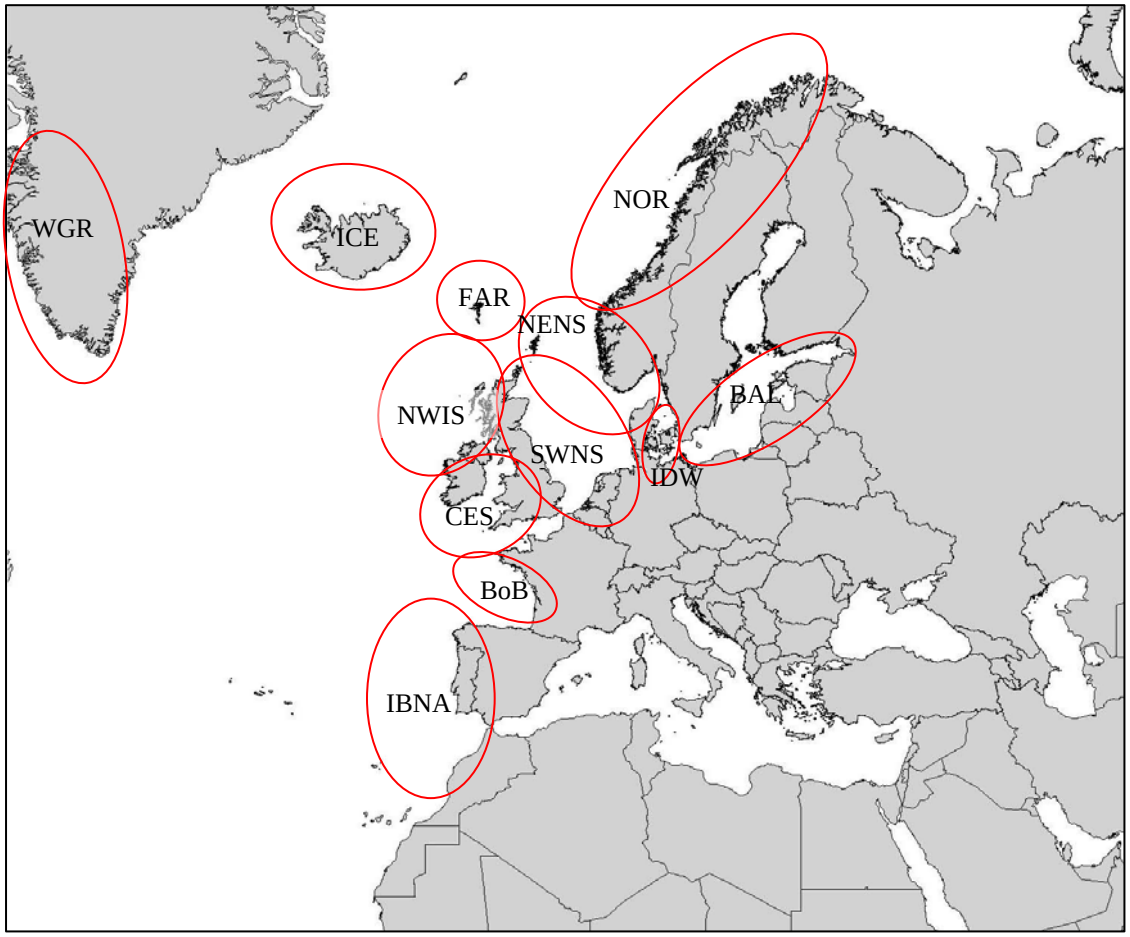

Figure 7. Map showing Recommended Management Units for Harbour Porpoise in the ASCOBANS Agreement Area and Environs

At present, a separate MU has been assigned to the Bay of Biscay although porpoises from this region have not been fully investigated (it may be marginal habitat for them anyway). There appears to be a small population occurring year-round along the French Biscay coast (V. Ridoux, *pers. comm.*), which is more likely to be linked to porpoises further north than to an Iberian population (as indicated by the results of Fontaine *et al.*, 2007a). Along the north Spanish coast, the shelf is very narrow and there does not appear to be a regular porpoise population, which may have created the conditions for genetic differentiation of the Iberian population. Along the Atlantic coasts of the British Isles and Ireland, various lines of evidence suggest that porpoises in South-west & Southern Ireland may be linked to South-west Wales and South-west England as well as offshore in the Celtic Sea. Further north, porpoises along the west coast of Ireland from Counties Clare to Donegal are comparatively uncommon and little investigated. For the time being, these are assigned to a separate MU along with western Scotland, although this needs further study. In the northern North Atlantic, the restricted shelf area around the Faroe Islands supports numbers of porpoises but information on their population structure also remains limited.

Recent analysis of genetic, stable isotope and toxicological data (Fontaine *et al*., 2007a, b; Bull *et al*., 2008) using individual-based approaches that do not require *a priori* delimitation of groups, has proven to be a promising tool for examining the possibly underlying processes shaping population structure. Their results suggest that in the northern European Atlantic, Irish Sea and North Sea, porpoises form a more or less continuous population. Significant isolation by distance among individuals was detected with local habitat-related variation in its strength (perhaps in relation to foraging strategy: e.g. benthic vs pelagic prey). This may mean that within this particular region, it is difficult, if not impossible, to demarcate management units on a geographical basis.

Future Research Priorities

There is a need to conduct more individual-based analyses along the lines of the above studies, and for further hypothesis testing using as many samples as possible whose locations are accurately known and that are spatially representative across the entire region. Any provisional spatial clusters could then be tested against one another. Possible comparisons might include West Greenland *vs* Newfoundland, western, southern and eastern Iceland; the Faroe Islands *vs* Iceland, northern Norway, western Norway, the Shetland Islands and north Scotland; Western Scotland *vs* North-west Ireland and South-west Ireland; the southern Irish Sea *vs* northern Irish Sea, West Scotland, Western Ireland, and South-west England; Orkney and North-east Scotland *vs* Eastern England, and the Danish, German, and Dutch North Sea; the Bay of Biscay *vs* English Channel, North-west France and Atlantic Iberia; and Northwest Africa *vs* Portugal, North-west and South-west Spain.

Very few porpoises remain in the Baltic Proper, but the possibility exists to examine historical museum specimens and conduct skull morphometrics and ancient DNA analysis in order to ascertain whether porpoises that lived in former times in the eastern Baltic differ from present-day porpoises in the western Baltic.

The satellite telemetry study of more than sixty individuals conducted in Danish waters over an eleven-year period, has been helpful in providing insight into

contemporary movement patterns. Tagging has relied upon incidental capture in pound nets, but the rescue of a sufficient number of live porpoises from most other types of fishing gear is unlikely to be practical without running the risk of causing undue distress. Of other approaches, it is recommended in particular that geometric morphometric studies be conducted upon a larger sample of material, collected from a comparable time period and spanning as wide a geographical area as possible. These should cover different age/sex classes, and where possible, make use of samples of known location (e.g. from bycatches).

A limitation in investigations of porpoise population structure has been knowledge of the precise locations and time periods for sample collections. Rarely have these been given in the literature, nor have the seasons been identified when the samples were collected. Even bearing in mind the fact that origins of stranded porpoises will always be imprecise, there are a number of studies, for example, where it is not clear whether samples derive from west, south or east coasts of Ireland, western or eastern sectors of the English Channel, or which sectors of the North Sea and Norwegian coasts. In future, it is recommended that maps are included showing the exact locations of all samples, and that some analyses are repeated using the MU divisions proposed above.

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8.3 Bottlenose Dolphin *Tursiops truncatus*

Peter Evans, Rus Hoelzel, Simon Ingram, Valentina Islas, Ada Natoli, and Vincent Ridoux

Introduction

The bottlenose dolphin has a worldwide distribution in tropical and temperate seas in both hemispheres. In the North Atlantic, it occurs from Nova Scotia in the west and the Faroe Islands in the east (occasionally as far north as northern Norway and Svalbard), southwards to the equator and beyond (Fig. 1).

Figure 1. North Atlantic Distribution of Bottlenose Dolphin

Along the Atlantic seaboard of Europe, the species is locally fairly common near-shore off the coasts of Spain, Portugal, north-west France, western Ireland (particularly the Shannon Estuary and Connemara), North-east Scotland (particularly Moray Firth south to the Firth of Forth), South-west Scotland, in the Irish Sea (particularly North and West Wales, including all of Cardigan Bay), and in the English Channel (Berrow *et al*., 2001; Lahaye and Mauger, 2001; Pineau *et al*., 2001; Evans *et al*., 2003; Reid *et al*., 2003; see Fig. 2). Smaller groups of bottlenose dolphins have also taken up residence at other localities – for example, around the Outer Hebridean island of Barra, and in the Inner Hebrides (Islay, Mull, Coll, Tiree and southern Isle of Skye) in West Scotland (Evans *et al*., 2003).

The species also occurs offshore in the eastern North Atlantic, particularly along the shelf edge (where it occurs often in association with long-finned pilot whales), as far north as the Faroe Islands and even Svalbard (Evans *et al*., 2003; Reid *et al*., 2003). In the Bay of Biscay, Certain *et al.* (2008) have shown that bottlenose dolphin preferential habitat was over the outer shelf and the shelf break.

In coastal waters, bottlenose dolphins often favour river estuaries, headlands or sandbanks where there is uneven bottom relief and/or strong tidal currents (Lewis and Evans, 1993; Liret *et al*., 1994; Wilson *et al*., 1997; Rogan *et al*., 2000; Liret, 2001; Ingram and Rogan, 2002). Offshore, the species occurs particularly along the continental shelf edge, seasonally entering near-shore waters around the Faroe

Islands, northern and western Scotland, western Ireland, in the Bay of Biscay, and around the Iberian Peninsula (Galicia and coast of Portugal) (Evans *et al*., 2003; Reid *et al*., 2003; Certain *et al*., 2008).

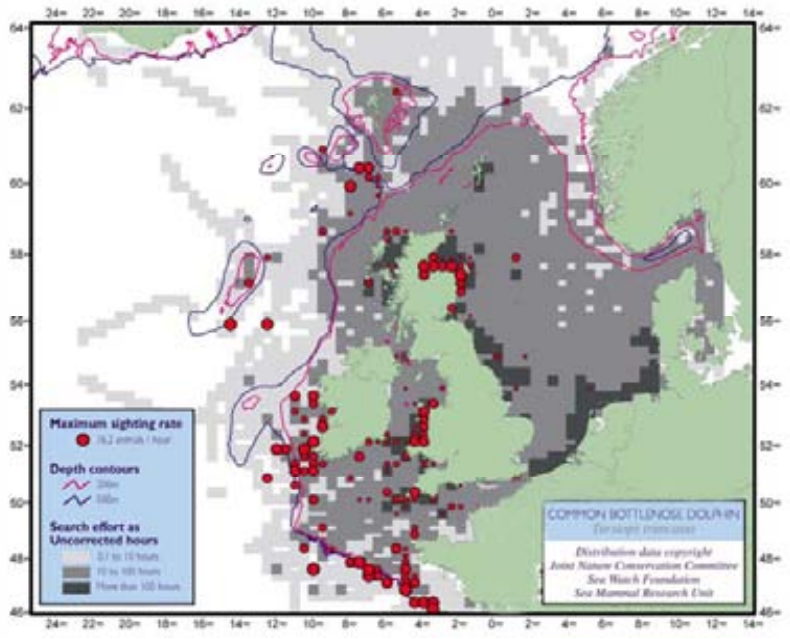
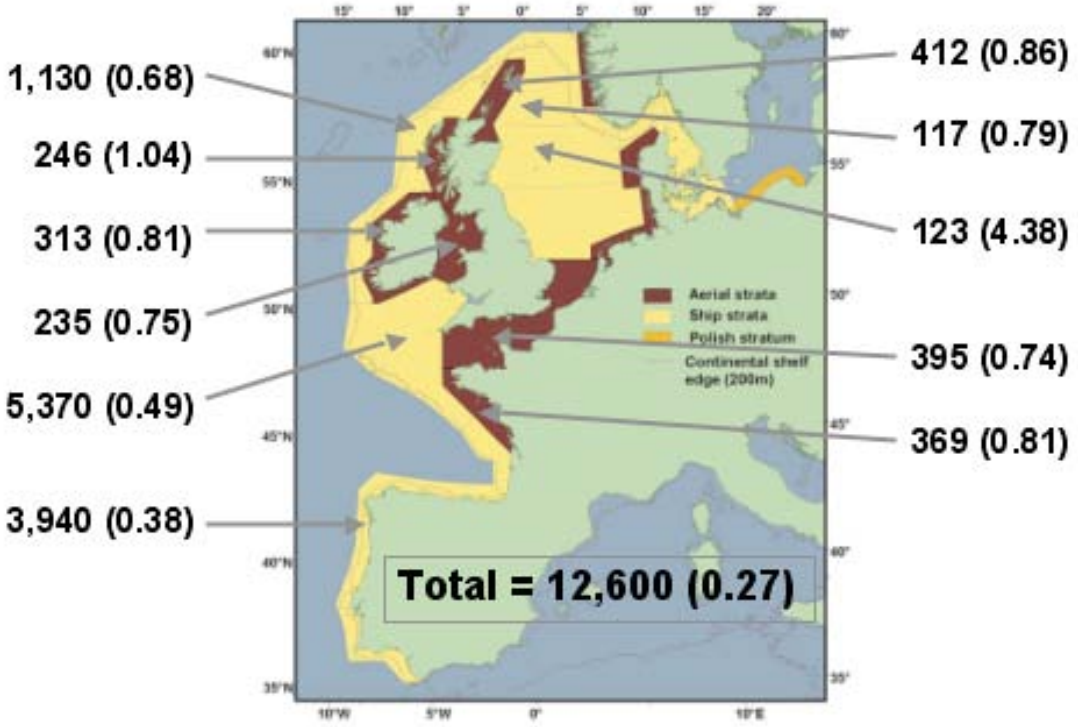


Figure 2. Sighting rates of Bottlenose Dolphins
[Records from 1979-98. Red circles are scaled in proportion to the number of animals observed per hour of observation. Sightings rates are standardised for observations made under different sea conditions but have not been corrected for differing efficiencies of the various people & vessels used to collect the data. The grey shaded cells indicate observation effort (from Reid *et al*., 2003)]



Figurre 3. Abundance estimates (and CVs) for bottlenose dolphins from SCANS II Survey, July 2005

SCANS II surveys of Northwest European shelf waters in July 2005 gave an overall abundance estimate of 12,600 (CV=0.27) (Hammond, 2008; Fig. 3), whereas, offshore, the CODA survey (July 2007) yielded an abundance estimate, uncorrected for g(0) and responsive movement, of 19,300 (CV=0.25) (P.S. Hammond, *pers. comm.*). These highlight the significant offshore population(s) of this species.

Regional inshore studies indicate a resident population of 129 bottlenose dolphins (95% CI = 110-174) in the Moray Firth (Wilson *et al.*, 1999), whilst the population in Cardigan Bay (within the Special Area of Conservation extending south to Fishguard) has been estimated at 213 bottlenose dolphins (95% CI = 183-279) (Baines *et al*., 2002; Evans *et al*., 2002). Neither population is closed, and individuals may join up for periods of time from elsewhere. In Western Ireland, estimates of between 113 and 140 individuals have been reported as occupying the Shannon Estuary (Ingram 2000; Ingram and Rogan, 2003, Englund *et al*., 2007, 2008). Elsewhere in Ireland, small groups (between 15 and 45) have been recorded on the west coast in Donegal Bay, Blacksod Bay, Killary Bay, Brandon Bay, and Kenmare River, and on the south coast around Cork Harbour and Youghal Bay (Ingram and Rogan, 2003; Ingram *et al*., 2001, 2003).

Between Normandy (North-west France) and Jersey in the Channel Islands, at least 66 individuals have been recognized by photo-ID (Lahaye and Mauger, 2001). Over a wider area, including the Bay of Mont Saint-Michel, Iles Chausey, and all of the Cotentin coast, around 170 individuals have been estimated from line-transect surveys and photo-ID (GECC, *unpubl.*). Further west, bottlenose dolphins can be seen along the Cancale coast and Gulf of St Malo, but no abundance estimates exist and these animals could partly be the same as those dwelling around Cotentin-Mont Saint-Michel Bay region. About 35 individuals inhabit the area around the island of Ouessant and the Achipelago of Molène, with a further 25 individuals around the island of Sein and Cape of Sizun (Liret *et al*., 2006; Oceanopolis, *pers. comm.*). Scattered sightings occur south to the Bay of Biscay, with regular groups along the coasts of Cantabria and Asturias, but no population estimates.

Skeletal Variation

Within the ASCOBANS region, no comparison has been made of skeletal variation from bottlenose dolphins collected in different areas. Elsewhere, studies have tended to show size differences between nearshore and offshore forms (Ross, 1977; 1984; Walker, 1981; Duffield *et al*., 1983; Ross and Cockcroft, 1990; Van Waerebeek *et al*., 1990; Mead and Potter, 1995), with near-shore individuals being larger in the eastern North Pacific (Walker, 1981), and smaller in the western North Atlantic (Mead and Potter, 1995). Mead and Potter (1995) also found that the offshore form had consistently wider nasal bones than the near-shore.

Genetic Analyses

The differences between coastal and offshore populations of bottlenose dolphins in the western North Atlantic (from Florida north to Nova Scotia), observed from morphology, diet and feeding ecology, parasite load, and haemoglobin profile (Mead and Potter, 1995; Hersh and Duffield, 1990), have been confirmed by genetic studies using microsatellites and mtDNA (Hoelzel *et al*., 1998). Putative populations on

either side of Florida were also found to be differentiated (based on mtDNA RFLP analysis), although not all samples compared may have been of the same morphotype (i.e. all coastal or all pelagic) (Dowling and Brown, 1993). A similar scenario has also been observed in the Gulf of Mexico (Sellas *et al*., 2005). An analysis of nine different populations from seven different areas of the world by mtDNA and microsatellite DNA markers found significant differentiation among all putative regional populations (Natoli *et al*., 2004). Dolphins in coastal habitats showed lower variability and were in most cases differentiated from a pelagic lineage, possibly suggesting local founder effects, at least in some cases. The western North Atlantic pelagic populations were found to be more closely related to eastern North Atlantic and Mediterranean bottlenose dolphins than they were to coastal populations in the western North Atlantic (Natoli *et al*., 2004).

A subsequent study focused on assessing the population structure of bottlenose dolphins across its European range analysed a total of 145 samples using microsatellite loci and sequences of the mtDNA control region (Natoli *et al*., 2005). It included samples from Scotland (n=20), South of England (n=6), Spain (n=18, Portugal (n=11) and compared them with samples from the Mediterranean Sea (n=74) and Black Sea (n=16). Within the Eastern North Atlantic, marked genetic differentiation was detected between the Scottish samples and those from the rest of the Eastern North Atlantic (F_{ST}=0.068, p<0.001), although no significant genetic substructuring was observed among the samples from the different areas of the Eastern North Atlantic. Across the range analysed five genetically different populations were found using both a Bayesian based method (Structure) and a classic F_{ST} statistics. These were: 1) Scotland, 2) other locations in the eastern North Atlantic, 3) western Mediterranean, 4) eastern Mediterranean, and 5) Black Sea. These appear to reflect different habitat characteristics in the different areas: the northern North Sea (Scotland) *vs* rest of eastern North Atlantic; Italian peninsula separating western and eastern Mediterranean; and Mediterranean Sea *vs* Black Sea. Both markers used gave consistent results showing that both males and females have a similar dispersal pattern and no sex-biased dispersal was detected (Natoli *et al*., 2005). Asymmetrical migration rates based on the mtDNA data showed some directional migration from the populations inhabiting the peripheral habitat areas, including Scotland.

In a study of population structure amongst bottlenose dolphins from the UK, sequence analysis of 549bp of the mitochondrial DNA control region identified eight unique haplotypes in a sample of 29 individuals (Parsons *et al*., 2002). Analysis of molecular variance suggested that the Moray Firth population in North East Scotland was genetically more closely related to Welsh animals (F_{ST}=0.15) than to its nearest neighbour population in West Scotland (F_{ST}=0.69). Furthermore, measures of within-population genetic diversity were markedly lower in the Moray Firth than any other sampled region. However, sample sizes were in all but one case very small: Moray Firth (n=15), W. Scotland (n=3), Wales (n=5), S. England (n= 1), and Ireland (n=5).

Since then, in a recent study with a larger sample size from Scotland (Islas, Graves and Janik, unpubl.), significant population structure was again found between the east coast of Scotland and Wales, and a higher separation between the west and east coasts of Scotland.

Parsons *et al*. (2002) demonstrated a low level of genetic diversity for the mitochondrial control region of the east Scottish coast bottlenose dolphin population. Local adaptation and then potential extinction of isolated groups may be a characteristic of this species, as may have been the case, for example, in the now extinct highly differentiated bottlenose dolphin population from the Humber estuary, North-East England, determined from ancient DNA studies and application of Bayesian inference (Nichols *et al*., 2007).

Further south, a genetic study of stranded bottlenose dolphins in Galicia, North-west Spain, by Fernández *et al*. (2009), using 451 base pairs of the mtDNA control region as well as microsatellites, found that in both cases the bottlenose dolphins that stranded in southern Galicia (n=32 for mtDNA haplotypes, and n=20 for microsatellites) were significantly different to those from northern Galicia (n=15 for mtDNA, and n=10 for microsatellites) as well as from Portugal (n=8 for mtDNA, and n=7 for microsatellites). There were also significant differences in microsatellite frequencies between southern Galicia and the northeastern corner of Spain (n=4), but not in mtDNA haplotype frequencies (n=3). However, these sample sizes were all very small.

Another recent genetic study analysed genotypes of 14 microsatellite loci obtained from skin samples from 26 bottlenose dolphins from the Shannon Estuary and from six stranded individuals from other locations along the west coast of Ireland (Miller *et al.*, 2009). They found that most loci (12 out of 14) showed alleles that were unique to either nside or outside of the estuary, indicating support for the photo-ID data that the Shannon Estuary population may be distinct from others along the west coast of Ireland.

Ecological Studies

Although a variety of techniques have been used in the western North Atlantic to study ecological aspects of bottlenose dolphin population structure, in North-west Europe these have largely been confined to photo-ID studies. Comparisons of images of recognisable individuals have shown no evidence for interchange between bottlenose dolphins in the Irish Sea with Western Scotland or the North Sea (Pesante *et al*., 2008), just one between Western Ireland and the Irish Sea (O'Brien *et al*., 2009), none between Barra in the Outer Hebrides and the Inner Hebrides (S. Ingram, *unpubl.* data), or between the southern coasts (Normandy and the Channel Islands) and the northern coasts of the English Channel (South coast of England) (Liret *et al*., 1998). Recently, however, a total of seven matches were obtained between bottlenose dolphins from West and East Scotland (Robinson, *et al*. 2009), although the East coast population appears to be largely isolated, with individuals ranging from Caithness as far south as the Firth of Forth and Northumberland, and no other matches as yet with the West coast or Irish Sea (Wilson *et al*., 2004; Pesante *et al*., 2008; Weir *et al*., 2009). Around Ireland, several long-distance movements have been confirmed (O'Brien *et al*., 2009), mainly up and down the west coast, but with one movement of c. 700 km being between Dublin Bay and Galway Bay, and others between Galway and Antrim on the north coast, and Galway and Cork on the south coast.

Along the French coast, an apparently isolated group lives around the Isle of Sein (Liret *et al*., 1997), and the degree of isolation of another group living around Molène, about 40 km northward has not been assessed. Further south along the French and

Spanish Biscay coasts, there have been insufficient photo-ID studies to determine whether discrete populations exist. In a recent past, an isolated group of six individuals used to live in Arcachon basin and was shown to be strictly resident by photo-identification (Ferrey *et al.*, 1993); it became extinct in the early 2000s. Elsewhere on the Iberian Peninsula, a small (n=26 individuals) isolated population has been studied for a number of years around the Sado Estuary of Portugal (Augusto, 2007), and recently a photo-ID study (with 24 dorsal fin profiles so far) was initiated off Sesimbra (Sousa *et al*., 2009). So far, no match between the resident Sado community and those from west central Portugal has been made.

Recommended Management Units

The limited information available at present suggests that bottlenose dolphins inhabiting the continental shelf edge and environs are best treated as a separate management unit. This is provisionally taken to include animals from around the Faroe Islands southwards along the shelf to the Iberian Peninsula. In particular, there may be a difference between truly oceanic areas and shelf break-outer shelf habitats.

The following near-shore populations are each proposed as separate management units (although it is quite possible that some areas have overlapping communities with different movement patterns): 1) North Sea (Eastern Scotland from Caithness to the borders with England); 2) Outer Hebrides (Island of Barra); 3) Inner Hebrides; 4) Irish Sea; 5) Shannon Estuary; 6) Western Ireland; 7) Southern England; 8) Channel Islands and Normandy coast (North France); 9) Brittany coast and islands (West France); 10) Southern Galicia; and 11) Sado Estuary (Portugal). Future studies may reveal further local populations along Irish, French, Spanish and Portuguese coasts.

Table 1. Supporting Evidence for Proposed Management Units in Bottlenose Dolphin
[MU = Management Units; AE = Atlantic Europe; NS = North Sea; OH = Outer Hebrides, IH = Inner Hebrides, IS = Irish Sea; SE = Southern England; NF = N France / Channel Islands, SHE = Shannon Estuary (Ireland); WEI = Western Ireland; BR = Brittany, SGA = S Galicia; SAE = Sado Estuary (Portugal)] √ = evidence for differentiation; x = evidence for no differentiation)

MU	mtDNA	microsat.	Photo-ID
AE	√	√	
NS	√	√	(√)
OH			√
IH			√
IS	x		√
SE			√
NF			√
SHE		√	√
WEI			√
BR			√
SGA	(√)	(√)	
SAE			√

Table 1 lists the proposed MUs, and the evidence upon which those classifications are based, and Figure 4 shows the geographical areas. It is important to emphasise that

these Management Units should be considered provisional and subject to updating on a regular basis as new information becomes available.

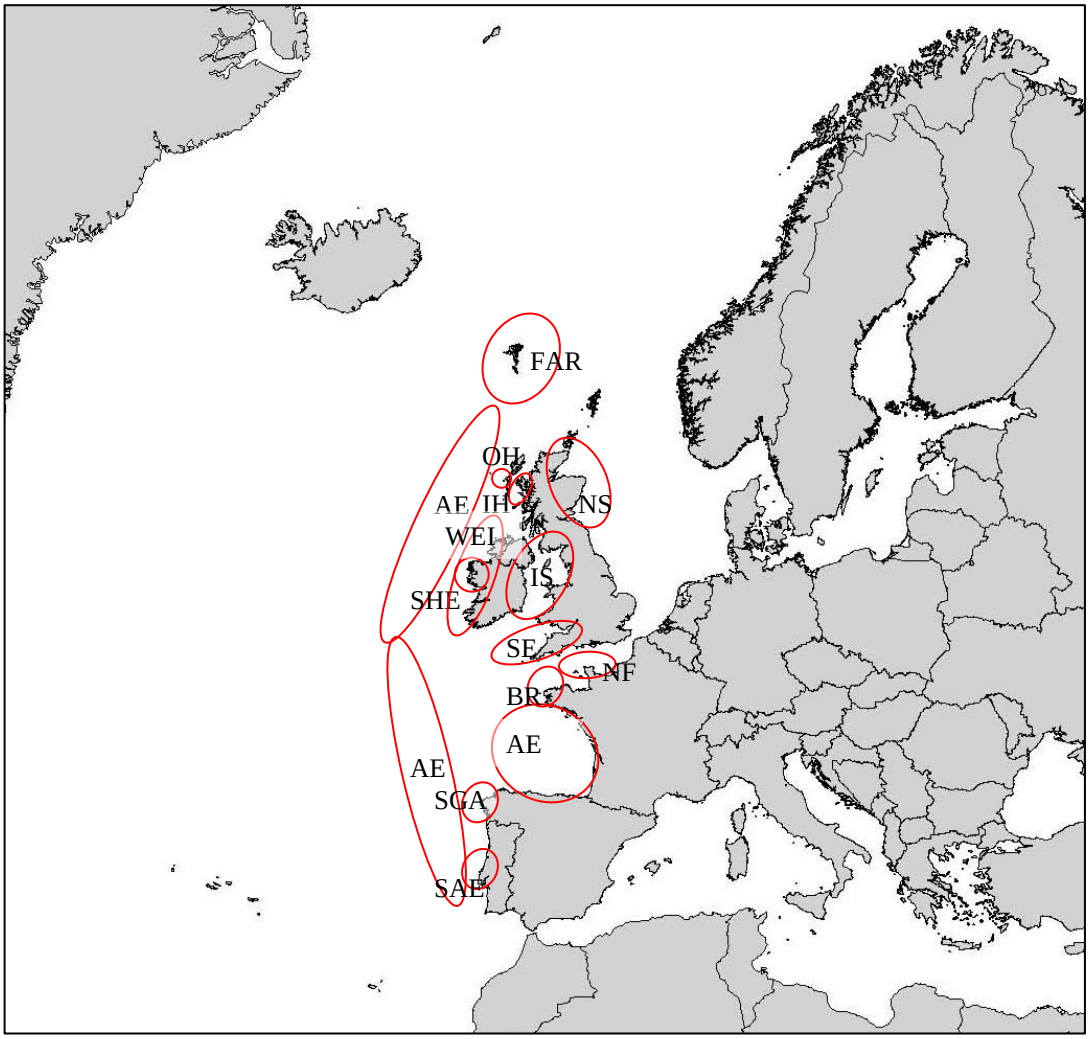


Figure 4. Map showing Recommended Management Units for Bottlenose Dolphin in the ASCOBANS Agreement Area and Environs

Future Research Priorities

There is an important need to investigate the sizeable offshore population of bottlenose dolphins, and to establish whether this forms one or more discrete units, and if there is interchange with any coastal populations. Those occurring in coastal regions may comprise more than one community (as indicated, for example, in Western Ireland and West Wales): a sedentary community that may be associated with a reliable food resource, for example in a bay or at an estuary mouth; and a more mobile community that ranges widely up and down a coastline, and a little offshore.

Photo-ID has been a useful tool to establish ranging movements of animals particularly in East Scotland, West Wales, around Ireland and in Northwest France. This needs to be repeated at the same level of intensity for coastal populations elsewhere.

Finally, other complementary approaches to investigating population structure should be used wherever practicable, including from dead animals, the measurement of skull characters, assessment of parasite and contaminant loads, and variation in life history parameters; and for both dead and living animals, stable isotope and genetic analyses.

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8.4 White-beaked Dolphin *Lagenorhynchus albirostris*

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Introduction

White-beaked dolphins are confined to temperate and sub-polar seas (7-13^{o} C) of the North Atlantic from South West and Central East Greenland, Svalbard and Barents Sea, south to about Cape Cod (USA) in the west and the Bay of Biscay in the east (see Figure 1 showing range of regular occurrence).

Figure 1. Distribution of White-beaked Dolphin
(depicting those areas where the species is thought to regularly occur)

Four principal centres of high density can be identified: 1) The Labrador Shelf including South-western Greenland; 2) Icelandic waters; 3) The waters around Scotland and North-east England, including the Central and Northern North Sea and north-west coast of Scotland); and 4) The narrow shelf stretch along the Norwegian coast, extending north into the White Sea (Kinze, 2008).

The species occurs over a large part of the northern European continental shelf, mainly in waters of 50-100 m depth, and almost entirely within the 200 m isobath (Reid *et al*., 2003; Evans and Smeenk, 2008; see Fig. 2).

The most recent (July 2005) population estimate, covering European continental shelf seas from South West Norway, south to Atlantic Portugal, gave an estimate of 22,700 (CV=0.42), with the majority in the North Sea and off North-west Britain (Hammond, 2008; Fig. 3).

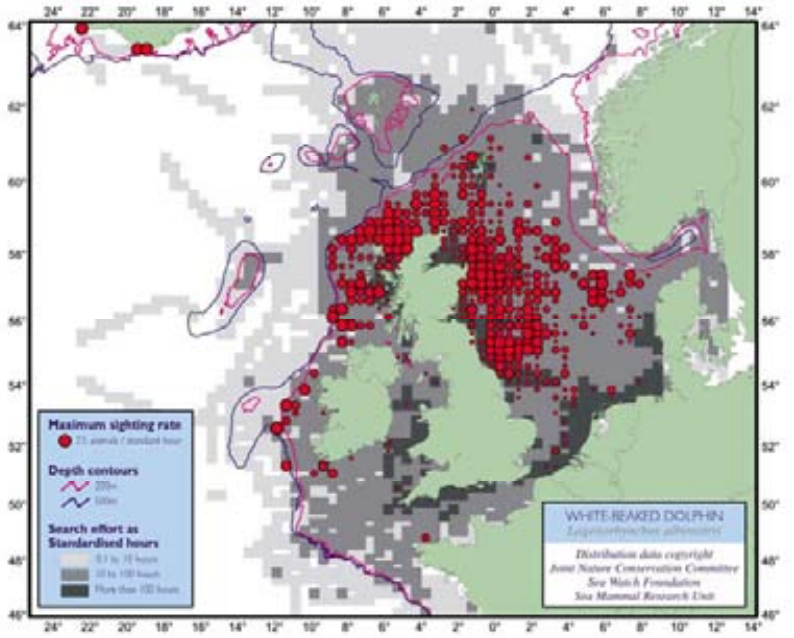
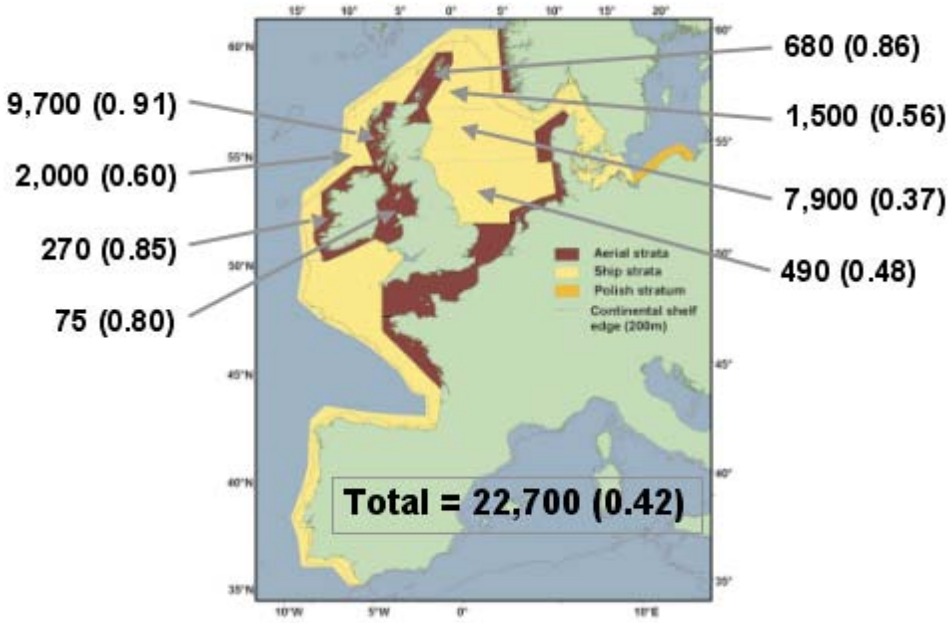


Figure 2. Sighting rates of White-beaked Dolphins
[Records from 1979-98. Red circles are scaled in proportion to the number of animals observed per hour of observation. Sightings rates are standardised for observations made under different sea conditions but have not been corrected for differing efficiencies of the various people & vessels used to collect the data. The grey shaded cells indicate observation effort (from Reid *et al.*, 2003)]



Figurre 3. Abundance estimates (and CVs) for white-beaked dolphins
from SCANS II Survey, July 2005

Skeletal Variation

Specimens from Eastern (North Sea and northern British Isles) and Western North Atlantic (Labrador Shelf) differ significantly in skull characters (Mikkelsen and Lund, 1994).

Life History Variation

Life history parameters have been estimated for a sample of 86 white-beaked dolphins originating from the Danish North Sea and Kattegat since 1980 (Galatius and Kinze, 2007). The males in the sample attained sexual maturity between 10 and 12 years of age (average 11.6), females between 6 and 10 years of age (average 8.7). Physical maturity was attained between 12 and 18 years in males, and 10 and 15 years in females. Females become physically mature on average at lengths of 251 cm, males at lengths of 271 cm, corresponding to mean ages of 15,6 yrs (95%CI: 9.8-23.1) and 11.4 yrs (95%CI: 7.7-18.1), respectively.

Females attain sexual maturity at a mean age of 8.7 yrs (95% CI: 5.1-14.6), males at 11.6 yrs (95% CI: 8.2-16.1). The mean lengths at sexual maturity were found to be 240 cm and 270 cm in females and males, respectively. There is a marked seasonality in the testes size of mature males. During the mating season (July and August), the combined testes mass increased six times from 500g to nearly 3000 g. The gestation period lasts about 11 months. Preliminary studies indicate a rather high annual ovulation rate of 0.7. Maximum age for females found was 34 yrs. Pregnant females were rarely encountered amoung stranded individuals, indicating longer periods of resting. These data represent the first detailed information for this species in the eastern North Atlantic.

Genetic Analyses

Genetic variation in white-beaked dolphins was evaluated using a fragment of the control region of the mtDNA (Banguera-Hinestroza *et al*., 2009). Overall, the genetic diversity of the species at the nucleotide level was extremely low (π =0.0056 ± 0.0004), comparable only to values reported in those cetacean populations with historically small population sizes, or that have been strongly affected by human activities (see, for example, Natoli *et al*., 2006; Bérubé *et al*., 1998).

Among the populations that were analysed, the highest variability was found in the population from the western North Atlantic (Canada), and the lowest genetic variability was in eastern North Atlantic populations.

Genetic differentiation among populations of white-beaked dolphin in the North Atlantic was evaluated using the control region of the mtDNA, and microsatellites, for some populations (Banguera-Hinestroza *et al*., 2009a). The study showed a clear genetic differentiation (using F_{ST} statistics) between the western North Atlantic population (Canada: n=7; NCBI GeneBank database n=6) and the three eastern North Atlantic populations examined (UK – n=38, the great majority of which were from northern and eastern Scotland or eastern England); Norway, all in the north between 71^{o} 26 N and 73^{o} 52’ E – n=33), and The Netherlands – n=38), as previously suggested by Mikkelsen and Lund (1994). F_{ST} values were as follows: UK v Netherlands, n.s., UK v Norway 0.092, UK v WNA, 0.10, Netherlands v Norway, 0.049, Netherlands v WNA, 0.108, and Norway v WNA, 0.082. There were fewer comparisons for microsatellites - UK v Netherlands, n.s.; Norway v UK, 0.019, and Norway v Netherlands, 0.029.

Among eastern North Atlantic populations, a clear genetic difference at the mitochondrial level (mtDNA) and nuclear level (microsatellites loci) was found

between UK populations (which included samples from Scotland, England and the Irish Sea) and the Norwegian population (samples from the northernmost region of Norway). The Norwegian population was also significantly different from animals sampled in the Netherlands (i.e. from the southern North Sea), but no differentiation was found between the Dutch and UK populations.

Ecological Studies
White-beaked dolphins have been studied in Faxaflói Bay in the Southwestern part of Iceland during the summers 1997–2006 (Rasmussen, 1999, 2004). Results from a photo-ID study showed that 12-20% had recognisable markings, with a maximum of nine resightings during a field season of three months duration (Rasmussen and Jacobsen, 2003). The species has also been studied in Icelandic waters using whale-watching boats as a platform, both in Faxaflói Bay and Skjálfandi Bay (Salo, 2004; Cecchetti, 2006; Magnúsdóttir, 2006). One individual white-beaked dolphin was tagged in Faxaflói Bay during the summer of 2006. That individual stayed within the 200 m depth curve and remained in the western part of Iceland (Rasmussen *et al*., 2007). This corresponds with the findings of Salo (2004), who found from the NASS data that white-beaked dolphins were more frequently observed in the western part of Iceland compared to the eastern part of Iceland. According to these observations, there could be a population of white-beaked dolphins in the western part of Iceland, and another one towards the Faroe Islands, but further studies are needed to confirm this.

Dietary Studies
The diet of the species seems to reflect the local abundance and availability of certain prey species, and as a consequence, geographical differences exist. In Danish waters cod and other gadoid fish were found to be the main prey items. White-beaked dolphins prey on larger cod (about 20 cm longer in total length) than do sympatric harbour porpoises. Similar dietary analysis from the Netherlands also found codfish as the most common prey fish, with whiting as the most common individual prey species, and a German study calculated that by numbers 79% and by mass contribution 94% of consumed fish were cod (Kinze *et al*., 1997). Analyses from Scottish waters showed that white-beaked dolphins additionally also consume cephalopods (Santos *et al*., 1994). For reasons given earlier, stomach contents analysis may reflect short-term dietary differences between individuals or groups, but alone cannot be used for identifying management units demographically separated.

Recommended Management Units
The above data showed that the Canadian population should be considered as a separate management unit. However, further management units for this region may be expected if more samples from other western North Atlantic regions are included in future genetic analysis.

In the eastern North Atlantic, evidence was found for considering individuals from the northernmost part of Norway as a distinct management unit. Given the genetic distribution of haplotypes found in other populations (for example along coastal areas of Scotland and England – Banguera-Hinestroza *et al*., 2009), individuals from Norwegian coastal areas (north to south) appear to form a continuous and differentiated population that may be considered as a single separate management unit. However, more sampling in the southern coastal areas of Norway will be

necessary to corroborate this hypothesis. This study also suggested the existence of one continuous population within British and southern North Sea waters, and therefore individual white-beaked dolphins belonging to this area could be considered as a distinct management unit. Photo-ID has also revealed matches between Scottish waters and the Danish North Sea and Skagerrak (C.C. Kinze, *pers. comm.*).

Table 1. Supporting Evidence for Proposed Management Units in White-beaked Dolphin (MU = Management Units; WNA = Western North Atlantic; IC=Iceland; NoN = Northern Norway; BI = British Isles and North Sea; √ = evidence for differentiation)

MU	Skeletal	mtDNA	microsat.	Photo-ID	Telemetry
WNA	√	√			
IC				(√)	(√)
NoN	√	√	√		
BI	√	√	√	(√)	

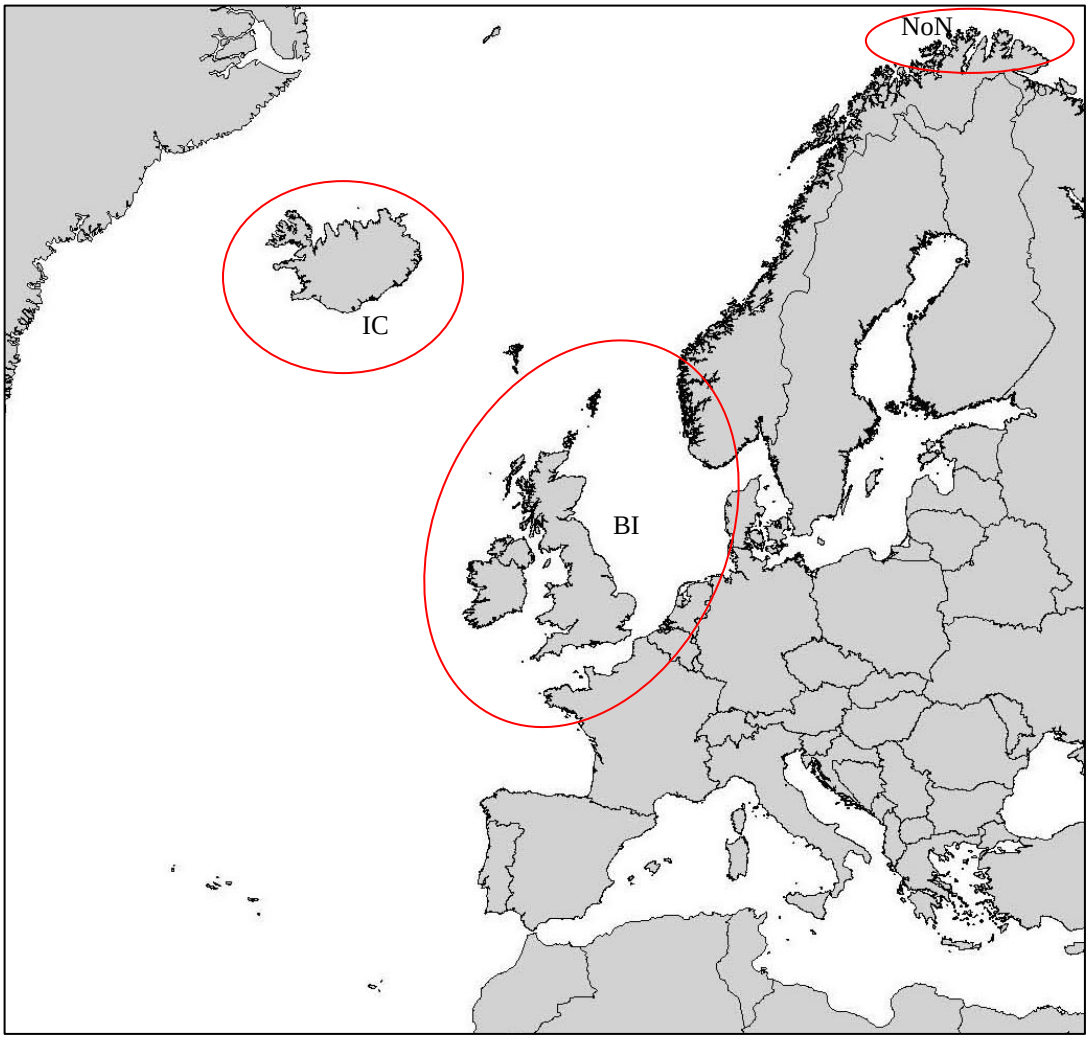


Figure 4. Map showing Recommended Management Units for White-beaked Dolphin in the ASCOBANS Agreement Area and Environs

To sum up, four management units are proposed for white-beaked dolphins based upon distribution patterns and the data obtained so far: 1) Western North Atlantic (Canadian waters, at least); 2) Icelandic waters; 3) northern Norway; and 4) a continuous management unit including the British Isles and all of the North Sea (Table 1; Fig. 4). The number of management units could increase significantly if more samples from other regions are analysed (e.g, Faroe Islands, Iceland, and western Ireland). For the time being, Ireland has been included within the British Isles MU.

Future Research Priorities

Sudies of genetic variability of white-beaked dolphins so far have shown that the populations of this species are highly vulnerable. As mentioned above, the extremely low nucleotide diversity (π = 0.0056 $\pm$0.0004), is probably due to a reduction in population sizes in the past, combined with the restricted habitat of this species to coastal areas highly affected by human activities (for example pollution and/or fisheries - see Jefferson *et al.*, 1993; Reeves *et al.*, 1999; Lien *et al.*, 2001) means that it should be a priority to study and protect populations of this species on both sides of the North Atlantic.

It is also of great importance to obtain and analyse samples from a geographical gradient on the two sides of the North Atlantic. Such samples should be analysed using several genetic markers (both nuclear and mitochondrial) in order to better understand the subdivision of these populations. In the western North Atlantic, samples from Canada and the east coast of the US (on a north-south gradient) will help to establish whether there is more than one management unit in this region. For the eastern North Atlantic, it is important to include samples from the southern and northern part of the North Sea, the Atlantic coast of Ireland, around Iceland and the Faroe Islands. This broad sampling will help not only to establish priority areas but also to elucidate how different events (fisheries, mass stranding, etc) are affecting the white-beaked dolphin populations.

In addition to the importance of a broad sampling regime for an accurate definition of management units, genetic studies on this species will provide a good opportunity to understand the evolution of marine species in the North Atlantic, and to elucidate the effects that past and future climatic changes may have upon the survival of the species and local populations in these northern latitudes. The genetic studies should also take place alongside ecological studies of the distribution and abundance of this species in coastal habitats.

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8.5 Atlantic White-sided Dolphin *Lagenorhynchus (Leucopleurus) acutus*

Eulalia Banguera--Hinestroza, Anders Galatius, Carl Chr. Kinze, and Peter Evans

Introduction

Atlantic white-sided dolphins are confined to temperate and sub-polar seas (7-12^{o} C) of the North Atlantic, mainly occurring offshore from SW Greenland, Iceland and the western Barents Sea south to Virginia (USA) in the west and the Bay of Biscay (47^{o} N) in the east (Fig. 1).

Figure 1. Distribution of Atlantic White-sided Dolphin
(depicting those areas where the species is thought to regularly occur)

The species is less common than white-beaked dolphin on the continental shelf, favouring the slope (mainly around 100-300 m depth) and deeper waters, particularly areas of high bottom relief and around deep submarine canyons (Fig. 2).

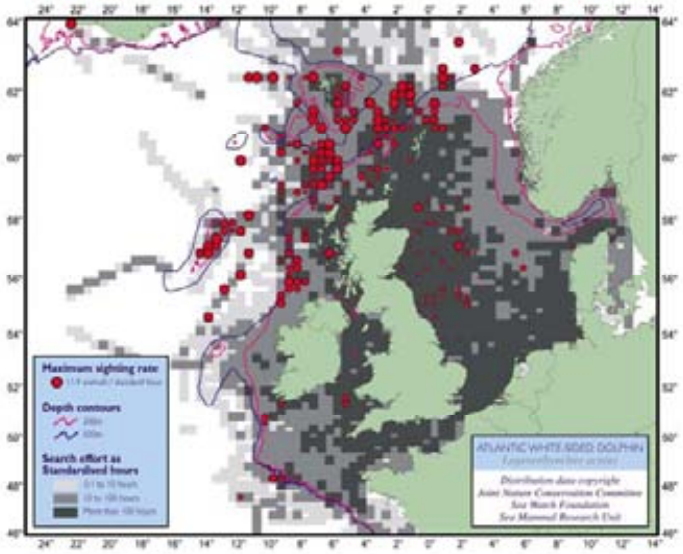

Figure 2. Sighting rates of Atlantic White-sided Dolphins
[Records from 1979-98. Red circles are scaled in proportion to the number of animals observed per hour of observation. Sightings rates are standardised for observations made under different sea conditions but have not been corrected for differing efficiencies of the various people & vessels used to collect the data. The grey shaded cells indicate observation effort (from Reid *et al*., 2003)]

The following appear to represent areas of relatively high density: 1) SW Gulf of Maine (40-42^{o}N) and continental slope at c. 39^{o}N; 2) Irminger Basin between SE Greenland and Iceland; 3) Iceland Basin south and east of Iceland; 4) Faroe Bank Channel and Faroe-Shetland Channel; 5) Halten Bank, west of Norway; and 6) Rockall Trough west of Scotland & Ireland (Selzer and Payne, 1988; Reeves *et al.*, 1999a; Evans *et al*., 2003; Reid *et al*., 2003; Salo, 2004; Evans *et al*., 2008). No comprehensive population estimates exist, and the July 2007 CODA survey of offshore NW European waters (between c. 44-61^{o}N) saw surprisingly few Atlantic white-sided dolphins and thus were unable to derive an abundance estimate (P.S. Hammond, *pers. comm.*). Further north, in Norwegian waters, an abundance estimate of c. 110,000 dolphins (white-sided and white-beaked, but likely to be mainly the former) was made from recent NAMMCO surveys (A. Bjørge, *pers. comm.*). Salo (2004) studied the sightings of white-sided dolphins from NASS (North Atlantic Sighting Surveys). She found that the species was more commonly observed in the eastern part of Iceland and towards the Faroe Islands than other parts of the region.

Skeletal Variation

No differences in skull characters were found between specimens from the Eastern and Western North Atlantic (Mikkelsen and Lund, 1994).

Genetic Analyses

The genetic variation of Atlantic white-sided dolphins was also evaluated using a fragment of the control region of the mtDNA (Banguera-Hinestroza *et al*., 2009). The nucleotide genetic diversity of this species was overall higher than the genetic diversity of white-beaked dolphins (π = 0.0095 ±0.0005). However, this variation is still lower than those reported for other dolphin populations (see Pichler and Baker, 2000). The genetic variability of this species showed a strong signature of past events in the DNA of this species, with evidence for a sudden demographic expansion after a reduction of population sizes in all the populations examined. Unique haplotypes were found in all different sampling areas that were analysed (Western Ireland,the Celtic Sea, West Scotland, Northern North Sea, and Western North Atlantic). On the other hand, Andersen (unpubl. data) compared 15 microsatellite loci and mtDNA for white-sided dolphins sampled from the Faroe Islands (n=123) and compared these with Scotland (n=41), but did not find any differences, so these probably should be considered as a single stock.

The study of genetic differentiation among populations using both mtDNA and microsatellite DNA loci revealed no differentiation between the Western North Atlantic (WNA) and samples from central to northern UK and western Ireland (Banguera- Hinestroza *et al*., 2009), nor among the different sampling areas in the Eastern North Atlantic. Sample sets included Western Ireland (N=22), the Celtic Sea (N=29), West Scotland (N=17) and Northern North Sea (N=20), and the Gulf of Maine (N=29). A separate set of 19th century samples from the WNA (N=26) was differentiated from the modern WNA samples from the Gulf of Maine; PHI_{ST} = 0.044. The sample from the south of England (English Channel) and Southwest of Ireland (Celtic Sea, N= 29) was also differentiated from the modern WNA sample (mtDNA, F_{ST} = 0.034), but the significance was marginal (p= 0.04).

The analysis of two temporally unrelated populations in the western North Atlantic (from Cape Cod and the Gulf of Maine) showed evidence for the following hypothesis: 1) the existence of several stocks of white-sided dolphins in the western North Atlantic, as previously suggested by Palka *et al*., 1997); and 2) the existence of a refugial population in the Gulf of Maine (the Gulf of Maine has already been suggested as a refugial area during the LGM by various authors - see Wares, 2002; Adams *et al*., 2006); and c) the existence of some level of genetic structure and differentiation between pelagic and coastal populations of Atlantic white-sided dolphin. This hypothesis should be the starting point for the continuation of genetic studies in this species.

Ecological Studies

Very little research has been undertaken on Atlantic white-sided dolphins in European waters (Evans and Smeenk, 2008). A limited number of biopsies have been collected from Icelandic animals, but so far, no analyses on these have been conducted to our knowledge. Stranded and by-caught white-sided dolphins from the southern North Sea (n=2) and South-west Ireland (n=4) have been examined for stable isotope levels, using muscle and liver (Das *et al*., 2003). Both carbon isotope ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) content were significantly depleted, by comparison with other marine mammal species examined, indicating the offshore habit and greater intake of invertebrates (such as oceanic cephalopods) by white-sided dolphins. Sample sizes were too small to make clear comparisons between the two regions.

Recommended Management Units

Given the results obtained so far with mtDNA analysis (Banguera-Hinestroza *et al*., 2009; Andersen, unpubl. data), at least four management units are proposed for the white-sided dolphin in the North Atlantic. As mentioned above for white-beaked dolphin, these units may change if the number of sampling regions is increased.

The four management units proposed are:

a) A North-eastern North Atlantic population including the northern North Sea;

b) A Central eastern North Atlantic population including the Celtic Sea and Western English Channel;

c) A Gulf of Maine population; and

d) The Cape Cod populations. Further genetic analysis is necessary to corroborate the existence of two management units along this Eastern seaboard.

Table 1. Supporting Evidence for Proposed Management Units in Atlantic White-sided Dolphin
(MU = Management Units; GoM = Gulf of Maine; CC = Cape Cod region;
NENA = North-eastern North Atlantic (Northern UK & Western Ireland);
CENA = Central eastern North Atlantic (Southern England & Celtic Sea)
(√) / √ = (weak) evidence for differentiation; x = evidence for no differentiation)

MU	mtDNA	microsat.	Stable isotopes
GoM	√	√	x
CC	√	√	x

NENA	√	√	
CENA	(√)		

Evidence for differentiation in the eastern North Atlantic is weak, and further genetic analyses, currently being conducted by Banguera-Hinestroza *et al*. (2009), may modify the present recommendations for MUs.

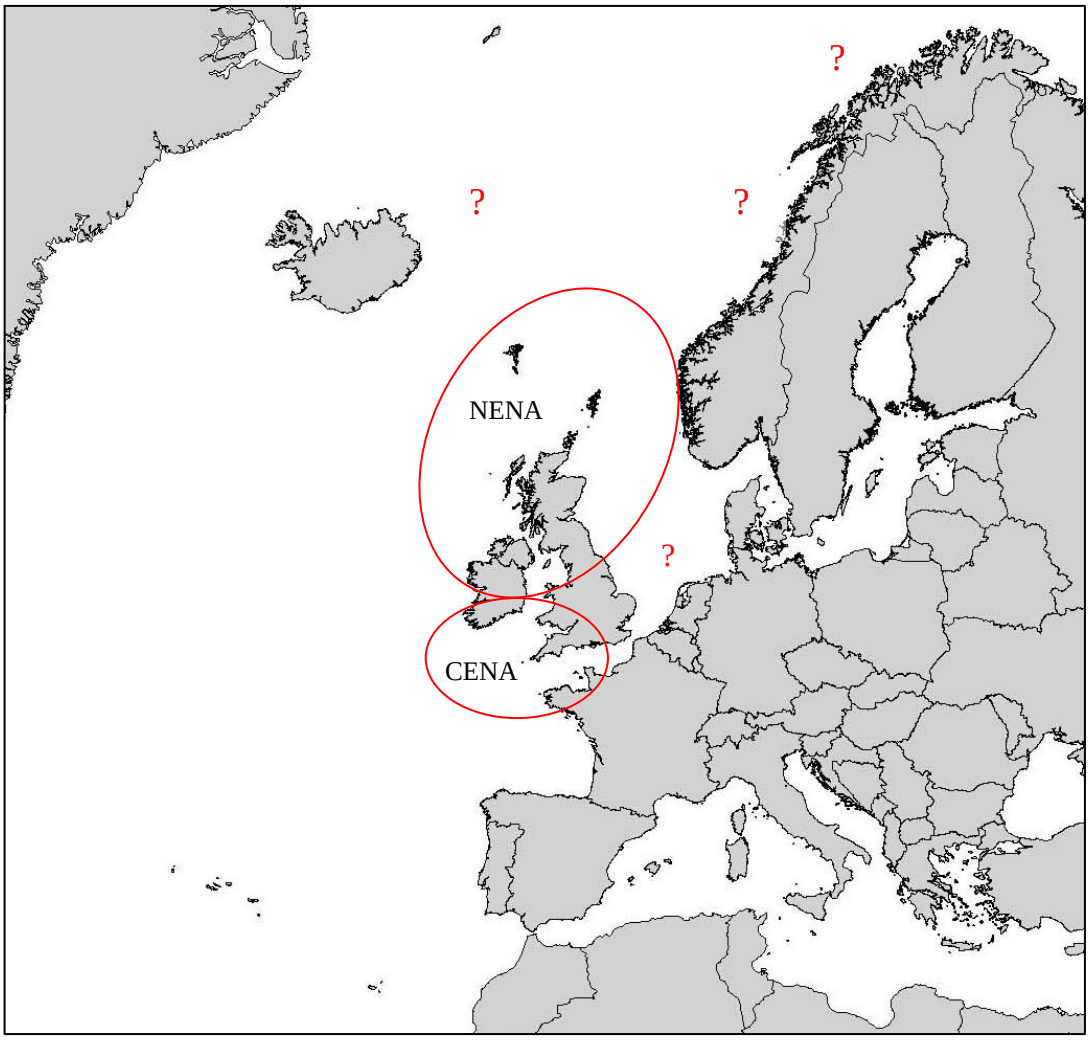

Figure 3. Map showing Recommended Management Unit for Atlantic White-sided Dolphin in the ASCOBANS Agreement Area and Environs

Future Research Priorities

In order to increase our understanding about subdivisions of Atlantic white-sided dolphin populations, it is imperative to increase the availability of samples for genetic studies. A clear strategy for obtaining samples from a broad range of regions should be launched in order to meet the conservation objectives for this species.

As indicated by the results presented above (Banguera-Hinestroza *et al*., 2009), there is some suggestion for the existence of pelagic and coastal stocks of white-sided dolphins. A sampling strategy along coastal areas of both regions (eastern and western North Atlantic), as well as from pelagic zones, will help to test this hypothesis and

establish whether or not pelagic white-sided dolphins should be considered in a different way to coastal white-sided dolphins.

A genetic study including new genetic markers, and increasing the available data, together with more studies about distribution and basic ecology of this species, will help not only to define appropriate management units, but also to improve our understanding above the evolution of this species and the forces influencing the population structure of North Atlantic species, like the Atlantic white-sided dolphin, that exhibit a broad range of distribution. In addition, the use of new phylogeographic approaches, will help to carry out comparative studies between Atlantic white-sided and white-beaked dolphins, as well as define different conservation strategies for both species and elucidate the existence of marine refugial zones in the North Atlantic.

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8.6 Short-beaked Common Dolphin *Delphinus delphis*

Sinead Murphy, Ada Natoli, Ana Rita Amaral, Luca Mirimin, Amelia Viricel, Florence Caurant, Rus Hoelzel and Peter Evans

Introduction

Two species of common dolphin are currently recognized in the North Atlantic – the short-beaked form, *Delphinus delphis*, and the long-beaked form, *D. capensis* (Heyning and Perrin, 1994; Rice, 1998; Perrin, 2009). The short-beaked common dolphin (hereinafter referred to simply as common dolphin) has a widespread distribution in the Northeast Atlantic, ranging from subtropical waters off Africa, into the Mediterranean Sea, northwards to approximately 65ºN latitude, west of Norway (Haug 1981; Weir *et al*., 2001; Reid *et al*., 2003; Bearzi *et al*., 2003; Murphy *et al*., 2008), and westwards to the mid-Atlantic ridge (Doksaeter *et al*., 2008). Evans *et al.* (2003) reported that sightings are rare in the eastern section of the English Channel and the southern North Sea (see also Fig. 2).

Figure 1. Overall Distribution of Short-beaked Common Dolphin
(depicting those areas where the species is thought to regularly occur)

In recent years there has been an increase in the number of sightings (and strandings) of common dolphins in north-west Scotland (Evans *et al.*, 2003; Macleod *et al*., 2005; Sea Watch, unpubl. data), which has been attributed to an increase in the sea surface temperature (SST) in the region (period examined 1948–2003), and a similar pattern has been observed in the North and Baltic Seas (Sea Watch, unpubl. data; H. Benke and C.C. Kinze *pers. comm*). An increase in strandings of *Delphinus delphis* along the Dutch and Danish coastlines also occurred between the 1920s and 1950s (Bakker and Smeenk, 1990), coinciding with a decline in strandings (1930s-1970s) along the Irish and English coast, and strongly suggesting a shift in the distribution of this species in western European waters at that time (Evans and Scanlan, 1989; Murphy, 2004; Murphy *et al*., 2006, 2008). This extension in distribution (and density) may have been related to changes in the distribution of prey, as a result of the negative phase of the North Atlantic Oscillation (Murphy, 2004). An earlier incursion of common

dolphins to the North Sea during the mid 1930s (reflected in the strandings record) was attributed to a stronger flow of Atlantic water and an associated invasion of squid, upon which the species was apparently feeding (Fraser, 1946).

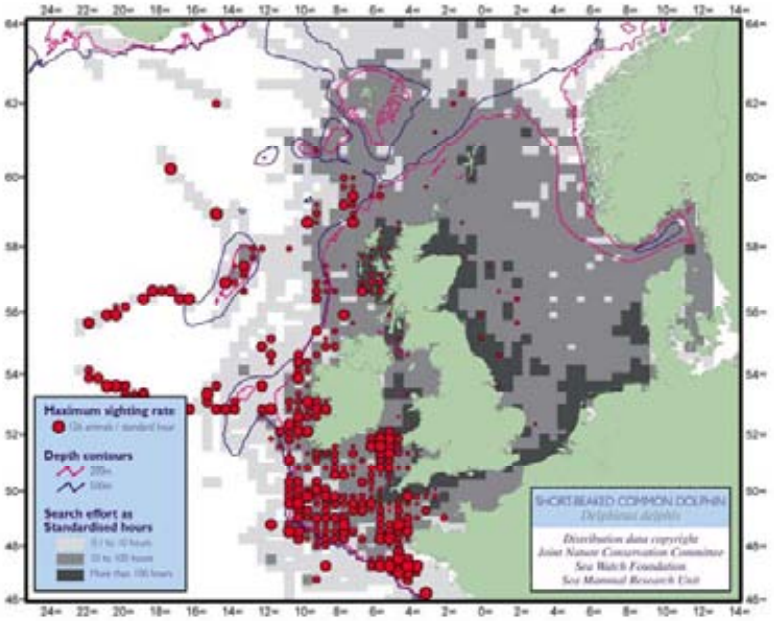

Figure 2. Sighting rates of Short-beaked Common Dolphins
[Records from 1979-98. Red circles are scaled in proportion to the number of animals observed per hour of observation. Sightings rates are standardised for observations made under different sea conditions but have not been corrected for differing efficiencies of the various people & vessels used to collect the data. The grey shaded cells indicate observation effort (from Reid *et al*., 2003)]

Seasonal movements have been documented in the Northeast Atlantic, with dolphins being more widely dispersed in offshore deeper waters during the summer (May-October) compared to the winter period (November-April), when there is a pronounced concentration in shelf waters of the western English Channel and further offshore parts of the Celtic Sea (WGMME, 2005). In shelf waters of the Irish Sea and west coast of Scotland, common dolphin abundance tends to be greatest in the summer months, although in recent years large schools have also been seen during winter off northern Scotland (Evans *et al*., 2003; Sea Watch, unpubl. data). Using sightings data along fixed ferry routes, Brereton *et al.* (2005) reported large numbers of common dolphins in the western English Channel during the winter months (December to February), with a reported 10-fold increase in sightings, whilst De Boer *et al*. (2008) from line transect surveys between January and March also concluded that the western English Channel was a very important winter habitat for the species. Kiszka *et al.* (2007) analysed sightings data obtained opportunistically onboard ferries operating predominately between July and October, and found aggregations to be larger in the northern Bay of Biscay than in the western English Channel. It has been suggested that the increased abundance of common dolphins at the shelf edge may be related to a preference for the concentration of its main prey species in this area, *Sardina pilchardus* and *Trachurus trachurus* (Meynier, 2004).

An earlier study by Forcada *et al.* (1990), using a diverse data set collected both opportunistically and on dedicated surveys from a variety of platforms, reported a bimodal distribution of common dolphins in the NE Atlantic. As a result, they

suggested the existence of two separate populations, one neritic and the other oceanic, but, since then, analysis of more extensive data suggests the species could be more or less continuously distributed across the North Atlantic (see Fig. 3).

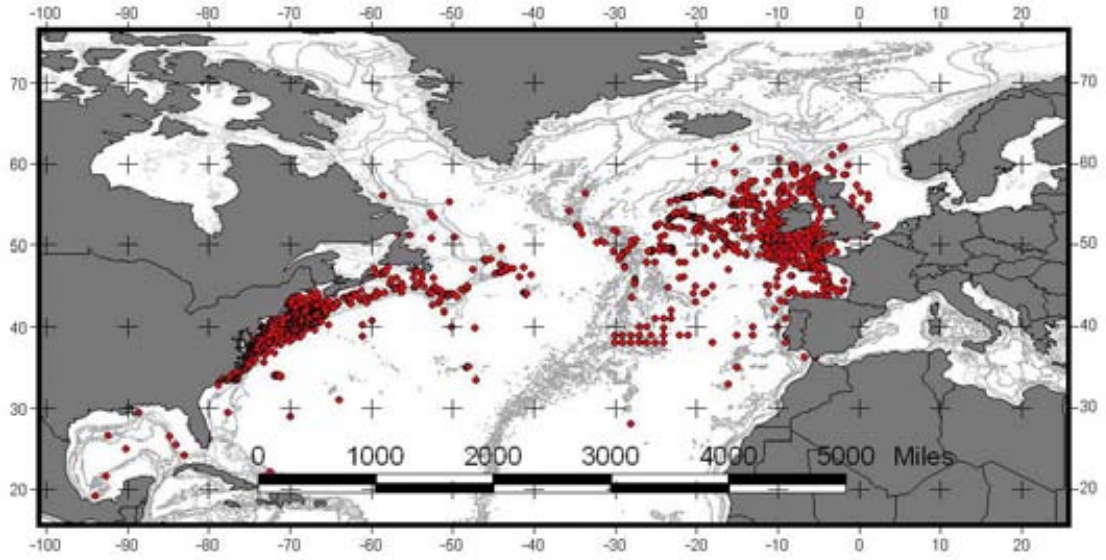

Figure 3a. Distribution of common dolphin sightings in the North Atlantic (data obtained between 1963 and 2007, but manly since 1980 and during summertime, by a large number of observer sighting schemes – see acknowledgements)

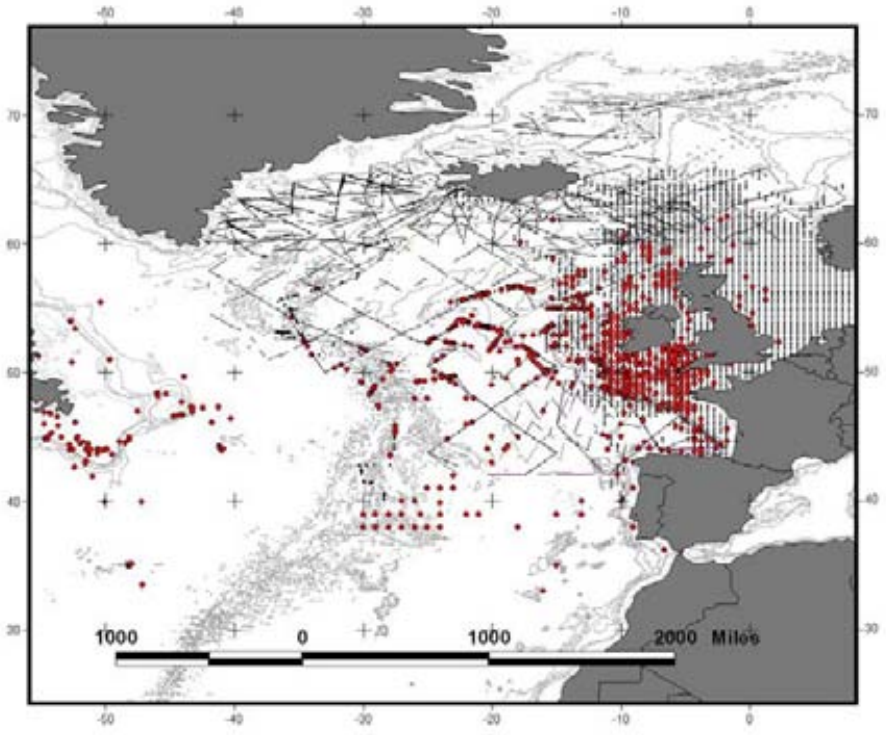

Figure 3b. Outline of available information on track lines and areas covered (black dots) by various surveys in the Northeast Atlantic

It appears that *D. delphis* is distributed, at least during the summer time, from coastal waters in the Northeast Atlantic to the mid Atlantic ridge, and as far south as the Azores. In fact, it may be distributed across the whole North Atlantic, between 35° and 60°N (partially covering a region heavily influenced by the Gulf Stream/North Atlantic Drift). However, due to a lack of observer effort, beyond the mid Atlantic ridge, between approx. 30-40°W (Fig. 3b), its full distributional range in the North Atlantic is not fully known.

There have been several abundance surveys of common dolphin in the region in recent years. The MICA survey, carried out in the summer of 1993, estimated an abundance of 61,888 individuals (95% C.I.: 35,461–108,010) in the continental shelf waters of the Bay of Biscay westwards to *c*. –20°W, and southwards to *c*. 43°N (area of operation of the French tuna driftnet fishery) (Goujon *et al*., 1993). The following year, the SCANS I survey (July 1994), covering an area from the Celtic shelf to *c*. 11°W and 48°S, produced an estimate of 75,449 individuals (CV=0.67; 95% C.I.: 23,900–248,900) (Hammond *et al*., 2002). Where the two surveys overlapped, in an area along the shelf edge (11°W-51°N to 8°W-48°N), Goujon (1996) estimated a total summer population of *c*. 120,000 common dolphins. However, the MICA and SCANS I surveys did not use a double-platform method, nor correct for animals missed on track line (g(0)) or, perhaps most importantly, responsive movement, and therefore estimates from these surveys should be viewed with caution.

In 2005, SCANS II re-surveyed the same area as SCANS I, but extended this to include also the Irish Sea, waters off western and Northern Ireland, west Scotland, and continental shelf waters off France, Spain and Portugal (Fig. 4). The total summer abundance for Northeast Atlantic shelf waters was *c*. 63,400 (CV = 0.46) (Hammond, 2008). Although, no common dolphins were sighted in the North Sea or Baltic during the SCANS II survey in July 2005, as mentioned previously there has been an increase in reported sightings and strandings of individuals in these seas in recent years. During August 2002, the ATLANCET aerial study surveyed an area of 140,000 km^2 of the continental shelf and shelf break in the Bay of Biscay (Ridoux *et al*., 2003). Estimates of 22,401 (95% C.I.: 11,253-27,652) common dolphins were made (Yanis, 2005).

The SIAR survey, rather surprisingly, estimated only 4,496 (95% C.I.: 2,414 – 9,230) common dolphins within an area of approximately 120,000 km^2 off western Ireland during the period 30 July to 22 August 2000 (Ó Cadhla *et al*., 2003). However, the noticeably lower abundance of common dolphins in the Atlantic margin may have resulted from the survey design and/or area covered (Ó Cadhla *et al*., 2003).

As part of the EU NECESSITY project, abundance was estimated for a defined management area (Figure 5) in relation to pelagic trawl fisheries in the NE Atlantic, which coincides with ICES Areas VI, VII, & VIII. As this area was not covered by a single survey, it was necessary to combine data from various surveys (including SIAR, SCANS I & II; MICA, NASS-95 E block, ATLANCET & PELGAS - see Burt, 2007). For surveys where the probability of detection on the trackline could not be estimated, it was assumed that $g(0)$ equals one. Responsive variables were latitude, longitude, slope, depth & distance from coast.

The estimated number of common dolphin schools was 28,791 (CV=0.24; 95% CI 15,370–42,210), and the estimated number of animals was 248,962 (CV=0.18; 95% CI 161,920–336,000) (Burt, 2007). It should be noted that this abundance estimate is specific to the management area described above, and does not cover the known range of the species. All sightings data used to calculate this abundance estimate were obtained during the summertime. Furthermore, the abundance estimate uses data obtained over a long temporal scale, and assumes that the density and distribution of common dolphins did not change during the 14-year sampling period (1993-2006).

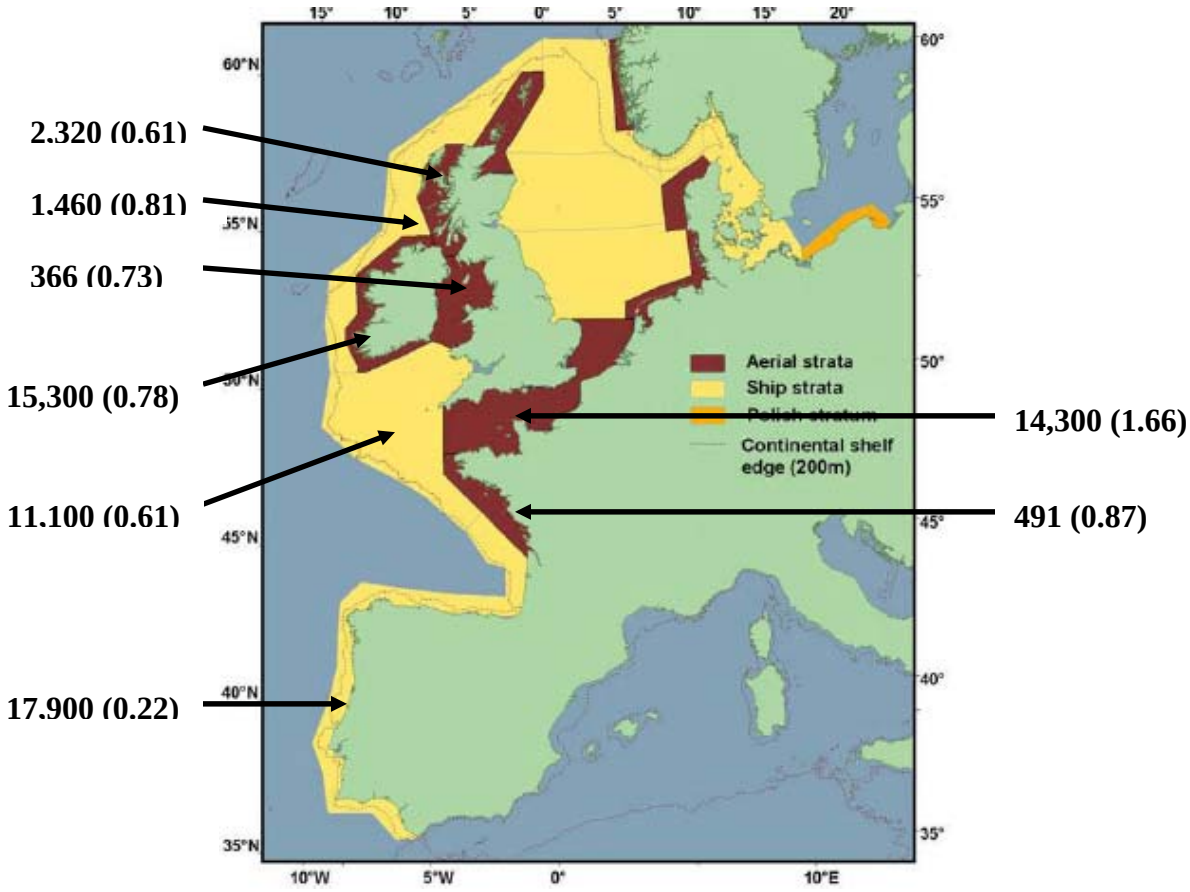

Figure 4. Abundance estimates (and CVs) for common dolphins from SCANS II Survey, July 2005

Besides the estimate of 248,962 common dolphins for the aforementioned defined management area, a recent study by Cañadas *et al.* (in press) calculated an abundance of 273,159 (CV = 0.26; 95% CI = 153,392–435,104) common dolphins for the W Block of the NASS-95 Faroese survey (see Fig. 5). Finally, the CODA offshore survey conducted in July 2007, covering the area depicted in Figure 6), estimated a total abundance of 162,300 (CV=0.46) (P.S. Hammond, *pers. comm.*).

The areas covered by the various surveys (SCANS I & II, MICA, NASS-95, SIAR, ATLANCET, and PELAGAS) are shown in Figure 5.

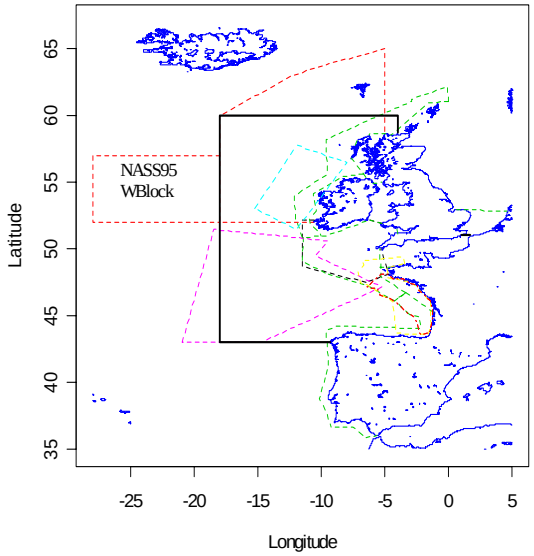

Figure 5. Plot of the region of interest (solid black line) and the regions covered by the surveys (dashed lines). The surveys are MICA (pink), SCANS-94 (black), NASS-95 (red), SIAR (cyan), ATLANCET (yellow), PELGAS (red in Bay of Biscay) and SCANS-II (green)

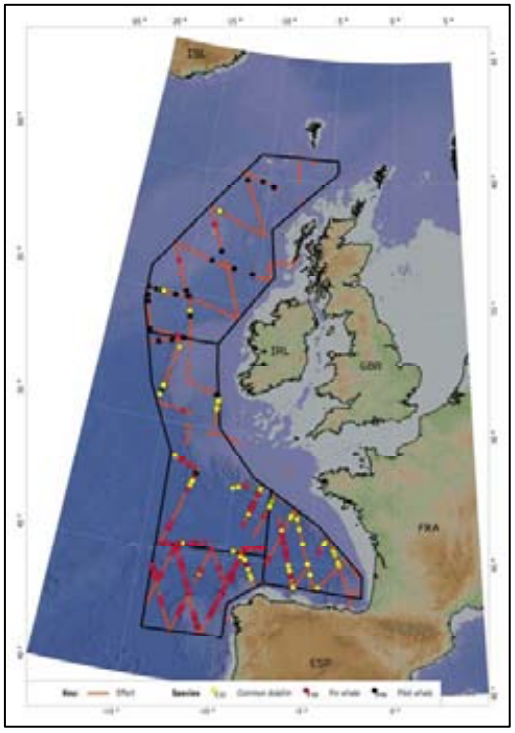

Figure 6. CODA Survey Area, July 2007

Genetic analysis

Based on genetic (Natoli *et al*., 2006; Amaral *et al*., 2007), and cranial morphometric analysis (Murphy *et al*., 2006), the common dolphin species reported to inhabit the Northeast Atlantic is the short-beaked form *Delphinus delphis*. Amaral *et al.* (2007), however, did identify a group of highly divergent individuals (five out of 69 animals) in this geographical region, the results being attributed either to introgressive hybridization between *D. delphis* and *D. capensis*, a separate species, or the existence of two divergent lineages within *D. delphis* (see below).

Several genetic studies have been undertaken to investigate common dolphin population structure in the Northeast Atlantic. These include Natoli *et al.* (2006), Viricel (2006) and Mirimin *et al*. (2007) using mtDNA control region sequences and nuclear microsatellite loci, and Amaral *et al.* (2007) on the mtDNA control region and cytochrome *b* sequence.

The main differences between these approaches are that mitochondrial markers (mtDNA control region and cytochrome *b*) are maternally inherited and deal with a four-fold smaller effective population size compared to nuclear by-parentally inherited markers. The lower mutational rate of the cytochrome *b* gene tends to make this marker more informative than the control region, in phylogenetic and taxonomic studies. By contrast, the mtDNA control region and the nuclear microsatellite loci, which are generally found in non-coding regions, show higher levels of polymorphism due to a relatively high mutation rate and so tend to be more sensitive in detecting fine-scale population structure.

As part of a broader phylogeographic study on common dolphin population structure across different oceans, Natoli *et al.* (2006) analysed both mtDNA and microsatellite loci in samples obtained from common dolphins inhabitating waters off Galicia (n=36 for mtDNA, and n=39 for microsatellites), in the Celtic Sea (n=29 for mtDNA, and n=41 for microsatellites), and in Scottish waters (n=21 for mtDNA, and n=26 for microsatellites), and observed low levels of genetic differentiation across a large geographical scale. Analysis of nine microsatellite loci showed low but significant genetic differentiation between the Scottish samples and those obtained from Galicia (NW Spain) and the Celtic Sea (F_{ST}Scotland-Galicia=0.012, F_{ST}Scotland-Celtic Sea=0.011, p=<0.05), but no significant genetic differentiation among the other putative populations. Using mtDNA analysis (369bp of the control region), samples from Galicia, the Celtic Sea, and Scotland did not exhibit any significant differentiation. The high number of shared haplotypes and the lack of any geographic clustering suggested a high level of gene flow between these areas. Neutrality tests based on Fu's Fs showed highly negative and significant values for the Scottish, Galician and Celtic Sea samples, suggesting possible population expansion (Natoli *et al*., 2006).

Genetic relationships between the Northeast Atlantic population and adjacent regions such as the Northwest Atlantic, eastern central Atlantic (including samples from Madeira, Azores and Canary Islands), and Mediterranean Sea, were also investigated (Natoli *et al*., 2006, 2008). Results from both mtDNA and microsatellite loci analysis showed no significant differentiation between samples from the Northeast Atlantic (n=80) and the eastern central Atlantic (n=14), suggesting high gene flow between these regions, and possibly the existence of a continuous population from Scotland to

the Canary Islands. A significant but low level of genetic differentiation was observed between the Northwest and the Northeast Atlantic populations, suggesting there may be some restriction in gene flow across the Atlantic Ocean. Genetic differentiation was more evident at the mtDNA level, whereas nuclear markers did not always indicate significant differentiation (for example, no significant differentiation was observed between the Northwest Atlantic and Scottish populations). This may indicate greater male-mediated gene flow. However, estimates of migration rate between these regions, based on a coalescent method, indicated possible bias in the long-term direction of migration for females especially from west to east. This could be consistent with oceanic patterns, although there are no data for any casual relationships (Natoli *et al*., 2006).

A similar pattern has been observed between common dolphins in the Western Mediterranean (Alboran Sea) and the adjacent Atlantic population (Portugal and Galicia), since significant differentiation was found only at the mtDNA level (Natoli *et al.*, 2008). Despite the presence of the strait of Gibraltar, the oceanographic characteristics of the Alboran Sea are similar to those of the Northeast Atlantic, and common dolphin in both these areas may have adapted to similar habitats, thus facilitating movements of individuals between these two populations (Natoli *et al.*, 2008). This seems not to apply to the Eastern Mediterranean (Ionian Sea) common dolphin population that shows significant differentiation from the other populations at both microsatellite and mtDNA levels (Natoli *et al*., 2008).

Using some of the same samples as Natoli *et al*. (2006, 2008) and cytochrome *b* gene sequences, Amaral *et al.* (2007) also investigated the existence of population structure of common dolphins in the Northeast Atlantic, by analyzing samples from animals inhabiting waters off Scotland, Galicia and western and southern Portugal. No significant genetic structure was detected between these putative populations. Neutrality tests, and the star-shape of the median-joining networks (MJ), suggested that the population is in expansion, results that are in accordance with the previous study by Natoli *et al.* (2006).

In a broader phylogenetic analysis of the cytochrome *b* gene sequences, a highly differentiated group of individuals (hereafter named Clade X) was identified (five out of 69 individuals; Amaral *et al.*, 2007). By comparing this group of individuals with the NE Atlantic *D. delphis* clade, a genetic distance of 1.59% was obtained. This is considerably higher than the genetic divergence (1.09) reported by Rosel *et al.* (1994), which was used to separate short-beaked common dolphins *D. delphis* from long-beaked common dolphins *D. capensis* off the Californian coast. It should be noted, however, that Natoli *et al.* (2006) reported a high differentiation among the populations described as long-beaked, instead of the expected monophyly, suggesting that these populations may have evolved from independent events converging on the same morphotype. By contrast, low genetic differentiation had been observed among the short-beaked populations across a large geographical scale (Natoli *et al*., 2006).

Recent research carried out by Amaral *et al.* (unpublished data) also identified the existence of four more individuals (clustered) within Clade X, by increasing the sample size from Galicia and including samples from common dolphins off the Irish coast. It was thought that by further increasing the sample size, it will likely result in the discovery of more individuals that will cluster within this group. With 12% of

sampled individuals assigned to Clade X (10 in a total of 83), this demonstrates the existence of a divergent evolutionary lineage within the genus *Delphinus* in this region, and therefore raises questions generally regarding the taxonomic status of common dolphins in the Northeast Atlantic (Amaral *et al*., unpubl. data).

Previous studies have failed to detect this divergent lineage, possibly because the cytochrome *b* gene sequence was never used before for this purpose. Since this gene retains more phylogenetically informative polymorphisms than the control region, it suggests that it may constitute a better molecular marker, and thus enable the distinction of different evolutionary lineages. High levels of differentiation between individuals of Clade X and *D. delphis* in the Northeast Atlantic were also reported in preliminary analyses of other molecular markers, such as the mitochondrial cytochrome *c* oxidase I gene, the 7 intron of the β-fibrinogen gene, and amplified fragment length polymorphisms (AFLPs) (A.R. Amaral, unpubl. data).

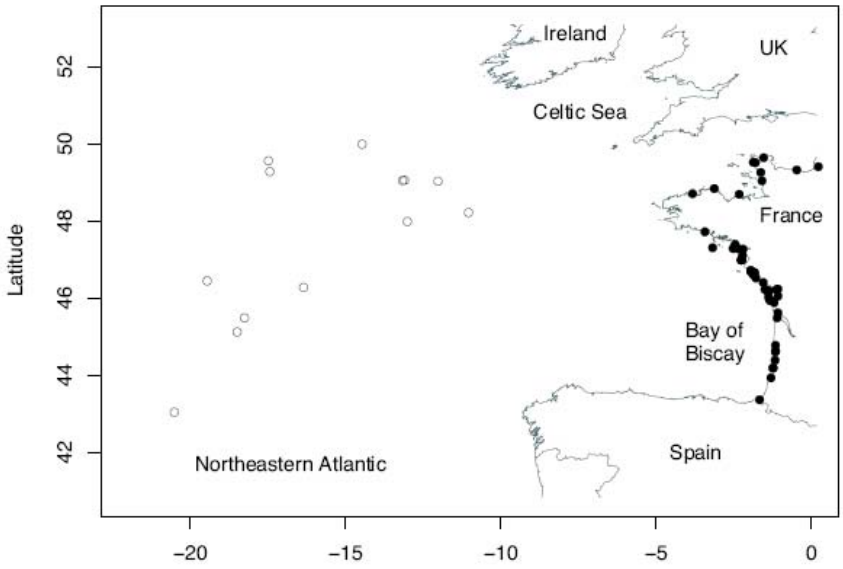

Figure 7. Stranding and by-catch locations of animals sampled by Viricel (2006)
Stranded animals = filled circles, by-caught animals in the French tuna driftnet fishery = open circles.

Viricel (2006) reported that common dolphins from the Bay of Biscay (BB), the English Channel (EC), and oceanic waters of the BB (Figure 7), appear to maintain sufficient levels of gene flow to prevent population subdivision, using both the mtDNA control region (933 bp) and seven (n = 149) to eleven (n = 134, excluding oceanic samples) nuclear microsatellite loci. A total of 98 unique control region haplotypes were defined by 90 variable sites. The EC and BB sample groups shared 12 haplotypes, whereas the oceanic Bay of Biscay group shared 2 and 5 haplotypes with EC and BB, respectively (Viricel, 2006). No significant differentiation between the three areas was detected using the frequency or distance-based approach in the AMOVA. Using nuclear microsatellite loci, most of the genetic variability was found within the three sample groups, with no differences between groups (using AMOVA and a Bayesian approach). However, Viricel (2006) also stated that the study had potentially low power to detect differences due to the high variability of the genetic markers analysed and relatively low sample size for the oceanic BB (n = 15). Furthermore, there may have been sampling biases due to seasonal migrations and sampling of stranded or by-caught dolphins.

Haplotype diversity of control region sequences was high in each group, and was 0.987 ± 0.0032 for the entire dataset (Viricel, 2006). This high haplotype diversity is similar to patterns observed in earlier studies for common dolphins in this region (see: Westgate, 2005; Natoli *et al.*, 2006), and suggests a large effective population size of common dolphins living in the North Atlantic (Westgate, 2005; Viricel, 2006). Evidence for a recent population expansion was provided by the unimodality of mismatch distribution, star-like shape of the MJ network, and large negative Fu's F*S* values (Viricel, 2006).

A recent study by Mirimin *et al*. (2007), as part of the EU-funded NECESSITY project, assessed genetic variability at 25 microsatellite loci and 556 base-pairs (bp) of the mitochondrial DNA control region. 152 common dolphins (stranded and by-caught) from four geographic areas (Ireland, western English Channel, France and Portugal) were genotyped at 20 microsatellite loci, and a total of 106 individuals from three geographic areas (Ireland, western English Channel and Portugal) were genotyped at 25 microsatellite loci (Mirimin *et al*., 2007). Sequences of the mtDNA control region from 13 stranded individuals sampled in Scotland, obtained from the recently published study by Amaral *et al.* (2007), were also included in the analysis.

Results from the AMOVA and from the other measures of genetic differentiation indicated non-significant levels of genetic structure among all sampled areas (*i.e*. most genetic variability resided within rather than between samples) (Mirimin *et al*., 2007). This lack of genetic structure was observed using both microsatellite and mtDNA control region markers, and for all estimators calculated. A retrospective power analysis conducted on the microsatellite dataset (using observed allele frequencies) revealed that this analysis had the power to detect degrees of genetic differentiation as low as F_{ST} = 0.005 (Viricel, Mirimin, unpublished data). Therefore, if differentiation between these areas exists, it must be relatively small. Similarly, results using the Bayesian approach (STRUCTURE) suggested that individuals from the sampled areas could belong to the same genetic stock (Mirimin *et al*., 2007). No significant genetic differentiation was detected when the sexes were analysed separately, signifying similar patterns of dispersal for male and female common dolphins (Mirimin *et al*., 2007).

Mirimin *et al.* (2007) also reported evidence of population expansion, which could have occurred following colonisation of the studied areas after the last glaciations, which ended some 10,000 years ago (Di Rienzo *et al*., 1994). Interestingly, the Scottish sample showed a unimodal distribution but not a significant negative F_{ST} value, which suggests that its marginal position in the distributional range may have led to smaller exchange rates of migrants to neighbouring aggregations (Mirimin *et al*., 2007).

Cranial morphometric analysis

Research undertaken on cranial morphometric analysis reported the existence of some population differentiation within the region (Murphy *et al*., 2006). Although 393 common dolphin skulls (from stranded and by-caught individuals) were measured, only mature specimens were included in the multivariate and discriminant analyses, and all individuals with >5 missing characters were removed from the dataset. Consequently, results should be interpreted with caution, due to small sample sizes

from each geographical area (Ireland, England & Wales, Scotland, Spain and Portugal).

Univariate analysis of variance and covariance (large sample sizes used) revealed that female Portuguese's dolphins differed significantly in orbit measurements from more northerly sampled areas (Murphy *et al*., 2006). MANCOVA indicated significant geographical variation in skulls in the mature male ($p=0.005$, $n = 58$) and female (0.001, $n = 52$) samples. In the discriminant analysis, both axes were significant in females. However, only discriminant function 1 was significant in males, and there appeared to be a slight segregation of male common dolphins from Ireland and Portugal away from England and Wales, Scotland, and Spain along function 1. Although sample sizes were small, female Portuguese common dolphins segregated from more northerly sampled animals in the discriminant analysis. Results could suggest that Portuguese female common dolphins may not interbreed with common dolphins from other areas in this study, and/or common dolphins off the Portuguese coast were mixing with common dolphins in the Mediterranean Sea, and/or common dolphins inhabiting waters further south of the sampled region (Murphy *et al*., 2006).

The inconsistent results from morphometric and recent genetic studies from the Northeast Atlantic could suggest that variations in morphological features - caused by adaptation to different habitats - may occur more rapidly than in genetic markers at the population level. However, a recent genetic study by Natoli *et al.* (2008) has reported directional movements of females from the western Mediterranean Sea (Alboran Sea) population into the Northeast Atlantic. As mentioned previously, based on microsatellite data (9 microsatellites), no significant genetic differentiation was detected between the Alboran and the Atlantic (Galicia and Portugal), but mtDNA analysis (426 bps) indicated significant differentiation.

Fatty acid analysis

During the EU-funded BIOCET project, blubber samples from common dolphins that stranded along the Scottish, Irish, French, and Spanish (Galician) coastlines were analysed (Learmonth *et al*., 2004). Comparison of the fatty acid profiles using the Kruskal-Wallis test indicated that for 14 of the 15 fatty acids (12:0, 14:0, 14:1n-5, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 20:1n-11, 20:5n-3, 22:1n-11 and 22:6n-3), the medians were significantly different between countries ($p\leq0.001$ for 13 of the 14 and $p<0.05$ for 18:2n-6). Principal components analysis resulted in no significant country-effect (Figure 8). There was however, some evidence of geographical separation along the y-axis (PC2), with samples from Scotland and Ireland generally having lower values than those from France and Spain.

Since mature female common dolphins were the largest group analysed for fatty acid profiles, these data were examined using Canonical Discriminant Analysis (CDA). CDA reported a significant geographical variation in fatty acid profiles ($p = 0.000$). Canonical discriminant functions 1 and 2 accounted for 53.9% and 37.0% of the variation, and the CDA plot shows an overlap in fatty acid profiles of common dolphins from Ireland and Scotland, whereas France and Spain appear to be separated from the other areas (Figure 9; Learmonth unpublished data). It should be noted, however, that these data were only obtained between 2001 and 2003, and a large proportion of the common dolphin samples from France were obtained from a single mass live stranding event at Pleubian, in 2002.

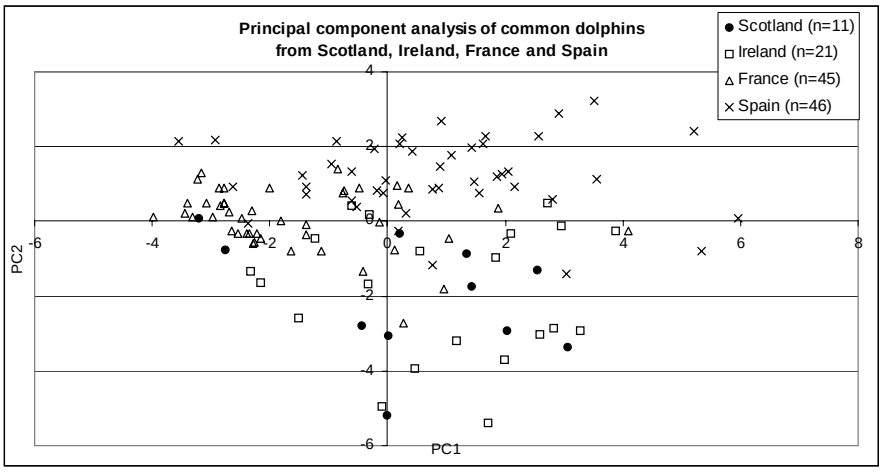

Figure 8. Principal components analysis based on the fatty acid profiles (15 fatty acids) of common dolphins from Scottish, Irish, French, and Spanish waters (2001-03). Taken from Learmonth *et al* (2004), and fatty acid profiles of both sexes, and all age classes were included in the plot

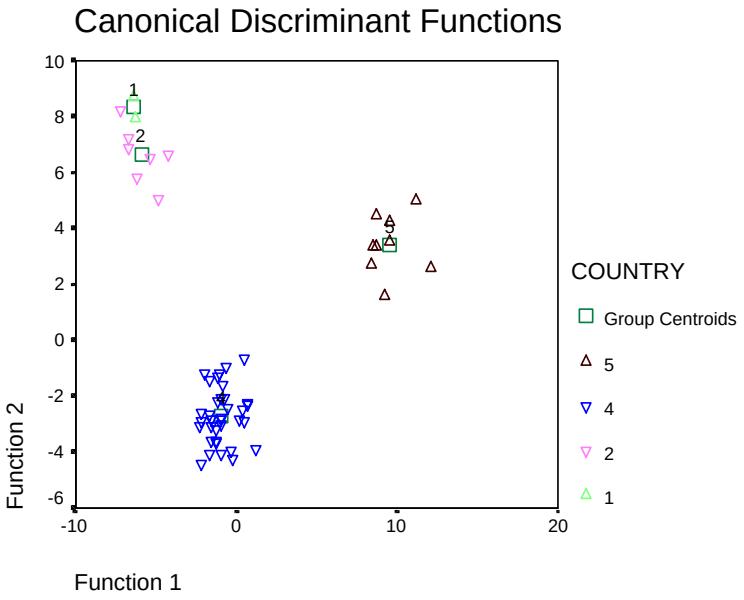

Figure 9. CDA plot of mature female common dolphin fatty acid profiles for each country. Country codes 1 = Scotland, 2 = Ireland, 4 = France, 5 = Spain.

Stable isotope analysis

To date, only limited stable isotope analysis has been undertaken on common dolphins in this region, and there have been no major investigations into deciphering the existence of ecological stocks using this technique. Das *et al.* (2003) investigated variations in carbon-13 using stable isotope analysis between common dolphins that stranded along the Irish and the French Channel coasts. Results suggested a more oceanic/offshore diet for Irish sampled common dolphins, although sample sizes were small. Mean muscle and liver carbon-13 were significantly more negative for animals off the Irish coast (n=8) compared to animals off the north French coast (n=14) (Das

et al., 2003). On the whole, common dolphin samples had, on average, lower carbon-13 values than more coastal species, such as white-beaked dolphins, harbour porpoises, and grey seals (Das *et al*., 2003).

Contaminant analysis

Lahaye *et al*. (2005) suggested the existence of two ecological stocks in the neritic and oceanic waters of the Bay of Biscay. This study was based on analysis of cadmium levels in kidneys of common dolphins caught in the summertime French tuna driftnet fishery in the mid 1990s (the oceanic stock) and from by-caught and stranded animals from French neritic waters, which died between 2001 and 2005 (Lahaye *et al*., 2005). Cadmium concentrations in oceanic by-caught dolphins were about four times higher than neritic ones ($p<0.0001$), with a rate of cadmium bioaccumulation with age five times higher in oceanic by-caught dolphins than in neritic ones. A comparison with data previously obtained by Holsbeek *et al.* (1998) for common dolphins stranded between 1977 and 1990 in the same area, suggests that cadmium levels in the kidneys of common dolphins from the Bay of Biscay have not changed substantially during these last 20 yr. Therefore, the observed differences of renal cadmium levels between stranded and by-caught animals are probably the result of the sample source rather than temporal variations. Based upon previous stomach contents analyses, the main prey occurring in the diet of common dolphins were analysed for their cadmium contents, and indicated that oceanic cephalopods constituted a major source of cadmium for the species. Since the half-life of cadmium in the kidney ranges between ten and fifteen years, it can be concluded that a long-term dietary segregation does exist between neritic and oceanic common dolphins from the Bay of Biscay. However, this long-term segregation does not exclude movements of common dolphins between offshore waters and the continental shelf, and thus the possibility of gene flow between "ecological" stocks. Moreover, this study was based on a small sample size from the proposed oceanic stock ($n=10$), and it was concluded that further analysis is needed to verify the existence of ecological stocks in the Northeast Atlantic.

Analyses of lead concentrations and isotopic composition (206Pb/207Pb) revealed no geographical differences between common dolphins that were found stranded along the north coasts of Brittany (northwest France) and Galicia (northwest Spain), suggesting movements between these areas (Caurant *et al*., 2006).

A recent contaminant study by Pierce *et al*. (2008) reported clear regional differences in polychlorinated biphenyls (PCB) levels in the Northeast Atlantic (using data obtained by the EU-BIOCET project). Analysis of samples obtained between 2001 and 2003 reported that female common dolphins off France (a large proportion from the Pleubian 2002 mass stranding event) and Galicia (northwest Spain) had significantly higher PCB concentrations in their blubber than females off Ireland, although the model also included a significant and generally negative effect of ''maturation'', i.e. lower POP concentrations at higher ovary weights (or increased ovarian activity). Although these results indicate the occurrence of spatial ecological variation, the transfer of PCBs from mothers to offspring during pregnancy and lactation may confound the use of these lipophilic markers for assessing ecological stocks.

Using both skull and mtDNA samples from continental shelf and slope waters of the Northwest and Northeast Atlantic, Westgate (2005, 2007) concluded that common dolphins in the two broad regions represented separate populations, despite low levels of genetic divergence. Thus it appears that *Delphinus* populations on each side of the North Atlantic have either not been separated for a very long period of time, or, alternatively, that a certain level of gene flow exists between both regions (Westgate, 2005). Natoli *et al.* (2006) also reported low F_{ST} values in the North Atlantic, suggesting some gene flow between populations. Further, using a larger sample size, and 14 microsatellite loci and mtDNA control region sequence data, Mirimin *et al.* (2009) found significant population structure between the two sides of the North Atlantic, with common dolphins incidentally captured off the south west coast of Ireland (Irish tuna-drift net fishery) and the western English Channel (UK bass pelagic trawl fishery) being genetically distinct from common dolphins by-caught off the US Atlantic coast. A genetically distinct population has also been described in the Mediterranean (Natoli *et al.*, 2008).

Recommended Management Units

The genetic data suggest that only one common dolphin (*D. delphis*) population exists in the Northeast Atlantic, ranging from waters off Scotland to Portugal, but with separate populations in the Northwest Atlantic, and Mediterranean Sea (Table 1; Figure 1).

Table 1. Supporting Evidence for Proposed Management Units in Short-beaked Common Dolphin (MU = Management Units; WNA = Western North Atlantic; ENA = Eastern North Atlantic; WMED = W Mediterranean (Alboran) Sea; SCO = Scotland; CEL: Celtic Sea; IRE = Ireland; ECH = English Channel; FRA = France; BoB_{NER} = Bay of Biscay neritic; BoB_{OFF} = Bay of Biscay offshore; GAL = Galicia; POR = Portugal; MAC = Macaronesia; √ = evidence for differentiation; x = evidence for no differentiation; (√), (x) = weak evidence; √ / x = conflicting evidence)

MU	mtDNA	microsat.	Cytochrome C	Skeletal	Fatty acids	Stable isotopes	Contamin.
WNA	√	√					
ENA	√	√					
WMED	√	x					
SCO	x	(√)	x	x	(√)		
CEL	x	(√)					
IRE	x	x		x	(√)	√	√
ECH	x	x		x			
FRA					(√)	√	√ / x
BoB_{NER}	(x)	(x)					√
BoB_{OFF}	(x)	(x)					√
GAL	√	(√)	x	x	(√)		√ / x
POR	√ / x	x	x	(√)			
MAC	x	x					

Within the Northeast Atlantic, low levels of differentiation across wide geographical areas, and a high number of shared haplotypes, have been reported in various studies. However, it should be noted that data from highly polymorphic markers such as microsatellite loci, need to be interpreted with caution, especially in the presence of very high within-population heterozygosity (see, for example, Hedrick, 1999).

As outlined earlier, recent results do suggest the existence of different evolutionary units within the Northeast Atlantic (Table 1). These individuals were reported stranded along the coasts of Portugal, Galicia, Scotland, and Ireland. It is worth emphasising that the genetic divergence between Clade X and NE Atlantic *D. delphis* samples is considerably higher (1.59) than the reported 1.09 value, which separated *D. delphis* from *D. capensis* off the Californian coast. However, analysis using a more phylogenetically informative nuclear marker, needs to be conducted in order to confirm this (following Moritz, 1994a, b, 1995).

The distributional range of the Northeast Atlantic population is not known. All samples analysed for genetic analysis in the Northeast Atlantic were obtained either from by-caught or stranded animals from continental shelf and slope waters, and the oceanic waters of the Bay of Biscay, and therefore the management unit/area for common dolphin in the NE Atlantic is confined to this region. No genetic samples have been obtained from animals inhabiting oceanic waters off the west coast of Ireland, the NASS W Block, or the mid Atlantic ridge.

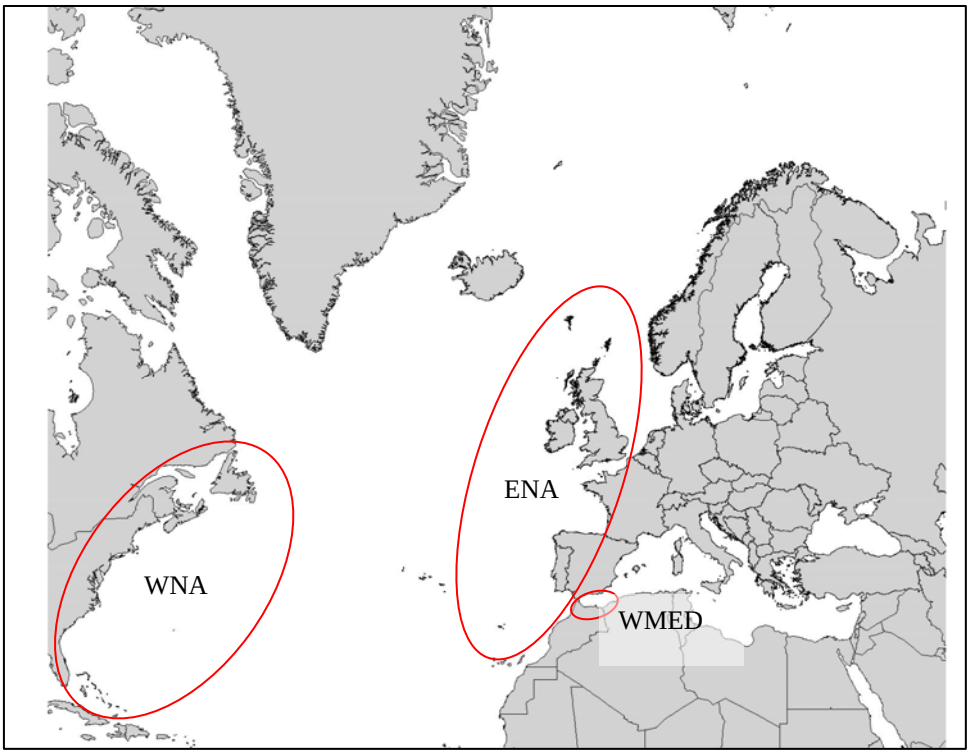

Figure 9. Map showing Recommended Management Units
for Short-beaked Common Dolphin in the North Atlantic & westernmost Mediterranean Sea

Analysis carried out to date suggests that the NE Atlantic population is in expansion. Common dolphins inhabiting waters off Scotland are in a marginal position in the distributional range, and there may be less exchange between these individuals with common dolphins inhabiting other regions in the NE Atlantic.

Directional movement of female common dolphins from both the NW Atlantic and Mediterranean Sea into the NE Atlantic population has been reported. On the other hand, where genetic differentiation has been observed, it was more evident at the

mtDNA level than through the highly variable nuclear markers. This suggests either that there is greater male-mediated gene flow, or that the mtDNA marker represents a much smaller effective population size. However, the high haplotype diversity of the mtDNA control region suggests a large effective population size of common dolphins living in the NE Atlantic.

Due to the low genetic differentiation in this species as a whole, it is proposed that common dolphins in the Northeast Atlantic should be managed using an ecological time scale, i.e. managing ecological stocks. However, although stable isotope and contaminant analyses suggest there may be some structuring of common dolphin populations within this region, with a possible existence of neritic and oceanic ecological stocks, at present there are insufficient data to verify this or to designate separate "ecological" management units.

Future Research Priorities

In order to further assess the population structure of common dolphins in the Northeast Atlantic, the following areas of research are recommended:

- Skin and blubber biopsy sampling of individuals inhabiting oceanic waters i.e. NASS W Block and the mid Atlantic ridge, for both genetic and ecological stock assessment studies.

- Further development of the common dolphin metadatabase created as an output of this workshop, summarising the samples and types of analyses conducted by the different research groups in the ASCOBANS region. To date, it contains information on the nuclear microsatellite loci, mtDNA control region and cytochrome *b* sequences that have been analysed, along with sample code, sampling location and sex and body length of individual, where data were available.

- Increasing the sample size of the cytochrome *b* study, in order to fully assess the existence of a separate evolutionary stock/species in the Northeast Atlantic (this is currently being undertaken: A.R. Amaral, *pers. comm.*).

- Develop and characterise other genetic markers such as SNPs to further test the hypothesis of a single genetic population in the Northeast Atlantic (this is also currently being undertaken: A.R. Amaral, *pers. comm.*).

- Undertake directed studies to assess the existence of ecological stocks within this region, using a large number of samples, obtained from all age/sex classes, and from a large geographical area.

- Stable isotope analysis (using material such as bone where deposition reflects primary food sources assimilated over periods of years) should be used in particular to assess the existence of inshore (neritic) and offshore (oceanic) ecological stocks within the NE Atlantic.

- Further studies should be undertaken using metals with a long half-life, such as cadmium, ensuring sample sizes (obtained from all age-sex classes) are

adequate, obtained over a large geographical area and from animals sampled during the same time-period.

- Tagging studies would also provide information on contemporary habitat use and site fidelity.

- It is clear that common dolphins exhibit seasonal movements within the NE Atlantic, and it may be that these are following the migratory patterns of primary prey. Information on the migratory patterns of potential fish prey should be collected to help determine the range of the population/stock.

Acknowledgements for Figure 3

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9. WORKSHOP ON POPULATION STRUCTURE OF THE HARBOUR PORPOISE IN THE BALTIC SEA

This one-day workshop on the population structure of harbour porpoises in the Baltic region was held on 10 October 2007 and chaired by Jonas Teilmann. The list of participants is indicated at the end of this report. It was divided into two parts. First, all available information on the issue was reviewed; and second, possibilities for a joint future project were discussed, and the main questions listed.

1. Review of Population Structure of Harbour Porpoises in the Baltic and Adjacent Waters

Several studies using various methods have tried to understand the population structure of harbour porpoises in the North East Atlantic and in particular the transition zone between the North Sea and the Baltic Sea. This transition zone consist of waters from the Skagerrak in the north through the Kattegat, the Danish Belt Seas, Øresund and the western Baltic Sea to the Baltic proper (Fig. 1). Until the first half of the 20th Century, a rather large population existed in the Baltic Sea, but a dramatic decline was observed during the past 50-100 years, and currently little is known about its distribution, size and status (Skora *et al*., 1988; Koschinski 2002; Andersen *et al*., 2001). It has been speculated that the harbour porpoises in the Baltic Sea left the area during winter to avoid the sea ice (reviewed by, for example, Teilmann and Lowry, 1996; Koschinski, 2002). Until the Second World War, catches of harbour porpoises during winter in the Little Belt were believed to originate from this seasonal migration (Kinze, 1995). Whether these catches play a role in the severe decline in the Baltic during the 20th Century is unclear. It is also unclear whether the speculated migration out of the Baltic during winter still exists (Koschinski, 2002). In 2002, ASCOBANS took the initiative to make a recovery plan for harbour porpoises in the Baltic Sea called the Jastarnia Plan (www.ascobans.org). One of the main priorities in the Jastarnia Plan was to "Analyse the stock affinities of harbour porpoises in the transition zone of the southwestern Baltic".

Studies on morphometric skull differences, contaminant levels, stable isotopes and genetics have tried to elucidate the population structure. However, results have been inconsistent, possibly due to small sample sizes, differences in area definition, and methods. Although direct comparison is not possible, some general patterns can be extracted.

Morphology

Kinze (1985) used non-metric characters to divide porpoises from the inner Danish waters and the Dutch coast into two groups. Börjesson and Berggren (1997) compared harbour porpoise skull measurements between the Swedish south and east coast (Baltic proper) to the Swedish westcoast (Kattegat and Skagerrak) and found significant differences in females but not in males. Huggenberger *et al.* (2002) analysed metric and non-metric characters in porpoise skulls and found differences between the North Sea, the Skagerrak/Kattegat/Belt Seas/western Baltic and the eastern Baltic (Fig. 1). They suggested a non-migratory separate population in the eastern Baltic (i.e. Baltic proper). Similar results were found in a preliminary study on 3D geometric analyses of skulls (Galatius and Kinze, in prep.). They tested recently collected samples from the Danish North Sea, inner Danish waters and samples from

the inner Danish waters, which had been collected in the 1940s. They found that all three groups were significantly different, and speculated that the 1940s samples may originate from the Baltic proper.

Contaminants
Bruhn *et al*. (1999) found differences in PCB loads between North Sea and Baltic Sea, while Berggren *et al*. (1999) found differences in PCB levels between the Swedish Baltic coast and the Swedish Kattegat/Skagerrak coast. Levels were generally higher in the Baltic, which could be explained by differences in geographical background levels or the diet of the porpoises. However, analyses of stable isotopes that might be used to determine differences in diet, did not show any significant results along the Swedish coastline (Angerbjörn *et al*., 2006).

Genetics
Analysing mitochondrial DNA restriction fragments, Wang and Berggren (1997) also showed significant differences between the Swedish Baltic coast and the Swedish Kattegat/Skagerrak coast. However, these findings have been discussed further in sections 7.14 and 7.15. Tiedemann *et al*. (1996) tested differences in mitochondrial DNA sequence patterns and found significant differences between the North Sea and the German/Polish Baltic coast. Andersen *et al*. (1997) used microsatellite markers and isozymes from the nuclear genome to detect differences between the inner Danish waters (Kattegat, Belt seas and western Baltic) and the North Sea, although fairly high gene flow was suggested. Andersen *et al*. (2001) later used 12 microsatellite markers to test population structure in the northeast Atlantic. They found that the Skagerrak porpoises clustered with the North Sea animals and that this was different from the inner Danish waters. However, they found no difference between the inner Danish waters and a sample from the Swedish south coast (Baltic proper). Preliminary results from Ralph Tiedemann, after analysing 316 porpoises using mtDNA markers and 217 porpoises using 15 microsatellite loci, indicate a separate Baltic proper population based on samples from the southern coast of the Baltic, while samples from the Swedish south coast had a closer relationship to the Kattegat and Belt Sea samples.

Satellite tracking
In Danish waters, a satellite telemetry study has shown that animals in the northern Kattegat, the Skagerrak and northern North Sea consist of one continuum of porpoises while the inner Danish Waters from the northern Kattegat south to the eastern part of the Baltic proper consisted of another group (Teilmann *et al*., 2008).

Together, these studies indicate a population structure with at least three components:

1) North Sea/Skagerrak extending down into the northern Kattegat (the North Sea/Skagerrak may require further subdivision, but this is outside the scope of this report).
2) Inner Danish waters from northern Kattegat, through the Danish Belts and including the Western Baltic Sea.
3) Baltic proper from the eastern border of the Western Baltic (Darss/Gedser underwater ridge) and eastwards.

The different studies reviewed above use different pre-determined area definitions that depend either on national borders, origin of available samples or the two underwater ridges (Darss/Gedser and Limhamn/Dragør) as a population border that may serve as semi barriers limiting movement of porpoises into the proper Baltic Sea. The various area definitions make it impossible to reach firm conclusions on precise population borders. To solve this problem, future projects using various methods should pool all existing samples from all areas into a single group, and then allow cluster analysis to structure the samples and define the borders.

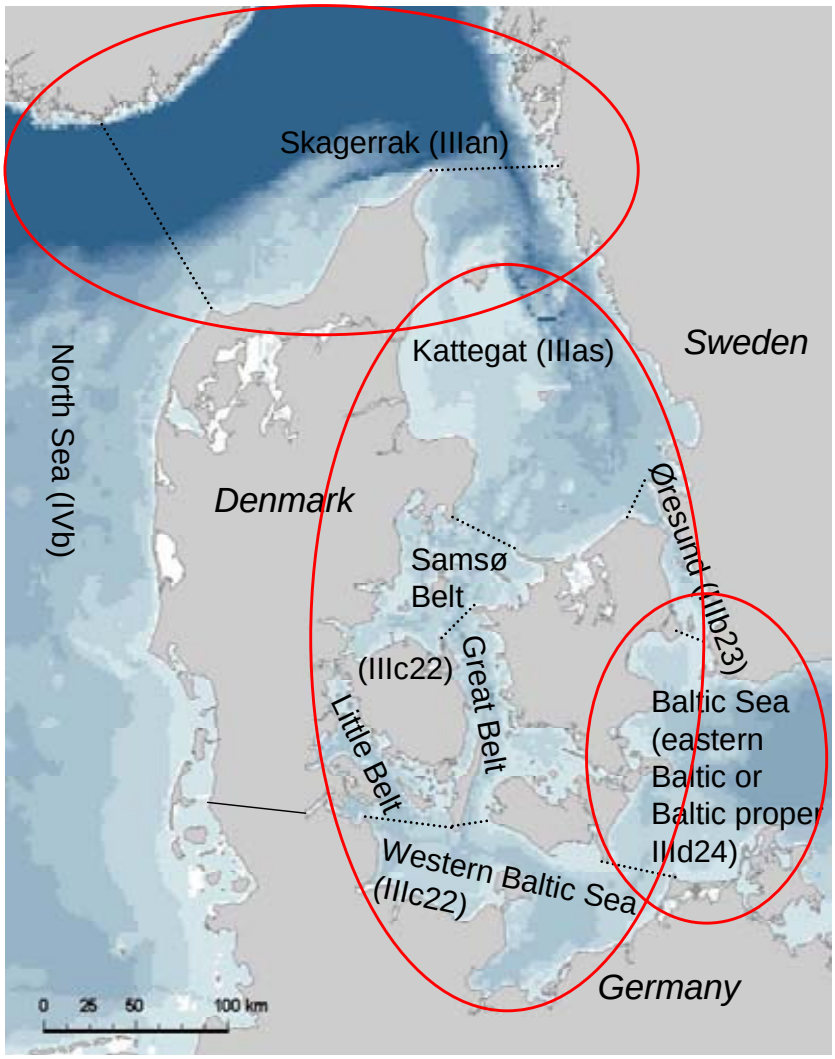

Figure 1. Map of the transition zone between the North Sea and the Baltic Sea. ICES squares used in other studies (e.g. Andersen *et al*., 2001) correspond to the following IIIan = Skagerrak, IIIas = Kattegat, IIIb23 = Øresund, IIIc22 = Samsø Belt, Great Belt, Little Belt and Western Baltic Sea pooled together, IIId24 = Baltic Sea (same as: Eastern Baltic or Baltic proper).

FUTURE PROJECT ON HARBOUR PORPOISE POPULATION STRUCTURE IN THE BALTIC SEA REGION

The second part of the workshop discussed possibilities for a joint future project. It was generally agreed that this was a good idea and that an application for funding should be made in the near future within the group attending the workshop. The hypotheses for this application, and the main uncertainties, were drafted and are listed below. Another important thing to consider is a joint database for all tissue samples collected in the Baltic region. This would help in designing the best study and preventing incorrect interpretations of the origins of the data. A list of fields that would be essential for a joint database is given below.

Hypotheses

1) Baltic immature porpoises migrate/disperse over a wide range (mix north/south and east/west), while adult porpoises (both females and male) are resident year round or during the breeding season.
2) The Baltic proper porpoises are separated from the Belt seas and Western Baltic. The transition zone between Dass and Rügen and Limhamn-Ystad will be treated as a separate sample to avoid mixing populations.
3) There is no substructure in the Baltic proper (east of Rügen/Ystad), for example Swedish south coast and Polish coast.
4) The differences observed are due to a cline by distance.
5) Porpoises in the Baltic migrate (or did migrate) west during winter and back again during summer.

Proposed studies to test hypotheses:

Re. points 1-3:

a) Higher genetic structure between adults/yearlings compared to immatures (using microsatellites - 17 loci, and mtDNA). Use all available samples (consider excluding strandings). Incorporate existing data from analysed samples but also analyse additional samples.
b) Higher morphometrics structure between adults/yearlings compared to immatures (geometric 2D and 3D). Measure as many skulls as possible. (Use both existing data and new measurements).
c) Test hypothesis on the behaviour of porpoises using data on satellite tagged animals from the adjacent areas in Kattegat/Belt seas and Skagerrak/North Sea.
d) Evaluate whether contaminant loads from other taxa show trends in time and space across the study area. Investigate short-term and long-term degrading components (turnover rate). Will the time span of the porpoise sampling be a problem? If not, test the hypothesis on existing data.
e) Evaluate the possibility of using stable isotopes (C, N, O), if variations within the region are sufficient to see differences.
f) Satellite tagging of harbour porpoises in the southwestern Baltic (in Denmark) should be carried out to obtain more information on movements in the area.
g) Spatially and temporally examine life history and health status (compare samples from the same period or the same location). Use contaminants, parasite loads, reproductive status, tooth ultra structure, and bone composition.

Re. point 4:

a) All genetic samples should be analysed in GeneLand (include samples from the whole region). Investigate for a morphometrics cline by geometrics.

Re. point 5:

a) Compare the historical Second World War samples from the Little Belt in Denmark with Polish tissue samples and museum skulls. Undertake a geometric comparison between eastern Germany, Poland, Baltic countries, Finland and Russia. Compare winter and summer samples.

Regarding stratification of area separation of age classes, the following was proposed:

Instead of using the Dass/Gedser and Limhamn/Dragør underwater ridges, which seem to have limited support, it was proposed to divide the area into four and test for differences. The four areas are east and west of the line between Rügen in Germany and Ystad in Sweden, as well as north/south of the midline between the Swedish southcoast and the German/Polish/Latvian coastline.

The samples should be divided into immatures (>10 months and >100cm), and adults (males>135cm and females>143cm, and yearlings<10 months, <100cm). Where sample size is large enough, strandings should be excluded (but test for differences between strandings and samples with a location through by-catch or biopsy).

Table of important fields to enter in a joint database for tissue samples in the Baltic Region:

ID number
Original number[1]
Country
Name of institute[2]
Contact person[3]
ICES area
ICES square
Name of location
Bycaugth[4]
Stranded[4]
Location of death or biopsy[5]
Month
Year
Sex
Length[6]
Age
Tissue available[7]
Skull
mtDNA[8]
Number of Microsatellite loci[8]
Geometric data[8]
Used in publication[9]

Footnotes

1 Give original number(s) if such numbers exist.
2 Institute that has the sample
3 Person that owns or has the right to distribute the sample.
4 Sample collected at the location of the by-catch or on the beach?
5 Lat/Long of by-catch or sample location.
6 Standard length (STD) from tip of rostrum to notch in tail.
7 Put X if sample for genetic analysis is available.
8 Indicate if analysis has been carried out.
9 Give reference

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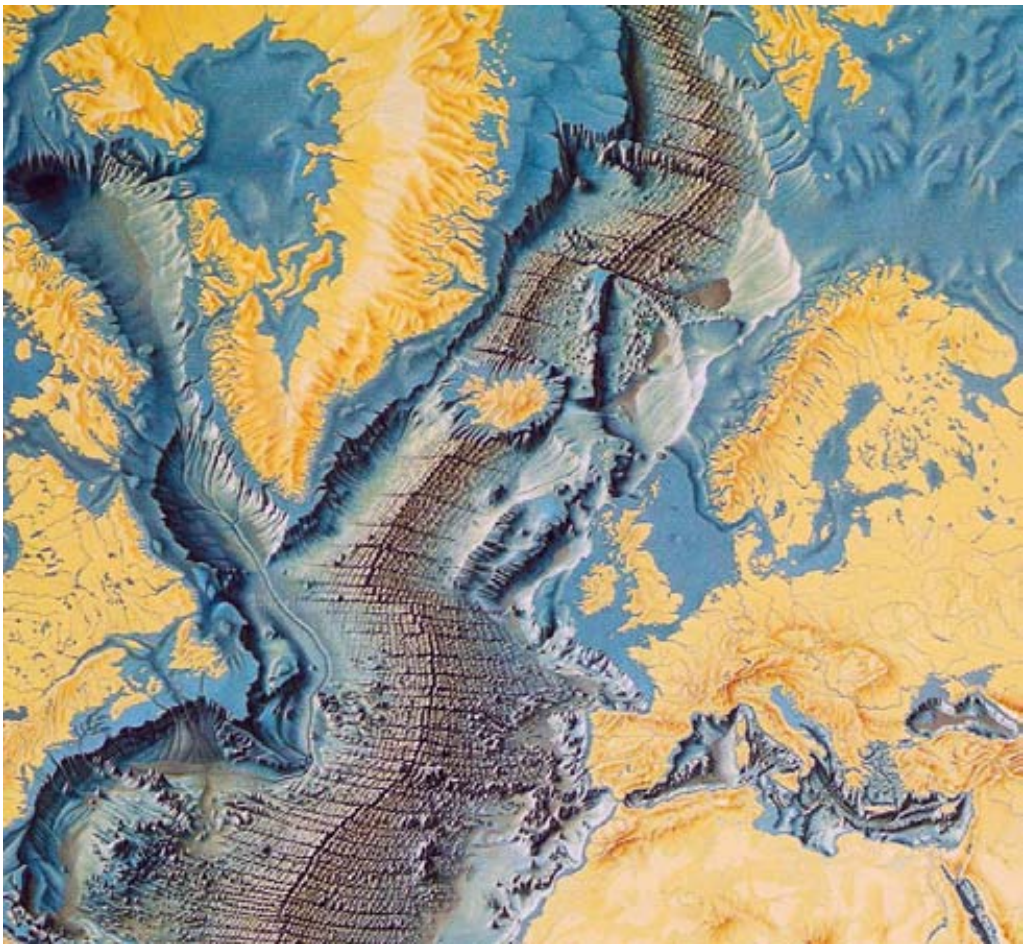
APPENDIX: North Atlantic Maps



a) Bathymetry

(Source: *Atlas of the Oceans*, 1990)

b) Current Circulation **c) Frontal Systems**

(Source: *Atlas of Cetacean Distribution in NW European Seas*, 2003)