Agenda Item 5.1

Implementation of the Triennium Work Plan (2010-2012) – Other Issues Review of New Information on Population Size, Distribution, Structures and Causes of Any Changes

Document 5-02

The use of ecological tracers for discriminating dolphin population structure: the case of the shortbeaked common dolphin *Delphinus delphis* in European Atlantic waters

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The use of ecological tracers for discriminating dolphin population structure: the case of the short-beaked common dolphin *Delphinus delphis* in European Atlantic waters.

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ABSTRACT

After a brief review of the variety of possible ecological tracers and some examples of successful uses, the case of the short-beaked common dolphin from the northeast Atlantic will be examined with the aim of contributing to define appropriate management units. The study of stomach contents suggests that common dolphins foraging in oceanic areas would differ from those foraging in neritic areas. Although extensive overlap existed, samples from Scotland and Ireland were generally separated from those from France and Spain. Muscle δ^{13} C and δ^{15} N values differed significantly between areas and pair-wise comparisons indicated two main groupings constituted of oceanic Bay of Biscay and Irish by-catches on one side and Iberian Peninsula and neritic Bay of Biscay on the other side. Significant differences were shown in Cd accumulation rates in the liver between offshore Bay of Biscay and Ireland (by-catch or stranded individuals) on one hand and neritic Bay of Biscay and Galicia on the other hand. Cadmium bioaccumulation in kidney of common dolphin showed significant differences between oceanic Bay of Biscay, Ireland and the Bay of Biscay and Galicia area. It is suggested to split northeast Atlantic common dolphin into three MUs: oceanic, northern neritic and southern neritic regions.

INTRODUCTION

Understanding population structure is a major issue for marine mammal conservation. In this aim, the identification of management units (MU) is central and crucial for monitoring the effect of human activity upon species abundance (Palsbøll et al, 2007). These MUs are usually defined as demographically independent populations whose population dynamics depend more on local birth and death rates than on immigration (Palsbøll et al, 2007). They can also be defined as "geographical areas with restricted interchange of the individuals of interest with adjacent areas" (Taylor & Dizon, 1999). A broad variety of methodologies has been used to document population structure in marine mammals and each has its specific time scales: generations to evolutionary times for genetic (mtDNA and microsatellites, major histocompatibility complex, single nucleotide polymorphism) and morphometric studies, days to life-time for ecological tracers (fatty acids, stable isotopes, trace elements, organic contaminants,...) and vital rates (fecundity, survival,...), days to years for behavioural studies (telemetry, photo-id,...). It is still debated which time scale is the most appropriate for management applications in which demographic processes are central. These different methods are reviewed by Lockyer (2003) in the case of the harbour porpoise (*Phocoena phocoena*) and were debated during the ASCOBANS/HELCOM small cetacean population structure workshop, held in October 2007 in Bonn (Evans & Teilmann, 2009).

Among these approaches, fatty acids, carbon and nitrogen isotopes, persistent organic pollutants and trace element loads are influenced by dietary intake and feeding habits. But these in turn might be influenced by distribution area and migratory or movement patterns. Although these compounds may be influenced by physiological or biological parameters (such as age, sex, number of pregnancies...), they can help in determining population structure by revealing segregation on a period that is linked to the half-life of the tracer and the turnover of the tissue in which the analyses are made. After a brief review of the variety of possible ecological

tracers and some examples of successful uses, the case of the short-beaked common dolphin from the northeast Atlantic will be examined with the aim of contributing to define appropriate management units. ECOLOGICAL TRACERS

General

Within a species, differences in feeding habits can be found between different geographical areas. Stomach contents only reveal very short-term differences that cannot alone be considered as indication of sub-population structure. However, food is the main route by which marine mammals incorporate bioavailable compounds from the environment into their tissues. These compounds arise from either biochemical processes (fatty acids, C and N stable isotopes), geochemical ones (trace elements) or anthropogenic ones (contaminants). Hence, these compounds can be considered as "ecological tracers" of food resources or habitats exploited by groups of individuals. Differences in tracer signatures found between groups of individuals representing subsets of a wider population may reveal some degree of ecological differentiation between these subsets. As a result of the physiological processes that govern the presence and concentration of each tracer in different tissues, the period of integration characteristic of any tracer- and tissue-specific signature will depend on the half-life time of the element and the turnover of the tissue. Between two sets of individuals, differences observed between tracer values for a given tissue corresponding to long integration periods would suggest long periods of differentiation that may support the existence of separate demographical units. Finally, because of the complexity of the processes involved, although a difference in a tracer signature suggests a difference in habitat or resource use at the corresponding time scale, the reverse is not necessarily true: a similarity in tracer signature does not always imply a similarity in habitat or resource use.

Compared to stomach content analysis that only correspond to a few days of integration (Figure 1), other tracers are integrated from weeks, when blood is analysed, to months, with fatty acids in blubber (Nordstrøm et al, 2008) or stable isotopes in muscle (Hobson & Clark, 1992). Longer periods of integration are available with cadmium (Cd) in kidney, which half-life is 10 to 38 years in man (Friberg, 1974; Kjellstrøm & Nordberg, 1978). In marine mammals, fossilisation of mercury (Hg) in liver through the formation of tiemanite (mercury selenide) corresponds to a life-time period of integration (Koeman et al, 1973; Martoja & Berry, 1980). Half-life duration of cadmium in human liver is more discussed, from about months (Pesch et al, 1989) up to 4 to 19 years (Kjellstrøm & Nordberg, 1978), but always much shorter than in the kidney. Finally, hard tissues and especially teeth and keratinous secretions like fur, whiskers and baleen plates, which generally do not remobilise accumulated elements, record the isotopic and elemental signatures of habitat use and dietary compositions that prevailed at the time when these tissues or secretions were produced (Hobson & Sease, 1998; Klevezal & Serezhenkov, 1996; Outridge & Stewart, 1999). Hence, they can provide records corresponding to their temporal patterns of production (life time in teeth, several years in baleen plates and the previous moult season for fur).

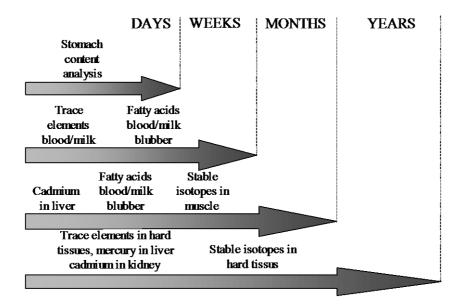


Fig. 1: Periods of integration covered by tracers according to the organ or tissue in which they are analysed (see text for references).

Examples

North Atlantic minke whales (*Balaenoptera acutorostrata*) were initially separated into ten management areas largely defined upon whale distribution on feeding grounds (Anon, 1993). Later on, the number of management units was reduced to four on the basis of regional variations of elemental, stable isotope and fatty acid signatures (Born et al., 2003; Møller et al., 2003). These four groups were consistent with those defined genetically (Andersen et al., 2003).

In the same way, numerous works were carried out on the eastern North Pacific killer whales (*Orcinus orca*). Two distinct types of killer whales termed "residents" and "transients" differ genetically (Hoelzel et al., 1998), acoustically (Barret-Lennard et al., 1996), morphologically (Ford et al, 2000) and in terms of their feeding ecology (Ford et al., 1998). A third one, called the "offshore" type, was based on their distribution in outer coastal waters between California and Alaska (Krahn et al., 2007). In order to provide further information on this third group, their long-term diet has been investigated through the analysis of stable isotope ratios of carbon and nitrogen, persistent organic pollutants and fatty acids in biopsy samples (Hermann, 2005; Krahn, 2007). The results provide evidence that the offshore killer whales constitute a third ecotype; they also differ genetically from the resident and transient killer whales (Barrett-Lennard, 2000).

Lastly, the Pacific walrus (*Odobenus rosmarus divergens*) was considered to be a single pannictic population for management purpose by the U.S Fish and Wildlife Service. However, Jay and collaborators (2008) investigated population structure by measuring trace element concentrations in whole teeth. Trace element profiles in teeth were significantly different between SE Bering sea and St Lawrence breeding areas, suggesting the existence of separate stocks.

COMMON DOLPHIN IN NORTHEAST ATLANTIC WATERS

Current knowledge

A synthesis on the short-beaked common dolphin was done at the ASCOBANS/ HELCOM small cetacean population structure workshop (Evans & Teilmann, 2009). This section largely takes up the main conclusions of this report.

The short-beaked common dolphin is generally considered to be the most common small cetacean inhabiting the temperate waters of the northeast Atlantic. Its distribution covers a large area, which extends from about 35 to 65°N, including the Mediterranean Sea, and westwards to the mid-Atlantic ridge (Evans & Tielmann, 2009). In the NE Atlantic, short-beaked common dolphins show a wide distribution, being observed over the continental shelf, the shelf-edge and in deep oceanic waters (Forcada et al., 1990). The bimodality of its distribution along a depth gradient in the northern Bay of Biscay suggested the occurrence of two different populations living in neritic *versus* oceanic waters respectively. However, more recent studies suggested that the species would be continuously distributed across the north Atlantic (Evans & Tielmann, 2009).

Common dolphin population structure has been investigated in several genetic studies using different markers including mtDNA and nuclear microsatellite loci (Natoli et al., 2006; Viricel 2006; Amaral et al., 2007; Mirimin et al., 2009). Evans & Tielmann (2009) concluded their synthesis with the assumption that "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" (Figure 2).

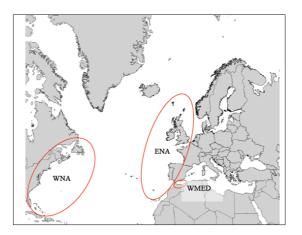


Figure 2: Map showing Recommended Management Units for short-beaked common dolphin in the North Atlantic Mediterranean Sea (Evans & Teilmann, 2009).

Due to the low genetic differentiation in this species as a whole and although, it is proposed that common dolphins in the northeast Atlantic should be managed using an ecological time scale, i.e. managing ecological stocks (Evans & Teilmann, 2009). Existing population structure might not be shown for two different reasons: (i) recurrent gene flow due to small number of individuals could maintain low levels of genetic differentiation on a long time scale without being sufficient to ensure demographic homogeneity on a shorter time range; (ii) the population split is too recent and not enough time has passed to generate significant differentiation. Hence, ecological tracers as defined above can help refining Management Units in the northeastern Atlantic on a finer time scale that may be more relevant to management issues than the evolutionary time scale.

Insights from ecological tracers

Various national (ERIKA oil spill monitoring project, fishery observer programme PROCET) or European research programs (BIOCET, PETRACET, NECESSITY) gave the opportunity to analyse different tissues from common dolphins either from by-caught (oceanic Bay of Biscay or offshore western Ireland) or stranded individuals along the coasts of Scotland, Ireland, France (Bay of Biscay) and Iberian Peninsula (Galicia and northern Portugal).

1. Stomach contents (few days)

The prey communities found in stomach contents may be considered as a descriptor of feeding stocks and inform on foraging areas used at a time scale of a few days. The European Atlantic waters may be divided into two major habitats: the neritic waters from the coast to the slope (depth < 200m) and the oceanic waters that extend off the slope. These habitats support two different fish communities, dominated by clupeids, scads and gadids in neritic waters and by myctophids in oceanic waters. Stomach contents analyses indicate that common dolphins collected offshore only exploit oceanic prey community (Berrow *et al.*, 2005 for oceanic Irish waters; Pusineri *et al.*, 2007 for oceanic Bay of Biscay) and samples obtained inshore only contain prey species of the neritic prey community (Berrow *et al.*, 2005 for coastal Irish waters; Meynier *et al.*, 2008 for neritic Bay of Biscay; Santos et al., 2004 and Silva, 1999 for Iberian peninsula). The complete absence of overlap in prey composition suggests that groups of common dolphins would forage in only one of the two main habitats rather than regularly switching back and forth. Hence, the study of stomach contents suggests that common dolphins foraging in oceanic areas would differ from those foraging in neritic areas.

2. Fatty acids in blubber (weeks)

Fatty acids (FAs) were analysed from blubber samples of individuals stranded along the Scottish, Irish, French and Galician coasts (Learmonth et al., 2004). Although extensive overlap existed, samples from Scotland and Ireland were generally separated from those from France and Spain along PC axis 2 (Figure 3) and significant differences were found between areas for 14 out of 15 FAs (Kruskal-Wallis test).

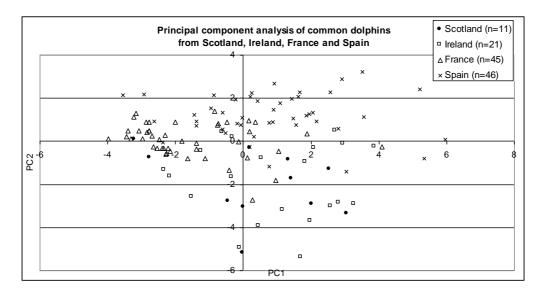


Figure 3: Principal component analysis based on the fatty acid profiles (15 fatty acids) of common dolphins from Scottish, Irish, French and Spanish waters (2001-03). For PC1 the proportion of variance explained was 0.368, for PC2 the proportion of variance explained was 0.198 (from Learmonth et al., 2004).

3. Stable isotopes in muscle (months)

Isotopic ratio of carbon (δ^{13} C) and nitrogen (δ^{15} N) were measured in the muscle of common dolphins from Iberian Peninsula (n =104), neritic Bay of Biscay (n = 64), oceanic Bay of Biscay (between *c*. 20°W and *c*. 10°W and between *c*. 42°N to *c*. 50°N; n =10), offshore western Ireland (by-caught individuals, n = 8), and Ireland stranded individuals (n = 19) (Figure 4). Muscle δ^{13} C and δ^{15} N values differed significantly between areas (K = 74.4, p < 0.0001; K = 101, P < 0.0001 respectively). Pair-wise comparisons indicated two main groupings constituted of oceanic Bay of Biscay and Irish by-catches on one side and Iberian Peninsula and neritic Bay of Biscay on the other side. Individuals from the more oceanic area exhibited a significantly lower δ^{13} C mean values [-18.44±0.49] than in other areas [-17.38±0.63 ‰ to -16.74±0.24 ‰] except offshore western Ireland [-17.76±0.30]. A similar pattern was observed for δ^{15} N with the lowest mean values found in the oceanic area [10.28±0.33]. Despite large sample size from the Iberian Peninsula (n = 104), variability in δ^{13} C isotopic signatures was high in this area, suggesting a higher inter-individual variability in habitat or resource utilisation.

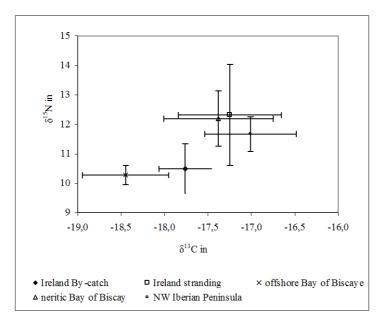


Figure 4: Muscle carbon and nitrogen isotopic signatures (‰) of short-beaked common dolphin (*Delphinus delphis*) from different areas within the northeast Atlantic (mean \pm sd).

4. Cadmium concentrations in liver (months to several years) and kidney (10 to 30 years)

Cephalopods were shown to be the main vector for Cd bioaccumulation in marine predators (Bustamante et al, 1998). Further, Lahaye and her collaborators (2005) investigated Cd exposure of common dolphins by comparing individuals from oceanic and neritic waters of the Bay of Biscay. Results showed that oceanic Cranchids and Histhiotheutids constituted a major source of Cd for oceanic common dolphins. As a result their Cd intake was found to be 12 times higher than in neritic dolphins. Thus, Cd was shown to be a relevant tracer for long-term dietary differentiation between oceanic and neritic dolphins.

Figure 5 shows the accumulation of cadmium (in $\mu g.g^{-1}$ wet weight) with age in common dolphins from the areas previously cited. Compared to stable isotopes determination, less individuals of Iberian Peninsula were analysed and they were only from Galicia (n = 31). Despite a variability that differed within each area, significant differences were shown between offshore Bay of Biscay and Ireland (by-catch or stranded individuals) on one hand and neritic Bay of Biscay and Galicia on the other hand (p < 0.0001) (Lahaye, 2006).

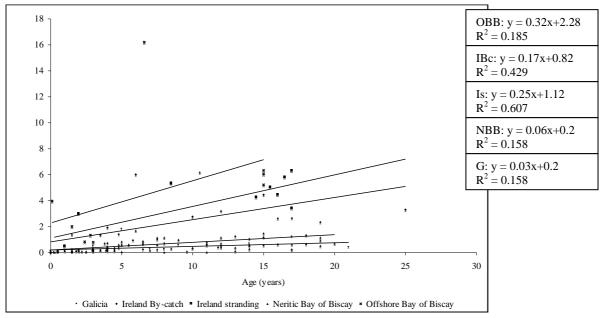


Figure 5: Bioaccumulation of cadmium with age in liver of short-beaked common dolphins (*Delphinus delphis*) from different areas within northeast Atlantic.

Cadmium bioaccumulation in kidney of common dolphin showed significant differences with areas ranked as follows, from the highest to the lowest accumulation rates : oceanic Bay of Bay of Biscay > Irish By-catch = Irish stranding >> Neritic Bay of Biscay > Galicia (p ranging between 0.0001 and 0.012) (Figure 6).

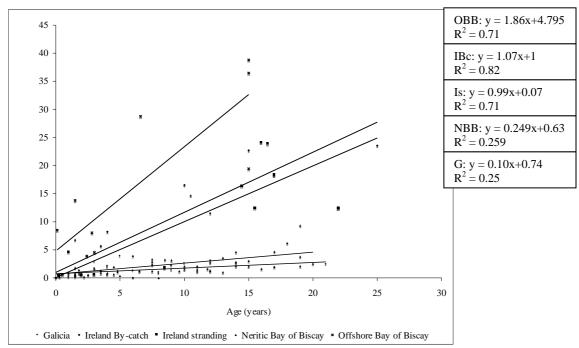


Figure 6: Bioaccumulation of cadmium with age in kidney of short-beaked common dolphins (*Delphinus delphis*) from different areas within the Northeast Atlantic.

SYNTHESIS AND CONCLUSIONS

Difficulties in interpreting data on ecological tracers relate to the fact that although they all inform on animals' environment and habitat (mainly through the diet), the nature of the information and the processes involved may be quite different. Moreover they are all influenced by other factors linked to metabolism and physiology. Fatty acids stored in the blubber depends on the diet for some of them (having been transferred from phytoplankton through the food chain), but are synthesised by the animal itself for others. Stable isotopes signatures provide information on diet and also feeding habitat (Hobson, 1999). Carbon isotope ratios mainly record the signature of primary producers, the signature being very similar among animals belonging to the same local food web irrespective of their trophic levels. Nitrogen isotope ratios mostly record trophic levels, but must always be compared to the nitrogen signature of local primary producers. Finally, trace elements record geochemical signatures but also dietary preferences that may differ according to the trace element being used. Because of this complexity, it is often considered than a difference observed in an ecological tracer between two sample sets would represent some difference in habitat or resource use at the time scale characteristic of the tracer under consideration, but the absence of difference in tracer values does not necessarily imply the absence of ecological differentiation.

In spite of these difficulties, some results can be pointed out from the different ecological tracers analysed. These observations are shown in figure 7. At a few days period of integration, results suggest that common dolphins foraging in oceanic areas differ from those that forage in neritic areas rather and do not regularly switch back and forth. Considering a period of a few weeks, extensive overlap appeared in FA composition in blubber among sample sets of neritic origin (oceanic ones have not been analysed for fatty acids), but samples from Scotland and Ireland were generally separated from those from France and Spain. When a longer period of integration is considered (isotopes in muscles, Cd in liver; several months to years), exchange between the oceanic and neritic areas seem to be limited to the northern part of the area of interest towards the Irish shelf. However, a significant difference appeared at a lifetime scale (Cd in kidney).

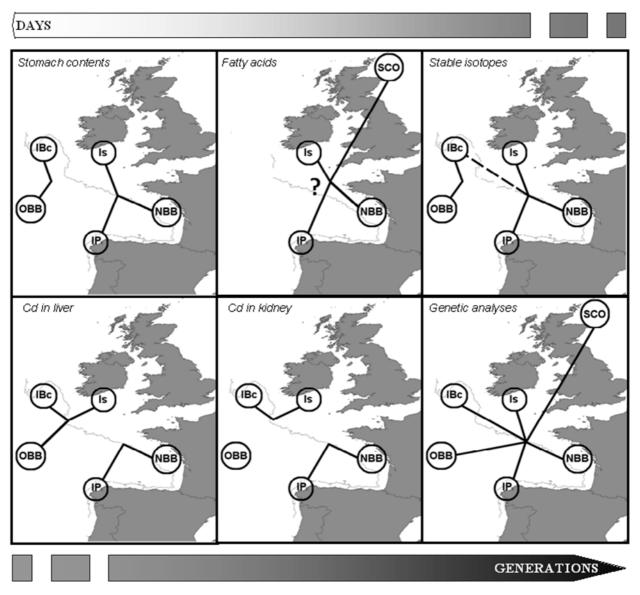


Figure 6 : mapping of the links between sampled groups, investigated through ecological tracers.

To sum up, the continental shelf would constitute an ecological boundary with some degree permeability in the northern part that remains undetectable over a long time period. Moreover, on this long time period, the northern part of the area would also be separated from the southern part with the Celtic Sea clearly separated from the Bay of Biscay and the north-eastern Iberian Peninsula.

In the northeast Atlantic, common dolphin populations have to face fishery activities that exert spatially limited by-catch pressures such as bass fisheries in the Bay of Biscay and in the Channel or Irish tuna fisheries (WGMME, 2005). These fisheries would imply an additional mortality in the areas where they operate. Moreover, the long-term tracers indicate a real segregation and suggest that this additional mortality cannot be compensated by individuals from other populations. Thus if the MU definition is admitted as "demographically independent populations whose population dynamics depend largely on local birth and death rates rather than on immigration" (Palsbøll et al, 2007), a short-term management and conservation of common dolphins would require the definition of three MUs in the northeast Atlantic (Figure 7).

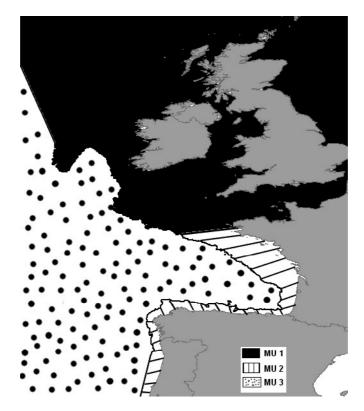


Figure 7 : Management Units suggested according to ecological tracers in the northeast Atlantic.

Genetic reflects groupings of individuals over generational time scales, whereas the analysis of ecological tracers can reflect groupings of individuals over the lifetime of individuals. The elemental composition of the teeth would be a relevant method to confirm the three MUs for common dolphins in the Northeastern Atlantic. A previous study (Caurant et al, 2006) investigated regional variation of lead concentrations in the whole teeth of different species of small cetaceans, but did not cover the whole area of interest. Trace element analysis in teeth of common dolphins from the northeast Atlantic would bring an additional tracer corresponding to a life-time integration period that would contribute to establishing population structure at a time scale that is relevant to demographic processes.

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