



UNIVERSITAT DE
BARCELONA

**Taxonomy, morphology and distribution of the
common dolphin, *Delphinus delphis* (shortbeaked
form) and *Delphinus capensis* (longbeaked
form), in West African waters**

**Taxonomía, morfología y distribución del delfín común,
Delphinus delphis (delfín de morro corto) y *Delphinus capensis*
(delfín de morro largo), en aguas del Noroeste Africano**

Ana Morais Pinela

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UNIVERSITAT DE BARCELONA



Facultat de Biologia
Departament de Biologia Animal
Programa de Doctorat de Biodiversitat

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Memoria presentada por

Ana Cristina Morais Pinela

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GENERAL INTRODUCTION





INTRODUCTION

TAXONOMY AND DISTRIBUTION

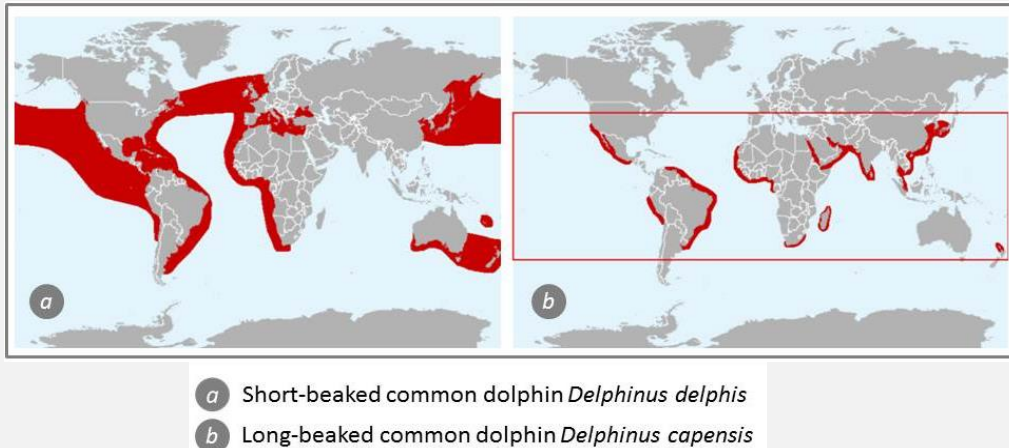
Although there is still an ongoing debate on how the members of the order Cetacea should be classified, there is agreement that it is a relatively modern taxon that contains at least 86 species (IUCN website). Dolphins belong to the superfamily *Delphinoidea* and to the family *Delphinidae*, which is thought to have appeared in the late Miocene, around 11 to 12 million years ago (Barnes *et al.*, 1985). Dolphins evolved in marine and aquatic waters and spread through the world having only as limitation the physiologically acceptable range of water temperatures that became evolutionary determined for each species. As a consequence, the family *Delphinidae* is the most diverse of all cetacean families, being composed of 35 species that distribute in all oceans and seas of the world (LeDuc, 2009).

The common dolphin (genus *Delphinus*) is one of the most representative examples of this evolutionary path. It is widely distributed, being present in the Atlantic, Pacific and Indian oceans, where it occupies from cold to temperate and subtropical waters (Evans, 1994; Heyning & Perrin, 1994). There are also populations of common dolphins living in enclosed basins, such as the Black Sea, and semi-enclosed basins, such as the Mediterranean Sea, the Gulf of California, the Sea of Japan, the Gulf of Thailand, the Persian Gulf and the Caribbean Sea (see BOX 1.; Heyning & Perrin, 1994; Jefferson *et al.*, 2008; Perrin, 2009). This enormous distribution range has led to the differentiation of a number of morphotypes which, until today, remain of unclear taxonomic status (Banks & Bronwell, 1969; Evans, 1982; Heyning & Perrin, 1994; White, 1999; Natoli *et al.*, 2006). Although in the past a number of species and subspecies of *Delphinus* were proposed for different areas, it has generally been considered until some decades ago that all forms belonged to a single species, *Delphinus delphis* (Hershkovitz, 1966). In many areas, however, two morphotypes were distinguished: a long-beaked form, mostly of inshore distribution; and a short-beaked form, of both offshore and inshore distribution (Banks & Bronwell, 1969; Evans, 1982; Heyning & Perrin, 1994; Natoli *et al.*, 2006; Rosel *et al.*; 1994).

**BOX 1. WORLDWIDE DISTRIBUTION OF THE COMMON DOLPHIN**

*Distribution of *Delphinus delphis*: warm temperate, subtropical, and tropical waters worldwide (Hammond et al., 2008a; © IUCN)*

*Distribution of *Delphinus capensis*: disjoint populations in warm temperate and tropical coastal waters (Hammond et al., 2008b; © IUCN)*



Currently, the genus *Delphinus* is accepted to include two species and four sub-species: the short-beaked common dolphin, *Delphinus delphis delphis* Linnaeus 1758, distributed in the continental shelf and the pelagic waters of the Atlantic and Pacific oceans; the long-beaked common dolphin, *Delphinus capensis capensis* Gray 1828, distributed in the nearshore tropical and temperate waters of the Pacific and South Atlantic oceans; the Black Sea common dolphin, *Delphinus delphis ponticus* Barabash 1935, distributed only in the Black Sea; and the Indo-Pacific common dolphin, *Delphinus capensis tropicalis* Van Bree 1971, distributed in the warm and temperate waters of the Pacific and Indian oceans (IWC, 2009; Perrin, 2009). This classification is based on the morphology of the specimens, both internal, such as cranial characters (e.g. beak length, rostral length/zygomatic width ratio, and tooth count) and external, such as total body length and coloration patterns (see BOX 2.; Heyning & Perrin, 1994; Jefferson & Van Waerebeek, 2002).

Differences between the short- and long-beaked forms, particularly on the ratio of rostrum length to zygomatic width, were found by Banks and Bronwell (1969), which assigned specimens with a ratio above 1.55 to *Delphinus bairdii* and below 1.53 to *D. delphis*. However, Van Bree and Purves (1972) found some specimens from other ocean basins with intermediate ratios of rostral length to zygomatic width. These findings were however attributed to the fact that the studied sample included immature animals and specimens were not separated by sex (Banks & Bronwell, 1969; Van Bree & Purves, 1972), thus leading to confounded sexual dimorphism, ontogenetic variation, and geographical variation with potential specific or



subspecific differences (Heyning & Perrin, 1994). Banks and Brownell (1969) results were later supported by Evans (1982) with multivariate analysis of skull measurements based on only mature individuals. Evans (1982) re-plotted rostral length on zygomatic width, obtaining more discrete clusters than Banks and Brownell (1969). Heyning and Perrin, in 1994, re-analysed the rostral length to zygomatic width ratio of mature *Delphinus* spp. specimens of the *D. capensis* type from South Africa (Ross, 1984) and found a range between 1.59 to 1.76. This led to the conclusion that South African animals had longer beaks than the long-beaked form of southern California. Regardless, there was considerable overlap in the range of values, which were more distinct than those of the short-beaked form of southern California. At present, it is thought that both forms of common dolphin inhabit South African waters (Best, 2007). In this area, the morphological measurements that distinguish both species vary, as in other parts of the world. Also, genetic evidences have not provided so far support for the separation of morphotypes along the southern African coast (Samaai *et al.*, 2005).

In the eastern North Pacific, the genus was separated into two species according to morphological and genetic characters of the two morphotypes: *Delphinus delphis*, or short beaked common dolphin, and *Delphinus capensis*, or long-beaked common dolphin (Heyning & Perrin, 1994; Rosel *et al.*, 1994; Kingston & Rosel, 2004). The specific name of the second species was taken from the first common dolphin ever described with a beak longer than usual, which had been found near Cape Town (Gray, 1828 *in* (Heyning & Perrin, 1994; Natoli *et al.*, 2006).

Heyning & Perrin (1994) gave for granted that the two forms would occur sympatrically in the west coast of Africa, just like it happened in the coast of California. According to the authors' distribution maps for this area, *D. delphis* would occur in European seas and off north-west Africa all the way south to Senegal, whereas, *D. capensis* was restricted to a small area between Western Sahara, south to approximately northern Senegal. These authors construed an approximate species range distribution based partly on published coloration patterns of common dolphin specimens (Cadenat, 1959). However, the distribution ranges proposed for both species have changed in the last decades and are currently thought to be more extended than previously proposed (see BOX 1.). Due to their high mobility and wide distributional range, movements of common dolphins are still unknown in most areas, although they are thought to be largely controlled by prey distribution (Young & Cockroft, 1994).

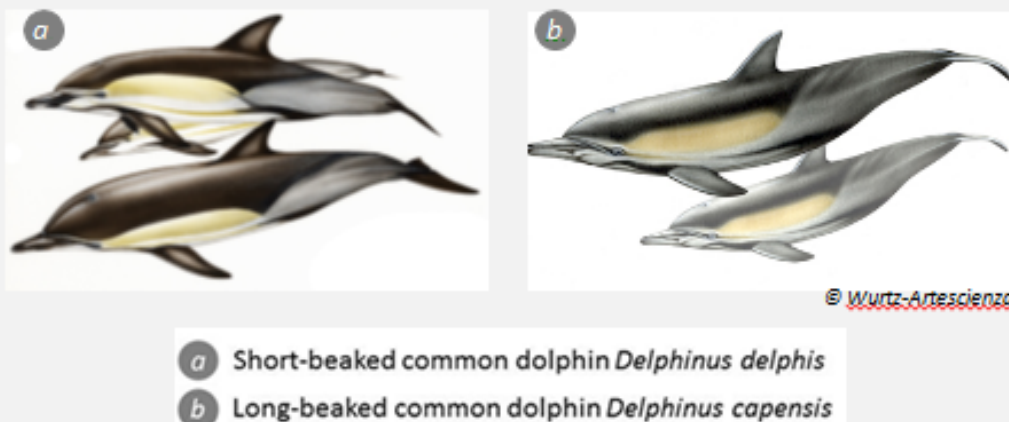
The two morphotypes (short- and long-beaked) have been proven to occur off the Atlantic coast of central Africa (Van Waerebeek, 1997). The range of the short-beaked form is believed to extend as far south as Senegal (Heyning & Perrin, 1994; Van Waerebeek, 1997; Rice, 1998), while the long-beaked is present from the waters off Mauritania to South Africa (Amaha, 1994; Van Waerebeek, 1997; Rice, 1998; Samaai *et al.* 2005). Thus, the waters off Mauritania are apparently a region of overlap in the geographical



distribution of the two forms, and indeed both morphotypes may occur sympatrically in this area, as it happens in California (Heyning & Perrin, 1994).

BOX 2. DESCRIPTION OF THE COMMON DOLPHIN

Common dolphins are marine mammals with a slender shape and a long beak sharply demarcated from the melon, in which the dorsal fin is high and slightly curved backwards. Common dolphins are distinguished from other marine mammal species by a unique crisscross color creating a four-part pattern: dark grey to black in the dorsal area, buff to pale yellow in the anterior thoracic patch, light to medium grey on the flank and white in the abdominal field. At their natural habitat, both species are difficult to distinguish, despite the fact that the color pattern of the short-beaked species is more crisp and colorful than that of the long-beaked. The long-beaked species has a more slender body shape and a longer beak than the short-beaked species, along with other demarcated external morphological differences, such as: a beak sharply demarcated from the melon, which is somewhat flat in appearance; a muffled coloration and a broader chin-to-flipper stripe; and the absence of white from the dorsal fin and flippers (which occurs in some short-beaked common dolphin populations). Additionally, the long-beaked common dolphins have 47 to 67 sharp pointed teeth in each tooth row, more than in the short-beaked common dolphins and any other delphinids species. Body size ranges from 164 to 201 cm and body mass to about 200 kg for the *D. delphis* (Perrin, 2009); whereas for the *D. capensis* body size reaches 2.54 m and body mass 235 kg (Jefferson *et al.*, 2008). Heyning and Perrin (1994) reported that body mass data suggest that the short-beaked form was heavier for a given body length than the long-beaked form. Several authors agree that variation in total length, external morphology, skeletal characters, and pigmentation has made it difficult to resolve the taxonomy of this genus (Heyning & Perrin, 1994).



Genetic examination of some common dolphin specimens from north-west Africa (Natoli *et al.*, 2006) showed that they were distinct from the same morphotypes in the North Pacific. Mitochondrial DNA together with nuclear DNA analysis, however, did not support the hypothesis of one single long-beaked lineage worldwide. No shared haplotypes and fixed mutations were found between long-beaked populations (South Africa, Pacific and Mauritania). At a local level, this study raised questions about the morphology and identity of the *Delphinus* complex off north-west Africa.



The worldwide distribution of the genus *Delphinus* is still not well-established because of taxonomic uncertainties (Rice, 1998). Although the distinction between the two species was made through the analyses of specimens from the eastern North Pacific, it was generally assumed that such discrimination could be extended to the two morphotypes of common dolphins inhabiting all oceans (Banks & Bronwell, 1969; Evans, 1982; Heyning & Perrin, 1994; Rosel *et al.*, 1994). However, Jefferson *et al.* (2009) warned that great care had to be taken in the identification of similar-appearing long-beaked delphinids, and that uncritical acceptance of records could lead to incorrect assumptions about ranges of the species involved.

There were several studies worldwide that reported an overlap in the species ranges that had been described for California (Van Bree & Purves, 1972; Amaha, 1994; Van Waerebeek *et al.*, 1994; Van Waerebeek, 1997; Jefferson & Van Waerebeek, 2002; Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010). This led to the proposal of intermediate forms between *D. delphis* and *D. capensis*. Even though the two-species concept had wide acceptance in the marine mammal community, recently this thesis has been re-evaluated, leading researchers to re-examine *Delphinus* spp. specimens from other areas of the world. In the areas where intermediate forms of short- and long-beaked morphotypes co-exist, it has been proposed that local speciation events could be acting at present to originate new species of *Delphinus* (Tavares *et al.*, 2010). At a more general level, Perrin (2009) proposed that more than two species of *Delphinus* spp. may exist worldwide, thus likely questioning present taxonomy.

ECOLOGY AND HABITAT USE

Resource specialization triggered by differences in habitat or in food availability locally, can lead to intraspecific differentiation in cetacean species (Hoelzel, 1998). As top trophic-level predators, cetaceans are consumers of most trophic levels, playing a very important role in the marine ecosystems (Bowen, 1997; Pauly *et al.*, 1998). Their interconnection with most levels of the marine trophic web makes them prone to first-hand experience the effects of changes in the environment, such as increasing sea-surface temperatures and changes in the abundance and distribution of prey (Bowen, 1997; Bearzi *et al.*, 2003).

When discussing the ecological role and foraging strategy of the common dolphin, it is necessary to define this species in terms of its prey selection. Elucidating specific types of prey has always been difficult for marine mammal scientists (Ambrose *et al.*, 2013), especially because most trophic ecology studies rely on faecal and/or stomach-content analyses. Generalist species are able to occupy and exploit a wide variety of habitats and display a high degree of dietary plasticity, preying on the most available prey and switching according to resources availability or scarcity.



Common dolphin's movements and migrations are usually associated to those of their potential prey' species, with diet varying according to the area and season being investigated (Young & Cockcroft, 1994; Santos *et al.*, 2004; Pusineri *et al.*, 2007; Brophy *et al.*, 2009; Ambrose *et al.*, 2013). Typically, they prey on small epipelagic and mesopelagic schooling fish that swim near the surface, such as sardines, mackerel and anchovies (Young & Cockcroft, 1994; Santos *et al.*, 2004; Pusineri *et al.*, 2007; Cañadas & Hammond, 2008; Otero & Conigliaro, 2012; Ambrose *et al.*, 2013).

In the eastern Pacific Ocean, *D. delphis* is known to migrate according to oceanographic conditions and prefers upwelling-modified waters (Ballance *et al.*, 2006), while *D. capensis* apparently prefers warmer and more coastal waters (Banks & Brownell, 1969). Most areas of distribution of *D. delphis* seem to coincide with moderate to strong upwelling, and the species appears to avoid warm, tropical waters. Isolated populations of *D. capensis* can be found in warm temperate and tropical coastal waters around the world, despite its overall distribution remaining unknown due to confusion with *D. delphis* (Rice, 1998; Sanino *et al.*, 2003). Previous observations in Chile (Sanino *et al.*, 2003) and Baja California (Niño-Torres *et al.*, 2006) indicated that the long-beaked form has a more coastal distribution than the short-beaked form (Perrin, 2009). Departing from this, it has been assumed that both forms of common dolphin inhabit South African waters, with the long-beaked having a more coastal distribution and the short-beaked having a more offshore one (Best, 2007). Contrarily, observations of *D. delphis* in Southern Australia (Bell *et al.*, 2002) revealed that dolphins with larger skulls and longer rostra tended to be closer to deep water, while those with smaller skulls and shorter rostra were distributed in shallow coastal waters.

The short- and long-beaked forms of common dolphins are known to occur sympatrically in several areas around the world (Heyning & Perrin, 1994; Van Waerebeek, 1997; Natoli *et al.*, 2006; Perrin, 2009). Heyning and Perrin (1994) anticipated that, to occur sympatrically, the two forms of common dolphin should have to exploit the environment in different ways. Indeed, there are fine-scale ecological differences in how the two species use southern Californian waters since there are indications that each one is linked to certain environmental factors at different times (Heyning & Perrin, 1994). The same could be happening in other areas where both morphotypes co-habit.

Natoli *et al.* (2006) proposed that the long-beaked form originated independently in different regions, suggesting that adaptation to local environments could be driving local speciation. In this scenario, it is likely that common dolphin' skull morphology could be reflecting adaptation to prey capture and to habitat use, influencing their distribution along the eastern Sub-tropical Atlantic ocean. Currently, there is almost no information on the ecology of common dolphins from north-west Africa and the published literature in other oceans does not show obvious differences in the diet of either short-beaked or long-beaked forms or *D. delphis* and *D. capensis* species (Ohizumi *et al.*, 1998). Thus, further research is



required, both off north-west Africa and elsewhere, to clarify potential allopatry and trophic niche segregation between these morphotypes.

THREATS AND CONSERVATION

Exploitation of marine ecosystems is causing a depletion of top-predators worldwide (Pauly *et al.*, 1998; Jackson & Sala, 2001; Myers & Worm, 2003). Cetaceans have a strong dependence on the environmental quality of their habitat, and suffer the impacts of overfishing directly through incidental kills in fisheries (by-catch), and indirectly through prey depletion and habitat degradation (Bowen, 1997; Bearzi *et al.*, 2003). Fisheries are replacing apex predators in most marine food webs and exercise a strong top-down control on these predators, making intensely exploited ecosystems highly fluctuant (Anderson *et al.*, 2008). There are inevitable consequences of fishing down the food web: increased ecosystem instability, unsustainable fisheries, and an inability for the ecosystem to support healthy and abundant populations of apex predators. Even when the prey and predator species do not overlap there is real danger of driving those predator species to the brink of extinction (Trites *et al.*, 2006).

The Mauritanian waters are one of the major African fishing grounds. Besides the large international fishing fleet that operates in the area, in the last decades Mauritania has developed a large industrial fisheries that competes with its traditional artisanal fishing, thus becoming one of the major fish-exporting African countries (Ababouch, 2000). As a consequence, this is identified as a priority area for marine conservation (Atta-Mills *et al.*, 2004; ter Hofstede & Dickey-Collas, 2006).

Accurate knowledge on biodiversity and ecosystem structure is needed to achieve conservation goals. By-catch of several cetacean species by industrial fisheries has been reported (Nieri *et al.*, 1999). However, more importantly, overfishing, overcapacity, habitat degradation, and inequitable access agreements have contributed to the decline in catches throughout West Africa (Atta-Mills *et al.*, 2004; Christensen *et al.*, 2004; Colman *et al.*, 2005; ter Hofstede & Dickey-Collas, 2006). Hence, it is of relevance to regularly estimate and monitor both the top-predator populations and the fish catch in the area. Despite the importance of assessing the impact of fisheries on the local ecosystem (Christensen *et al.*, 2004; Jouffre & Inejih, 2005), no studies have been conducted involving marine mammals in north-west Africa, and their possible interactions with fisheries operating in the area are largely unknown.



OBJECTIVES

The general aim of the present thesis is to investigate the taxonomy, morphology, habitat use, and distribution of the common dolphin (genus *Delphinus*) in the eastern Sub-tropical Atlantic Ocean (NW Africa). Attaining this aim involves understanding the factors that influence the above biological traits and would contribute to the knowledge on the genus *Delphinus* in this poorly studied area.

Specific objectives are:

- 1) To examine the local marine trophic network and the relationship between apex predators (marine mammal species, in general, and common dolphins, in particular), and selected representatives from various trophic levels;
- 2) To confirm the presence of the two morphotypes of common dolphin in the study area (short- and long-beaked forms);
- 3) To discriminate between the two morphotypes and investigate whether differences are of taxonomic relevance;
- 4) To infer distribution (offshore vs. inshore) and niche segregation (trophic level exploited) of the two morphotypes;
- 5) To investigate differences in morphology and habitat use between NW Africa common dolphins and other areas, in particular the eastern North and South Atlantic Ocean.

The latter objective is of particular relevance because the short- and long-beaked forms of common dolphins have been proposed to occur sympatrically in the eastern Sub-tropical North Atlantic and therefore this allows a unique opportunity to ascertain the adscription of the African forms of the genus.



SPECIFIC OBJECTIVES BY CHAPTER:

CHAPTER 1.

It describes the isotopic landscape for the local eastern Sub-tropical Atlantic marine ecosystem by examining the trophic network and the relationship between apex predators (marine mammal species, in general, and common dolphins, in particular), and representatives from other trophic levels.

CHAPTER 2.

It studies the foraging ecology and potential intra-specific differences of common dolphins in different Atlantic populations through the analyses of stable isotopes. The ultimate objective is to define populations units of relevance to management and conservation, not only in the study area but also in other regions.

CHAPTER 3.

It investigates the relationship between skull morphometrical variation and the carbon and nitrogen stable isotope signatures to assess population structure, niche segregation (through determination of trophic behaviour) and taxonomic status of the common dolphins in this area.

CHAPTER 4.

Using two-dimensional geometric morphometrics, it examines geographic population differentiation through analysis of skull size and shape variability. This study provides a quantification and effective visualization of morphological evidences of isolation and/or differentiation between closely-related species /populations of common dolphins along the Eastern Atlantic Ocean.

CHAPTER 5.

It resolves the taxonomic uncertainties around the *Delphinus* complex by means of a two-dimensional geometric morphometric analysis of the skull with the objective of detecting morphological variation within the common dolphin population from the eastern Sub-topical Atlantic, allowing insight into the evolutionary and ecological processes of phenotypic diversification.



SUPERVISORS' REPORT

Dr. Alejandro Aguilar and **Dr. Assumpció Borrell** co-supervisors of the PhD student **Ana Cristina Morais Pinela** certify that in the present thesis entitled "*Taxonomy, morphology and distribution of the common dolphin, *Delphinus delphis* (short-beaked form) and *Delphinus capensis* (long-beaked form), in West African waters*" five manuscripts were published or are in preparation to be submitted in prestigious scientific Journals included in the *Science Citation Index* (SCI).

The contribution of the PhD student to each of the manuscripts as well as the impact factor is detailed below, as stated in the 2013 *Journal Citation Reports* (JCR) and published by the *Institute for Scientific Information* (ISI):

1. Stable isotopes reveals habitat partitioning among the marine mammals off NW Africa and unique trophic niches for two globally threatened species.

Pinela AM, Borrell A, Cardona L, Aguilar A. 2010.

Marine Ecology Progress Series 416: 295-306.

Impact factor: 2.640

Study design: **AMP**, AA, AB, LC

Sampling: **AMP**, AA, AB, LC

Data analysis: **AMP**, LC

Scientific writing: **AMP**, AA, AB, LC

2. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope values in common dolphins (*Delphinus spp.*) worldwide, with particular emphasis on the eastern North Atlantic populations.

Pinela AM, Borrell A, Aguilar A. 2015.

Rapid Communications in Mass Spectrometry 29: 855-863.

Impact factor: 2.642

Study design: **AMP**



Sampling: **AMP**, AA, AB

Data analysis: **AMP**

Scientific writing: **AMP**, AA, AB

3. Common dolphin morphotypes: niche segregation or taxonomy?

Pinela AM, Borrell A, Aguilar A. 2011.

Journal of Zoology 284: 239-247.

Impact factor: 1.947

Study design: **AMP**, AA, AB

Sampling: **AMP**, AA, AB

Data analysis: **AMP**, AA, AB

Scientific writing: **AMP**, AA, AB

4. Geographic variation in size and shape components of the skull of common dolphins (genus *Delphinus*) from the eastern Atlantic Ocean using landmark-based geometric morphometric methods.

Pinela AM, Velázquez-Vacca A, Borrell A, Aguilar A. 2015. (*In prep.*)

Study design: **AMP**

Sampling: **AMP**

Data analysis: **AMP**, AVV

Scientific writing: **AMP**, AA, AB

5. Taxonomic status of the common dolphin (genus *Delphinus*) in the eastern Sub-tropical Atlantic Ocean.

Pinela AM, Borrell A, Aguilar A. 2015. (*In prep.*)

Study design: **AMP**

Sampling: **AMP**

Data analysis: **AMP**

Scientific writing: **AMP**, AA, AB



For the record we also certify that none of the co-authors of the manuscripts comprising this thesis has used, implicitly or explicitly, the present scientific work for the development of their own Doctoral thesis.

Barcelona, 7th of May of 2015

Dr. Alex Aguilar Vila

Departament de Biologia Animal

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METHODOLOGICAL APPROACH

STUDY AREA

The present study focuses on the eastern Sub-tropical Atlantic Ocean, one of the most productive areas in the world (Ferreira, 1978), where two important currents meet: the cold Canary Current and the warm Guinea Current. This generates a quasi-permanent upwelling zone with high biological productivity (Wells & Bleakley, 1995; Longhurst, 1998; Shumway, 1999) which supports a highly diverse and abundant fauna of marine mammals (Marchessaux & Campredon, 1988; Robineau & Vely, 1998; Nieri *et al.*, 1999). Given the mix of waters, boreal species in the area can be found alongside tropical and subtropical species (Robineau & Vely, 1998). However, available information on cetaceans of this region is limited reports on strandings, sightings and catches (Duguy, 1976; Maigret 1980 a, b, 1981; Marchessaux & Campredon, 1988; Van Waerebeek, 1997; Van Waerebeek *et al.*, 1999).

This thesis focuses on aspects of the biology and ecology of common dolphins (*Delphinus* spp.) from the North-west coast of Africa. Yet, for comparative purposes other populations of well-established common dolphin *taxa* were also investigated. The study areas varied according to the objectives being investigated and the methods applied. However, given their geographical proximity with the core study area, two populations were investigated in most studies throughout the thesis: that from the eastern north Atlantic along the Iberian Peninsula (Atlantic waters of Spain and Portugal), and that from the eastern south Atlantic (Atlantic waters of South Africa). The Iberian Peninsula was particularly useful for comparison not only by its proximity but also because it is well studied and enjoys a wealth of data and information available.

SAMPLING

The biological material studied consisted of bone tissue obtained from skeletal remains, and the whole skulls of *Delphinus* specimens collected in northern-west Africa, mainly Mauritania, the eastern Atlantic coast of the Iberian Peninsula and South Africa. Also, to study the Mauritanian marine ecosystem, samples from other species of marine mammals, marine turtles, fish, cephalopods, marine plants, macroalgae and phytoplankton were collected. Large part of this materials were already available in the



specimens collections of the University of Barcelona, the Parc National du Banc d'Arguin and other institutions in Mauritania and South Africa (details are given in the Research Papers section) but specific fieldwork was conducted to collect further material along the coast of north-west Africa.

METHODOLOGIES

Because investigation of intra and inter specific population variability requires a multiplicity of approaches, several different methods were used in the present study. These mainly included stable isotope analyses of carbon and nitrogen in bone, as well as measurement-based and 'landmark'-based (geometric) morphometric analyses of the skull.

A brief description of the main methods used in the present study is given below, but the reader is referred to each scientific manuscript for in-depth details.



STABLE ISOTOPES

Stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) have been used to study animal ecology since the late 1970s, mostly as dietary tracers (Kelly, 1999), since several biochemical processes in nature results in changes in isotopic values of elements in primary producers but also throughout the food web (Deniro & Epstein, 1978, 1981). In marine mammals, applications have particularly focused on the trophic ecology of populations (*e.g.* Hobson *et al.*, 1997; Lesage *et al.*, 2001; Drago *et al.*, 2009; Gómez-Campos *et al.*, 2011). Also, variation of stable-isotope ratios in different populations of the same species have been used to infer population structure in cetaceans (*e.g.* Abend & Smith, 1995; Walker *et al.*, 1999; Borrell *et al.*, 2006; Borrell *et al.*, 2013; Giménez *et al.*, 2013; Vighi *et al.*, 2014) and pinnipeds (*e.g.* Smith *et al.*, 1996).

Environmental differences such as light intensity, nutrient concentrations, and species composition affect the relative abundance of the heavy to light stable isotopes of both carbon (measured as $\delta^{13}\text{C}$) and nitrogen (measured as $\delta^{15}\text{N}$) (Walker *et al.*, 1999). Therefore, populations from different geographic locations, even when they have similar diets, can display dissimilar isotopic signatures (Das *et al.*, 2003; Borrell *et al.*, 2006; 2013), and thus be differentiated using stable isotope measurements. This differentiation is possible, since the isotope profile of consumers reflects those of its prey in a predictable manner due to selectivity of lighter isotopes during consumer's metabolic process (Deniro & Epstein, 1978, 1981). Therefore, stable isotope analysis offers an integrated measure of all prey assimilated over a



comparatively protracted period of time (Tieszen *et al.*, 1983; Michener & Schell, 1994; Hobson, 1999). As different tissues have different elemental turnover rates, each tissue can represent a different period of dietary integration and different spatial scales (DeNiro & Epstein, 1978, 1981; Tieszen *et al.*, 1983; Hobson & Clark, 1992; Kurlle & Worthy, 2002). Consequently, the choice of an appropriated tissue for analysis is of extreme importance in the use of stable isotope analysis in ecology. In the present study we selected bone because, owing to its low turnover rate, it averages several years of dietary history (Hobson & Clark, 1992).

Carbon isotopes

$\delta^{13}\text{C}$ value in a consumer is close to that of the diet and is preferentially used to indicate the relative contribution to the diet of different potential primary sources in a trophic network (Smith *et al.*, 1996). In the marine environment, $\delta^{13}\text{C}$ is related to inshore or benthic food webs *versus* offshore or pelagic food webs (Deniro & Epstein, 1978; France, 1995). Hence, it discriminates between habitats where phytoplankton is the only source of organic carbon (pelagic and offshore habitats), and those where macrophytes are a relevant source of organic carbon (vegetated onshore benthic habitats; Rubenstein & Hobson, 2004; Fry, 2006). Even though $\delta^{13}\text{C}$ shows little change between trophic levels, suffering little or no enrichment, it can be a useful indicator of primary productivity supporting trophic webs (Deniro & Epstein, 1978).

Nitrogen isotopes

$\delta^{15}\text{N}$ value is commonly taken as an indicator of trophic level (Cabana & Rasmussen, 1996) because it increases from prey to predator due to the preferential excretion of the light isotope by metabolic processes involved in the synthesis of protein, production of urea, and acid uric in consumers (Caut *et al.*, 2009). In consequence, $\delta^{15}\text{N}$ typically shows a stepwise increase per trophic level within a food chain (Cabana & Rasmussen, 1994), with an average isotopic difference of 3-5 ‰ between consumers and their prey, despite the fact that these differences may vary according to the tissue being analyzed and other factors such as food composition, type of aminoacids involved, etc (Deniro & Epstein, 1981; Hobson & Clark, 1992; Michener & Schell, 1994). Therefore, $\delta^{15}\text{N}$ can be used to indicate the trophic level in a given marine ecosystem, although it cannot give any information about specific items in the diet.



MORPHOMETRICS

Morphometrics are the set of techniques that involve any quantitative measurement and analysis of morphological traits to study the variation in shape and its co-variation with other variables (Bookstein, 1991; Dryden & Mardia, 1998). Although morphometrics have many applications, their major goal is to gauge morphological similarity and difference. Traditionally, the principle of morphological similarity has been commonly used to classify populations, species or higher-level taxonomic groups (Evin *et al.*, 2008). At lower taxonomic levels, morphological similarity can be difficult to detect and sometimes traditional morphometrics is inadequate to differentiate between groups (Adams *et al.*, 2004). On the contrary, modern geometric morphometrics, (GM; Rohlf & Marcus, 1993) is appropriated to investigate the evolution of forms among and within species (Monteiro, 1999; Gannon & Rácz, 2006; Loy *et al.* 2011; Galatius *et al.*, 2012), as well as functional morphology and ecological divergence (Adams & Rohlf, 2000; Claude *et al.*, 2004; McKinnon *et al.*, 2004; Woodward *et al.*, 2006).

Measurement-based morphometrics

This type of morphometrics is the one initially developed and mostly analyzes lengths, widths and ratios. Thus, all the data involved are measurements of size (Marcus, 1990). However, despite the many measurements, these produce few independent variables because the majority of measurements of size are highly correlated (Bookstein *et al.*, 1985) so it can be difficult to separate information on shape from that on size (Zelditch *et al.*, 2004). However, measurement-based morphometrics is still useful when either absolute or relative sizes are of particular interest, or when size measurements are of relevance, such as in studies of functional morphology. Nevertheless, measurement-based morphometric data contains little information about the spatial distribution of shape changes across an organism, and their statistical power to distinguish shapes is much lower (Adams *et al.*, 2004).

To distinguish between the two forms of common dolphins from the NW coast of Africa, some cranial measurements will be taken as described by Heyning & Perrin (1994). Both morphotypes have skulls which are disjunct in rostral length, significantly different in proportionate length of the rostrum (Banks & Brownell, 1969; Perrin, 1984; Heyning & Perrin, 1994), and differ in the number of tooth in the upper jaw (Banks & Brownell, 1969; Evans, 1982; Heyning & Perrin, 1994).



Landmark-based geometric morphometrics

This type of morphometrics was developed in the 1970s on the other hand, is the quantitative representation and analysis of morphological shape using geometric coordinates instead of measurements (Rohlf & Marcus, 1993; Toro Ibacache *et al.*, 2010). Landmark-based geometric morphometric methods begin with the collection of two- or three-dimensional coordinates of biologically definable landmarks (Zelditch *et al.*, 2004). Direct analysis of these coordinates as variables would be inappropriate as the effects of variation in position, orientation, and scale (known as ‘superimposition’) of the specimens is still present (Rohlf & Slice, 1990); thus, the non-shape variation must be mathematically removed prior to the analysis (Adams *et al.*, 2004). Once non-shape variation has been eliminated, the variables become shape variables and may be used for statistical analyses and graphical representations of shape may be generated for comparison of samples or groups (Adams *et al.*, 2004). Figure 1 illustrates the simplified steps to conduct morphometric analyses using a GM approach.

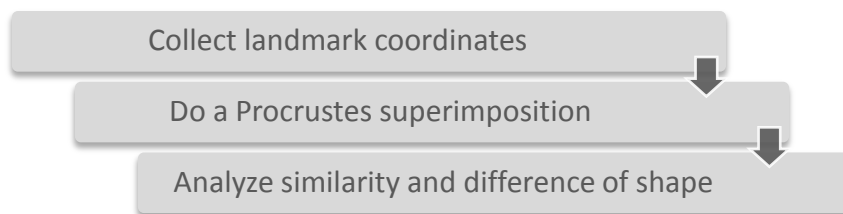


Figure 1 – Simplified steps using a geometric morphometric approach.

Geometric analyses of landmark coordinates do solve many of the problems confronting traditional methods of measurement (Adams *et al.*, 2004; Zelditch *et al.*, 2004). The advantages of geometric representations are: 1) results can be presented visually as a shape rather than as a table of numbers; 2) data can be easily collected from digital photographs; 3) size is mathematically removed from the analysis to focus on pure shape (Zelditch *et al.*, 2004).

In all species of delphinids, moderate to strong patterns of morphological variation over short geographical distances seem to be typical (Mitchell, 1970; Perrin, 1984). Such geographical variation and the usual small size of samples analysed have hampered studies of ‘*alpha*-level’ taxonomy because samples collected over wide areas are needed to distinguish between intraspecific and interspecific differences, which would be the focus of ‘*gamma*-level’ and ‘*beta*-level’ taxonomies, respectively (Perrin, 1984; Heyning & Perrin, 1994; Rosel *et al.*, 1994; Natoli *et al.*, 2006). In our study, methods of geometric analyses are applied to investigate variation within and between the short- and long-beaked morphotypes (Evans, 1982;



Perrin, 1984; Heyning & Perrin, 1994). With this objective, two-dimensional geometric morphometric analyses, based on photographs of the skull, were conducted in order to assess geographic variation in morphology, to investigate potential discrimination between the short- and long-beaked morphotypes and, with this information, to infer the taxonomic status of the species in the study area.

A photograph taken from the perspective of someone on a sailboat, looking down at the water. The boat's hull, painted in shades of green and blue, is visible on the right side of the frame. A large, light-colored sail is partially visible at the top. The ocean is a deep blue-green, with white foam from the boat's wake churning in the foreground. The sky is a clear, bright blue.

RESEARCH PAPERS

CHAPTER 1



RESUMEN

La abundancia de isótopos estables de carbono ($\delta^{13}\text{C}$) y nitrógeno ($\delta^{15}\text{N}$) en el hueso de 13 especies de mamíferos marinos de la costa noroeste de África fue investigada para evaluar sus posiciones en la red trófica y sus hábitats preferidos. Además, se recogieron muestras de productores primarios y de posibles especies de presa en la zona de estudio para caracterizar el paisaje isotópico local. Esta caracterización ha indicado un aumento de $\delta^{13}\text{C}$ desde alta mar hasta cerca de la costa y que $\delta^{15}\text{N}$ era un buen indicador de nivel trófico. Por lo tanto, las especies más costeras fueron *Monachus monachus* y *Sousa teuszii*, mientras que el *Physeter macrocephalus* y la *Balaenoptera acutorostrata* fueron las más pelágicas. $\delta^{15}\text{N}$ ha revelado que los mamíferos marinos ubicados en el nivel trófico más bajo fueron *B. acutorostrata*, *Stenella coeruleoalba* y *Delphinus* sp., y que los que ocupan el más alto fueron *M. monachus* y *P. macrocephalus*. El nivel trófico de *Orcinus orca* fue similar a *M. monachus*, lo que sugiere que *O. orca* se alimenta de peces. La conservación de las especies costeras y amenazadas (*M. monachus* y *S. teuszii*) a lo largo de la costa NW de África debería ser una prioridad, porque estas especies, siendo los principales superpredadores, no pueden ser reemplazados por otros mamíferos marinos.

SCIENTIFIC JOURNAL REFERENCE

Pinela AM, Borrell A, Cardona L, Aguilar A. **2010**. Stable isotope analysis reveals habitat partitioning among marine mammals off the NW African coast and unique trophic niches for two globally threatened species. *Marine Ecology Progress Series* 416: 295-306. (DOI: 10.3354/meps08790) Impact factor = 2.64

PDF available in the APPENDIX, page 195 (Appendix 1)



1

Stable isotope analysis reveals habitat partitioning among marine mammals off the coast of NW Africa and unique trophic niches for two globally threatened species

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ABSTRACT

Stable isotope abundances of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in the bone of 13 species of marine mammals from the northwest coast of Africa were investigated to assess their positions in the local trophic web and their preferred habitats. Also, samples of primary producers and potential prey species from the study area were collected to characterise the local isotopic landscape. This characterisation indicated that $\delta^{13}\text{C}$ increased from offshore to near-shore and that $\delta^{15}\text{N}$ was a good proxy for trophic level. Therefore, the most coastal species were *Monachus monachus* and *Sousa teuszii*, while the most pelagic were *Physeter macrocephalus* and *Balaenoptera acutorostrata*. $\delta^{15}\text{N}$ indicated that marine mammals located at the lowest trophic level were *B. acutorostrata*, *Stenella coeruleoalba* and *Delphinus* sp., and those occupying the highest trophic level were *M. monachus* and *P. macrocephalus*. The trophic level of *Orcinus orca* was similar to that of *M. monachus*, suggesting that *O. orca* preys on fish. Conservation of coastal and threatened species (*M. monachus* and *S. teuszii*) off NW Africa should be a priority because these species, as the main apex predators, cannot be replaced by other marine mammals.

KEY-WORDS: marine mammals, stable isotopes, trophic ecology, habitat use, NW Africa.



INTRODUCTION

Exploitation of marine ecosystems is causing a rapid depletion of top predators worldwide (Pauly *et al.*, 1998a; Jackson & Sala, 2001; Myers & Worm, 2003), and, as a result, marine food webs are undergoing extraordinary changes in their structure and function (Jackson *et al.*, 2001; Emslie & Patterson, 2007). Fisheries are replacing apex predators in most marine food webs and exercise a formidable top-down control on these predators, but major differences in the behaviour of fisheries and that of marine apex predators (Trites *et al.*, 2006) make intensely exploited ecosystems highly fluctuant (Anderson *et al.*, 2008). An ecosystem-based management system has been advocated to avoid those problems, but this approach is often hindered by a poor knowledge of the basic biological traits of most apex predators.

The waters of the NW coast of Africa are among the most productive in the world (Ferreira, 1978; Feidi, 1996). In these waters, two important currents meet: the cold Canary current and the warm Guinea current, generating a quasi-permanent upwelling zone that triggers a high biological productivity (Wells & Bleakley, 1995; Longhurst, 1998; Shumway, 1999). Such productivity supports a highly diverse and abundant fauna of marine mammals, (Marchessaux & Campredon, 1988; Robineau & Vely, 1998; Nieri *et al.*, 1999) which typically include, given the mix of waters, boreal species as well as tropical or sub-tropical species (Robineau & Vely, 1998). Additionally, the region shelters the last remnant populations of some scarce or highly endangered species, such as the Mediterranean monk seal, *Monachus monachus* (González *et al.*, 1997), the Atlantic humpback dolphin, *Sousa teuszii*, (Van Waerebeek *et al.*, 2004) and the African manatee, *Trichechus senegalensis* (Padial & Ibáñez, 2005). Mauritania is one of the major African fishing nations and has developed, alongside with the traditional artisanal fishing, industrial fisheries, becoming one of the major fish exporting African countries (Ababouch, 2000). Over-fishing, overcapacity, habitat degradation and inequitable access agreements have contributed to the decline in catches throughout West Africa (Atta-Mills *et al.*, 2004, Hofstede & Dickey-Collas, 2006). Assessing the impact of fisheries on the local ecosystem is of great interest (Jouffre & Inejih, 2005) but no studies have been conducted involving marine mammals.

Available information on the marine mammals of the region is limited to stranding and sighting reports in the case of cetaceans (Duguy, 1976; Maigret, 1980a, b, 1981; Marchessaux & Campredon, 1988; Van Waerebeek *et al.*, 1999), although a few demographic parameters are known for Mediterranean monk seals (González *et al.*, 1997; Gazo *et al.*, 2000). Nevertheless, the trophic relationships among the marine apex predators of the region have never been investigated, and their possible interactions with the fisheries operating in the area are largely unknown.

The methods traditionally used in the study of the trophic ecology of marine mammals have been faecal or stomach-content analyses. These methods present limitations because they only show recently eaten preys, and results can be severely biased due to differences in prey digestion (Hobson *et al.*, 1996).



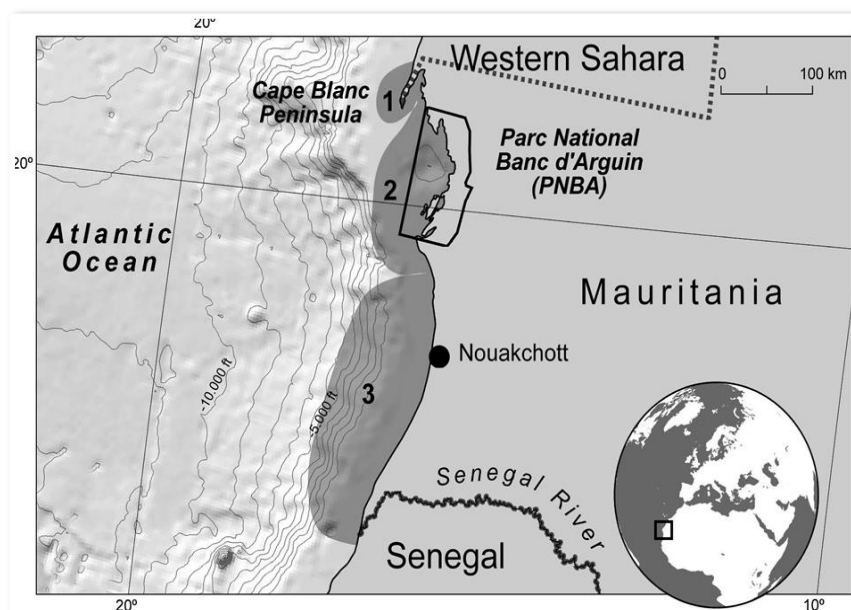
Conversely, stable isotope analysis offers an integrated measure of all preys assimilated over a comparatively protracted period of time (Michener & Schell, 1994; Tieszen *et al.*, 1983; Hobson, 1999). In marine mammals, stable isotopes of carbon and nitrogen have been extensively used to investigate the trophic ecology of populations (*e.g.*, Hobson *et al.*, 1997; Lesage *et al.*, 2001; Drago *et al.*, 2009). The relative abundance of heavy to light carbon isotopes ($\delta^{13}\text{C}$) has been used to discriminate between habitats where phytoplankton is the only source of organic carbon (pelagic and offshore habitats) and those where macrophytes are a relevant source of organic carbon (vegetated onshore benthic habitats) (Rubenstein & Hobson, 2004; Fry, 2006; Cardona *et al.*, 2007). In contrast, the relative abundance of heavy to light nitrogen isotopes ($\delta^{15}\text{N}$) is commonly taken as an indicator of trophic level (Cabana & Rasmussen, 1996) because this abundance increases from prey to predator due to the preferential excretion of the light isotope (Caut *et al.*, 2009).

In this study, stable isotope profiles of carbon and nitrogen were used to investigate the habitat use and the trophic level of the marine mammals off the northwest coast of Africa to determine these species' positions in the marine food web.

METHODOLOGY

Study site and sampling

The study was conducted in the Islamic Republic of Mauritania, located in the northwest region of Africa. Its coastline extends latitudinally more than 700 km from Cape Blanc to the Senegal River (Figure 1). The northern part of the coast is composed of rocky cliffs with scattered small, tide-dependent and sandy beaches (habitat 1 in Figure 1); the intermediate region consists of tidal mudflats inhabited by the seagrasses *Halodule wrighti* and *Zostera noltii* and shallow waters that extend several kilometres offshore and support dense sub-littoral meadows of the seagrass *Cymodocea nodosa* with scattered deep unvegetated water channels in between (habitat 2 in Figure 1, Parc National du Banc d'Arguin); and the southern part is a practically continuous sandy beach with scattered sub-littoral patches of the seagrass *Cymodocea nodosa* (habitat 3 in Figure 1, La Grand Plage). Despite these heterogeneity, there are no major differences among the three regions in the distribution of the potential prey for marine mammals, whose abundance depends mainly on the depth of the coastal waters and the variations of the sea surface temperature related to the upwelling (Hofstede & Dickey-Collas, 2006).

**Figure 1**

Map of the study site with the bathymetry shape (in feet), the over line of the continental shelf, and the three habitat types indicated by dark grey areas with numbers (from habitat 1 to habitat 3, see text for habitat characterization).

Bone samples from 13 species of marine mammals (243 individuals, Table 1) were collected opportunistically between 1990 and 2008 along the Mauritanian coastline. Except for the *M. monachus*, which all samples came from natural mortalities, we have no information on the cause of death of the marine mammals used in this study. Bone was the selected tissue because, due to its low turnover rate, reflects the diet of animals through several years (Hobson & Clark, 1992). The bone collected from each skull was a small fragment of turbinate bones from the nasal cavity because this fragment was easy to crush, and its sampling did not damage the skull for subsequent studies. Furthermore, samples from seven species of potential prey (fish and cephalopods), one species of sea turtle, seven species of benthic macrophytes (benthic primary producers) and phytoplankton (pelagic primary producers) were also collected to characterise the isotopic landscape off the coast of Mauritania (Table 1).

The samples of leaf and thallus, collected from seagrasses and macroalgae respectively, were dried with sodium chloride, while the dorsal white muscle of the fish, the mantle of the cephalopod, and the muscle of the turtle were stored in a freezer at $-20\text{ }^{\circ}\text{C}$ until analysis. Samples of skull and other bone tissues were stored without preservatives at ambient temperature ($15\text{--}25\text{ }^{\circ}\text{C}$). Phytoplankton (whole organisms in collective samples) was refrigerated at $4\text{ }^{\circ}\text{C}$, brought to the laboratory, filtered in a pre-combusted GF/C filter and processed for isotopic determination.



Stable isotopes analyses

Stable isotope abundances (expressed as delta notation, δ), in which the relative variations of stable isotope ratios are expressed in per mil (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where X is the value of the heavy isotope of the sample (^{13}C or ^{15}N), R_{sample} is the ratio of the heavy to the light isotope of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$), and R_{standard} is the ratio of the heavy to the light isotope in the reference standards, which were the Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for carbon and the atmospheric nitrogen (air) for nitrogen.

For the pre-treatment of the samples, approximately 1 g of tissue was rinsed with distilled water, dried for 3 days at 60°C and powdered with mortar and pestle. Because lipids are depleted for ^{13}C as compared with other molecules, and thus can mislead the analyses by decreasing the $\delta^{13}\text{C}$ signal (DeNiro & Epstein, 1977), they were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution (Bligh & Dyer, 1959). When conducting stable isotope analysis in bone, several authors have carried out preventive demineralisation of the tissue by treating it with either a 0.5 or 1 M hydrochloric acid (HCl) solution (Bocherens *et al.*, 1997; Newsome *et al.*, 2006). However, a test using a subset of the samples ($n = 20$) revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not differ between demineralised and untreated samples, so this procedure was not followed. However, the seagrasses and macroalgae were treated with a 0.5 M HCl solution to eliminate inorganic carbonates that could bias the results (Ng *et al.*, 2007). Phytoplankton was also treated with 0.05 M HCl to remove carbonates following the protocol of Ogawa and Ogura (1997). Some authors agree that it is possible that HCl treatment adversely affects the nitrogen isotopic signature (Bunn *et al.*, 1995), thus primary producers samples (seagrasses, macroalgae and phytoplankton collective samples) were separated in two sub-sample used for carbon (after the HCl treatment) and for nitrogen (without the HCl treatment).

Finally, approximately 1 mg of bone, 0.5 mg of white muscle from fish and of mantle from cephalopods, 4 mg of homogenised seagrasses and of macroalgae and 12 mg of the homogenised phytoplankton with filter were weighed in tin capsules (3.3 x 5 mm), combusted at 900°C and analysed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan, Thermo Finnigan, Bremen, Germany). Atropine was used as a standard to check the elemental composition for carbon and nitrogen. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the IAEA (International Atomic Energy Agency), namely polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$) and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios, namely $(\text{NH}_4)_2\text{SO}_4$



(IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$) and KNO_3 (IAEA NO_3 , $\delta^{15}\text{N} = +4.7\text{‰}$), were used for calibration at a precision of 0.3‰.

Data analyses

The assumption of normality in the distributions of the sample groups was checked with the Kolmogorov-Smirnov test. The assumption of homogeneity of variances between sample groups was checked with Levene's test.

Differences in mean bivariate isotope ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) among marine mammal species were assessed by a multivariate analysis of variance (MANOVA) because they displayed a normal distribution. Univariate analysis of variance (ANOVA) was conducted separately for both isotopes. If a statistically significant interaction was found between marine mammal species for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using an ANOVA, additional pairwise comparisons were made using a generalized linear model because it admits data with no homogeneity of variances. *Post-hoc* analyses were made by means of least significant differences tests (LSD) based on estimated marginal means. As a method of estimation, the maximum likelihood (ML) was used in all cases. Normality distribution and identity as a link function was always used (for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), since data followed a normal distribution. Goodness of fit values (Akaike's information criterion, AIC) were used to compare different models. The confidence interval level was set at 95% for all comparisons. The *post-hoc* pairwise comparisons by generalized linear model are more flexible and less conservative statistical tool than the *post-hoc* pairwise comparisons by general linear model (GLM) because several types of distribution and models can be chosen to fit the data. The *post-hoc* test was conducted for the species represented by more than one sample (SCOE, GGRI and BACU were excluded from the analysis).

Unless stated, data are presented as mean \pm standard deviation (SD) throughout the manuscript, except when a species is represented by a single individual. All the statistical analyses were conducted using the SPSS ver.15 software.

RESULTS

The isotopic landscape

Macroalgae presented lower $\delta^{13}\text{C}$ values in comparison with seagrasses but presented higher $\delta^{13}\text{C}$ values in comparison with phytoplankton (Figure 2, Table 1). The $\delta^{13}\text{C}$ of green turtles was close to that of the seagrasses that they consume (Cardona *et al.*, 2009), whereas the $\delta^{13}\text{C}$ of fish and cephalopods were between those of phytoplankton and benthic primary producers. As expected, coastal fish (*Plectorhynchus mediterraneus*, *Caranx rhonchus* and *Sardinella aurita*) displayed higher $\delta^{13}\text{C}$ values than did more offshore



species (*Pagellus bellotii*, *Scomberomorus tritor* and *Mullus barbatus*), but none of the sampled species was truly oceanic. The $\delta^{15}\text{N}$ of macrophytes was highly variable and ranged from 2.6‰ (the seagrass *Cymodocea nodosa*) to 5.4‰ (the macroalgae *Sargassum* sp.). Phytoplankton displayed higher $\delta^{15}\text{N}$ values than did any benthic macrophyte. As expected, green turtles were more enriched for $\delta^{15}\text{N}$ than were benthic primary producers, and the zooplanktophagous fish *S. aurita* was more enriched for $\delta^{15}\text{N}$ than was phytoplankton. Likewise, fish including only small invertebrates in their diets (*M. barbatus* and *S. aurita*) presented lower $\delta^{15}\text{N}$ values when compared with fish consuming large invertebrates and fish (*C. rhonchus*, *S. tritor* and *P. mediterraneus*). Such an isotopic landscape indicates that the bone of oceanic marine mammals should present lower $\delta^{13}\text{C}$ values than that of coastal marine mammals, especially those inhabiting seagrass meadows. Furthermore, the $\delta^{15}\text{N}$ is a good proxy for trophic level in the considered system, although species inhabiting seagrass meadows might present lower $\delta^{15}\text{N}$ values in comparison with oceanic species at the same trophic level.

Table 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of each species collected along the Mauritanian coast. Data are given as mean \pm SD (‰), minimum and maximum isotope value (min.-max.), identification codes (ID code), and number of individuals per species (N). Bold values represent mean \pm SD (‰), minimum and maximum isotope value for each category

Species	ID code	N	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
			mean \pm SD	min.-max.	mean \pm SD	min.-max.
Pelagic primary producers						
Phytoplankton	PHYTO	3 [†]	-24.4 \pm 3.5	(-26.9)-(-20.3)	8.9 \pm 0.3	8.6-9.2
Benthic primary producers		34	-14.6 \pm 0.8	(-19.9)-(-5.3)	3.8 \pm 0.18	1.8-5.8
Seagrasses						
<i>Cymodocea nodosa</i>	CNOD	5	-6.3 \pm 1.1	(-7.5)-(-5.3)	2.6 \pm 0.8	1.8-3.8
<i>Halodule wrightii</i>	HWRI	5	-11.8 \pm 0.8	(-13.0)-(-10.7)	4.3 \pm 1.1	2.8-5.8
<i>Zostera noltii</i>	ZNOL	5	-10.5 \pm 1.3	(-7.5)-(-5.3)	2.9 \pm 0.2	2.7-3.2
Macroalgae						
<i>Asparagopsis</i> sp.	ASPA	2	-19.7 \pm 0.4	(-19.9)-(-19.4)	3.7 \pm 0.5	3.3-4.1
<i>Dictyota</i> sp.	DICT	5	-18.8 \pm 0.2	(-19.1)-(-18.7)	4.7 \pm 0.4	4.3-5.3
<i>Padina</i> sp.	PADI	2	-16.1 \pm 1.3	(-17.0)-(-15.2)	3.4 \pm 0.9	2.7-4.0
<i>Polysiphonia</i> sp.	POLY	5	-19.6 \pm 0.2	(-19.9)-(-19.4)	3.6 \pm 0.4	3.2-4.1
<i>Sargassum</i> sp.	SARG	5	-17.7 \pm 0.2	(-17.9)-(-17.5)	5.1 \pm 0.4	4.4-5.4
Fish		43	-15.4 \pm 0.91	(-18.0)-(-14.1)	13.1 \pm 1.73	9.7-16.0
<i>Caranx rhonchus</i>	CRHO	11	-14.8 \pm 0.29	(-15.5)-(-14.4)	14.1 \pm 0.50	13.5-14.8
<i>Mullus barbatus</i>	MBAR	10	-16.2 \pm 0.77	(-17.5)-(-15.0)	13.1 \pm 0.83	11.6-14.2
<i>Pagellus bellotii</i>	PBEL	9	-15.7 \pm 1.25	(-18.0)-(-14.2)	14.3 \pm 1.07	13.0-16.0
<i>Plectorhinchus mediterraneus</i>	PMED	1	-14.1		14.2	
<i>Sardinella aurita</i>	SAUR	10	-15.0 \pm 0.39	(-15.7)-(-14.3)	10.4 \pm 0.66	9.7-11.0



<i>Scomberomorus</i>						
<i>tritor</i>	STRI	2	-15.7 ± 0.21	(-15.8)-(-15.5)	14.1 ± 0.32	14.0-14.4
Cephalopods						
<i>Loligo sp.</i>	LOLI	1	-15.1		14.4	
Marine turtle						
<i>Chelonia mydas</i>	CMID	4	-9.3 ± 2.2	(-11.6)-(-6.8)	7.8 ± 1.2	6.0-8.6
Marine mammals						
		243	-11.7 ± 1.23	(-15.9)-(-8.1)	13.4 ± 1.16	10.8-17.1
<i>Balaenoptera</i>						
<i>acutorostrata</i>	BACU	1	-15.9		11.8	
<i>Delphinus sp.</i>	DDEL	95	-12.4 ± 0.81	(-15.3)-(-9.7)	12.7 ± 0.83	10.8-15.9
<i>Globicephala</i>						
<i>macrorhynchus</i>	GMAC	9	-11.5 ± 0.53	(-12.5)-(-10.7)	14.0 ± 0.39	13.5-14.6
<i>Globicephala melas</i>	GMEL	2	-11.1 ± 0.42	(-11.4)-(-10.8)	14.1 ± 0.27	13.9-14.3
<i>Grampus griseus</i>	GGRI	1	-12.0		13.8	
<i>Monachus</i>						
<i>monachus</i>	MMON	12	-10.4 ± 0.63	(-11.2)-(-8.9)	14.5 ± 0.70	12.9-15.4
<i>Orcinus orca</i>	ORCA	3	-11.9 ± 1.39	(-13.2)-(-10.4)	14.5 ± 0.52	14.0-15.0
<i>Phocoena</i>						
<i>phocoena</i>	PPHO	42	-11.7 ± 0.94	(-14.0)-(-9.7)	13.6 ± 0.76	11.6-15.4
<i>Physeter</i>						
<i>macrocephalus</i>	PMAC	2	-13.9 ± 2.11	(-15.4)-(-12.4)	15.9 ± 0.06	15.9-16.0
<i>Sousa teuszii</i>	STEU	11	-9.7 ± 0.93	(-11.5)-(-8.1)	13.3 ± 1.33	11.7-15.3
<i>Stenella</i>						
<i>coeruleoalba</i>	SCOE	1	-12.0		11.9	
<i>Stenella frontalis</i>	SFRO	4	-12.1 ± 0.30	(-12.4)-(-11.8)	13.2 ± 1.02	12.3-14.6
<i>Tursiops truncatus</i>	TTRU	60	-11.1 ± 1.23	(-15.4)-(-8.3)	13.9 ± 1.30	11.4-17.1

[†]: collective samples.

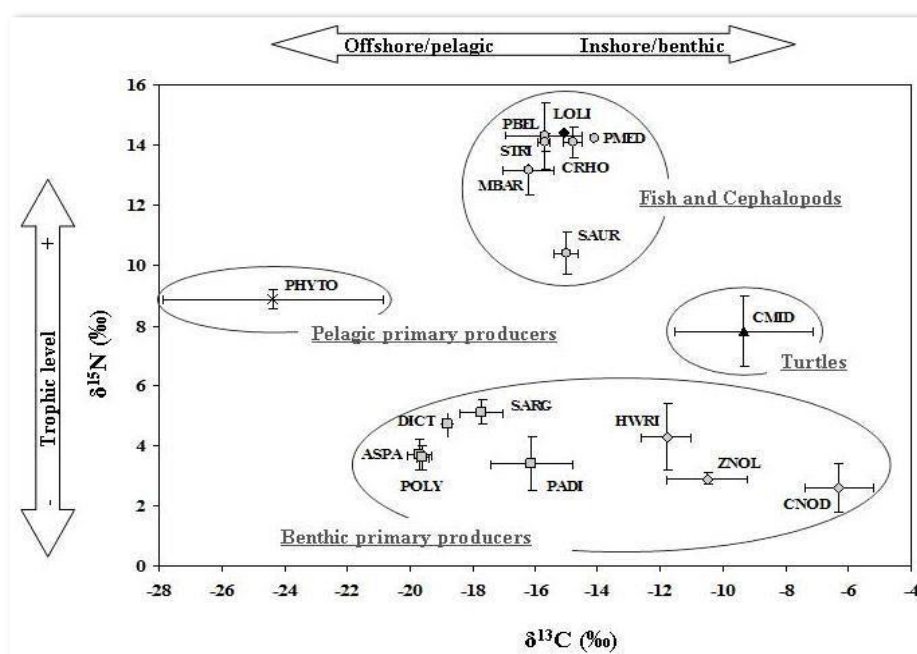


Figure 2

Mean (\pm SD, ‰) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values for all trophic groups considered, except marine mammals: phytoplankton (cross), seagrasses (grey diamond), macroalgae (grey square), fish (grey circle), cephalopod (black diamond) and sea turtle (black triangle).



Stable isotopic composition of marine mammals

Statistically significant differences exist among the bivariate isotopic signals of the considered species of marine mammals (MANOVA test: Wilk's lambda = 0.413, $F = 14.131$, $p < 0.001$). ANOVA indicate that both nitrogen and carbon were involved in those differences ($\delta^{13}\text{C}$: $F = 16.982$, $p < 0.001$; $\delta^{15}\text{N}$: $F = 11.256$, $p < 0.001$; Table 2). The results of the *post-hoc* test for pair-wise comparisons between species for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are illustrated in Table 3.

Table 2 Summary of the results of the ANOVA to test for differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the marine mammal species investigated in the study area

	SS	df	MS	F	P	r^2
$\delta^{13}\text{C}$ (‰)						
Model	140.791	9	15.643	16.982	< 0.001	0.399
Intercept	7009.343	1	7009.343	7608.979	< 0.001	
Species	140.791	9	15.643	16.982	< 0.001	
Error	211.875	230	0.921			
Total	33183.103	240				
$\delta^{15}\text{N}$ (‰)						
Model	94.977	9	10.553	11.256	< 0.001	0.306
Intercept	10178.900	1	10178.900	10857.200	< 0.001	
Species	94.977	9	10.553	11.256	< 0.001	
Error	215.631	230	0.938			
Total	43505.029	240				

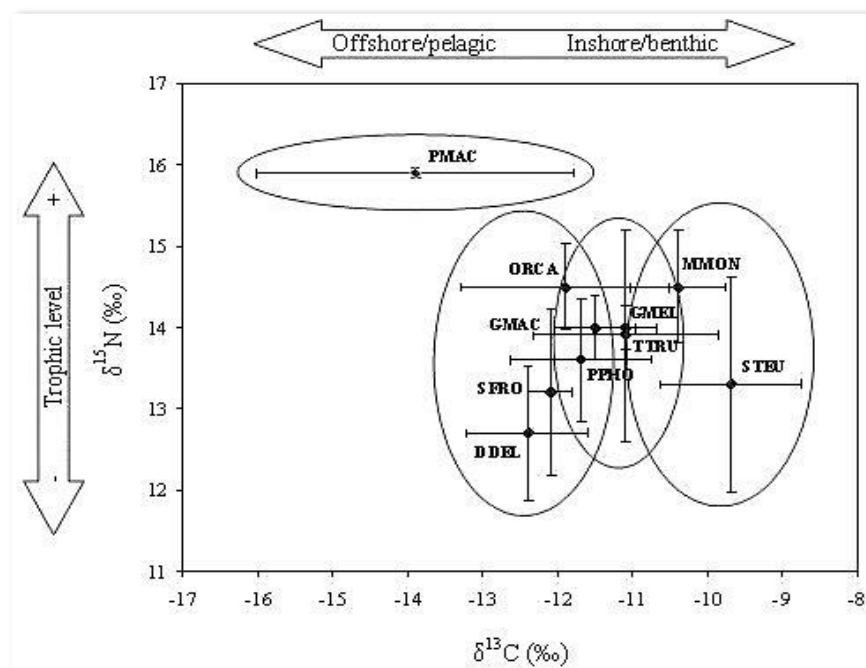
As revealed by the $\delta^{13}\text{C}$ values, four major groups of marine mammals exist as delineated by their distribution along the coastal-oceanic axis (Figure 3). Carbon isotope values together with the results of the *post-hoc* pairwise comparisons allowed us to group species according to their similitude's of carbon isotopic values. The most coastal group, characterised by values of the $\delta^{13}\text{C}$ close to those of seagrasses, included *M. monachus* ($\delta^{13}\text{C} = -10.4$) and *S. teuszii* ($\delta^{13}\text{C} = -9.7$).



Table 3 Results of the post-hoc test for multiple comparisons between the marine mammal species collected along the Mauritanian coast. Significance level of the p -value for carbon isotopes are reported below the diagonal ($\delta^{13}\text{C}$) and those for nitrogen isotopes above the diagonal ($\delta^{15}\text{N}$): p -values significant for a significance level of 0.05 are highlighted in **bold**

	DDEL	GMAC	MMON	PPHO	STEU	TTRU	SFRO	GMEL	ORCA	PMAC
DDEL		0.000	0.000	0.000	0.052	0.000	0.334	0.049	0.002	0.000
GMAC	0.008		0.271	0.259	0.104	0.778	0.154	0.940	0.458	0.009
MMON	0.000	0.008		0.006	0.004	0.064	0.020	0.577	0.988	0.042
PPHO	0.000	0.523	0.000		0.351	0.118	0.399	0.513	0.128	0.001
STEU	0.000	0.000	0.076	0.000		0.055	0.829	0.305	0.060	0.000
TTRU	0.000	0.199	0.026	0.001	0.000		0.143	0.825	0.314	0.003
SFRO	0.585	0.284	0.002	0.435	0.000	0.033		0.291	0.077	0.001
GMEL	0.060	0.588	0.332	0.363	0.054	0.960	0.218		0.633	0.048
ORCA	0.357	0.567	0.017	0.805	0.000	0.155	0.732	0.378		0.091
PMAC	0.024	0.001	0.000	0.001	0.000	0.000	0.029	0.003	0.018	

The most oceanic group, characterised by highly negative values of the $\delta^{13}\text{C}$, included *Physeter macrocephalus* ($\delta^{13}\text{C}=-13.9$). *Balaenoptera acutorostrata* ($\delta^{13}\text{C}=-15.9$) probably belonged to that group, although only one individual had been sampled. The remaining nine species were between these two groups, with *Delphinus* sp., *Globicephala macrorhynchus*, *Phocoena phocoena*, *Stenella frontalis* and *Orcinus orca* presenting lower $\delta^{13}\text{C}$ values than *Globicephala melas* and *Tursiops truncatus* (Table 1 and Figure 3).

**Figure 3**

Mean (\pm SD, ‰) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of the marine mammal species collected in Mauritania. The identification codes and number of individuals for each species are presented in Table 1; and ellipses group the species that did not display significant differences on $\delta^{13}\text{C}$ (p -value > 0.05).

Regarding the $\delta^{15}\text{N}$ values, significant differences were observed between the most $\delta^{15}\text{N}$ enriched species (*P. macrocephalus*) and those species less enriched (e.g., *Delphinus* sp. and *S. teuszii*, Table 1, 3 and Figure 3). The only specimens of *B. acutorostrata* and *Stenella coeruleoalba* analysed were also highly depleted for $\delta^{15}\text{N}$: 11.8 and 11.9, respectively. Differences in $\delta^{15}\text{N}$ values among species reveal differences in trophic level: species with higher $\delta^{15}\text{N}$ values are at a higher trophic level than species with lower $\delta^{15}\text{N}$ values. However, as previously stated, coastal species might present lower $\delta^{15}\text{N}$ values in comparison with oceanic species at the same trophic level. Accordingly, *P. macrocephalus* was the species with the highest trophic level, while *B. acutorostrata* and *S. coeruleoalba* were the species with the lowest trophic levels. It is important to notice that the mean trophic level of *O. orca* ($\delta^{15}\text{N}=14.5$) was equal to that of *M. monachus* ($\delta^{15}\text{N}=14.5$) and lower than that of *P. macrocephalus* ($\delta^{15}\text{N}=15.9$). However, it is worth noting that direct comparison can be slightly biased due to the fact that *M. monachus* is a much more coastal species than *O. orca*.

DISCUSSION

Although the use of stable isotopes as diet tracers is a powerful technique, interpreting the results is not always straightforward because the method is more reliable when large differences exist between the isotopic signals of the considered sources. Many researchers do not assess the variability in $\delta^{15}\text{N}$ among



the primary producers because it is assumed that they have similar $\delta^{15}\text{N}$ values. Although this is true in some ecosystems and regions, exceptions are common (Drago *et al.*, 2009). The present study reveals a high variability in $\delta^{15}\text{N}$ among macrophyte species from the same region and also between macrophytes and phytoplankton, with differences often larger than the average increase expected per trophic level (Minagawa & Wada, 1984; Caut *et al.*, 2009). As a consequence, variability in the $\delta^{15}\text{N}$ of marine mammal bone may reflect not only differences in trophic level but also shifts among foraging grounds with primary producers differing in $\delta^{15}\text{N}$ values. In the Mauritanian ecosystem, coastal marine mammals are expected to have lower $\delta^{15}\text{N}$ values than their oceanic counterparts because macrophytes display lower $\delta^{15}\text{N}$ values than particulate organic matter. In species feeding on a variety of prey species with different nitrogen baselines, diet composition is difficult to establish simply through stable isotope analysis of nitrogen. Fortunately, the $\delta^{13}\text{C}$ of primary producers from Mauritania varied more consistently among groups, with phytoplankton less enriched in ^{13}C than macroalgae, and macroalgae less enriched in ^{13}C than seagrasses. This allowed us to interpret the enrichment in ^{13}C of the bone of marine mammals as evidence of more coastal foraging habitats.

Therefore, *S. teuszii* and *M. monachus* appear to be the most coastal species, a result consistent with published information about the distribution of these species off Mauritania. The main area of occurrence of *S. teuszii* is the Banc d'Arguin, a large system of shallow waters and channels extending along the northern coast of the country (Maigret *et al.*, 1976; Maigret, 1980a; Van Waerebeek *et al.*, 2004). *M. monachus* is found in the Cape Blanc Peninsula, although it has also been observed to travel several miles offshore to obtain its prey (Gazo & Aguilar, 2005). Despite the similarity in the $\delta^{13}\text{C}$ of *M. monachus* and *S. teuszii*, the former was far more enriched for $\delta^{15}\text{N}$. This result is also consistent with stomach content analysis, as *S. teuszii* consumes primarily detritivorous and zooplanktophagous fishes abundant near shore (Cadenat & Paraiso, 1957; Van Waerebeek *et al.*, 2004), whereas *M. monachus* principally eats lobsters, octopus and benthic, carnivorous fish (Marchessaux, 1989). However, increasing fish landings have resulted in stocks, especially those of demersal fish and invertebrates (crustaceans and cephalopods), being overexploited or severely depleted (Christensen *et al.*, 2004; Jouffre & Inejih, 2005), most likely affecting the feeding behaviour of *M. monachus* and *S. teuszii*.

Also, the $\delta^{13}\text{C}$ values suggest that *B. acutorostrata* and *P. macrocephalus* are the most oceanic species in the region; *B. acutorostrata* has been observed both in coastal and offshore waters in the Western Sahara (Van Waerebeek *et al.*, 1999). Data about the distribution of *P. macrocephalus* off Mauritania are limited (Maigret, 1980b), although the species is oceanic over most of its world range (Rendell *et al.*, 2004). The diet of these species off NW Africa is unknown, but elsewhere *P. macrocephalus* preys mostly on large, oceanic squids with a high trophic level (Clarke *et al.*, 1976; Pauly *et al.*, 1998b; Ostrom *et al.*, 1993; González *et al.*, 1994), which is consistent with its high $\delta^{15}\text{N}$ in the study area. In



contrast, *B. acutorostrata* primarily consumes schooling fish (Das *et al.*, 2003; Hassani *et al.*, 1997; Pauly *et al.*, 1998b; Macleod *et al.*, 2007). This kind of prey could explain its semblance with *S. coeruleoalba* with respect to $\delta^{15}\text{N}$ values, although only one specimen of the latter was analysed.

The remaining species lie between the former two groups, spaced along a continuous gradient. *T. truncatus* is found in both coastal and offshore habitats along the Mauritanian coastline (Robineau & Vely, 1998), which probably explains the large intra-specific variability in the $\delta^{13}\text{C}$ values and suggest the occurrence of persistent individual differences in foraging habits or even the existence of coastal vs. offshore populations/ecotypes in the area, like elsewhere (Segura *et al.*, 2006).

Nevertheless, the mean $\delta^{13}\text{C}$ value of *T. truncatus* is close to that of *G. melas*, a typical deep-water species in the North Atlantic (MacLeod *et al.*, 2007) that is also known to occur over the continental shelf off Banc d'Arguin and off the Grande Plage (Maigret *et al.*, 1976; Robineau & Vely, 1998). *G. melas* displays a slightly higher $\delta^{13}\text{C}$ value than *G. macrorhynchus*, a species usually inhabiting deeper water over the upper slope in the North Atlantic (Davis *et al.*, 2002), although nothing is known about its distribution off Mauritania. Finally, these three species present lower $\delta^{13}\text{C}$ values than those of *S. teuszii* and *M. monachus* and higher values than those of typical oceanic species, such as *Delphinus* sp. and *P. macrocephalus* (Robineau & Vely, 1998; Rendel *et al.*, 2004). However, *T. truncatus*, *G. melas* and *G. macrorhynchus* also show similar $\delta^{15}\text{N}$ values, suggesting that they possibly forage similar prey. Published dietary data, only available for *T. truncatus* off Mauritania, identified the detritivorous grey mullet, *Mugil cephalus*, as their main prey item (Robineau & Vely, 1998). This observation does not seem to correlate with the typical prey of the two species of *Globicephala* (Overholtz & Waring, 1991). Possibly, only a fraction of the population of *T. truncatus* regularly consumes grey mullet, while most of the population has a diet similar to that of *Globicephala* spp. This scenario has been reported, for instance for northern Spain, where *G. melas* and *T. truncatus* feed mainly on neritic cephalopods. Likewise, in the Gulf of California the coastal ecotype of *T. truncatus* displayed values of the $\delta^{13}\text{C}$ similar to those of coastal prey items such as the mullet *Mugil cephalus*, while the offshore ecotype of *T. truncatus* displayed values of the $\delta^{13}\text{C}$ more similar to those of pelagic fish and medium-sized squids (Segura *et al.*, 2006).

The species next to the previous three in the decreasing order of $\delta^{13}\text{C}$ is *P. phocoena*. It inhabits cold-temperate and sub-polar regions in the North Atlantic, generally showing affinity for shelf waters (Skov *et al.*, 1995). Mauritania is the southern limit of the distribution range of the species probably because of the coastal upwelling, which produces relatively cold waters (Smeenk *et al.*, 1992). Sightings suggest that *P. phocoena* lives close to the coast in the non-upwelling season and moves offshore in the onset of the upwelling season (Marchessaux & Campredon, 1988; Smeenk *et al.* 1992; Robineau & Vely, 1998). The diet of *P. phocoena* off NW Africa is unknown, but the average $\delta^{15}\text{N}$ is similar to that of *T. truncatus*, which is consistent with a fish-based diet (Fontaine *et al.*, 1994). *O. orca* has been reported in both coastal and



oceanic areas off Mauritania, except in the very shallow waters of the Parc National du Banc d'Arguin (Duguy, 1976; Maigret, 1981). However, the $\delta^{13}\text{C}$ values reveal a preference for the deeper parts of the continental shelf and the shelf break, as they are close to the values for *Delphinus* sp. The Mauritanian population of *O. orca* appears to be the "offshore" ecotype, which is an ecotype thought to eat fish (Ford *et al.*, 2000; Saulitis *et al.*, 2000). This is in agreement with visual observations that suggested that *O. orca* avoided other marine mammals, including the monk seal *M. monachus* (Aguilar A., *unpublished results*). The average $\delta^{15}\text{N}$ of this species suggests a diet based on carnivorous fish, as reported for southern Spain (De Stephanis *et al.*, 2008). *Grampus griseus* is poorly reported in NW Africa (Robineau & Vely, 1998), but isotopic values revealed an oceanic habitat preference that occurs seaward of the continental slope and is concentrated along the upper continental slope (Baumgartner, 1997; Davis *et al.*, 2002).

Delphinus sp. is seldom observed onshore in Mauritania (Robineau & Vely, 1998) and displays a typical oceanic behaviour. *S. frontalis* also typically inhabits the shelf break (Nieri *et al.*, 1999) because the quasi-permanent upwelling may act as a thermal barrier and prevent it from approaching the inshore coastal zone (Robineau & Vely, 1998). Likewise, *S. coeruleoalba* is oceanic over most of its range (Aguilar, 2000; Davis *et al.*, 2002). Accordingly, these three species are more depleted for $\delta^{13}\text{C}$ than *M. monachus*, *S. teuszii* and *T. truncatus*. *Delphinus* sp. and *S. coeruleoalba* presented some of the lowest $\delta^{15}\text{N}$ values of all marine mammals, which is similar to what was observed for other marine trophic webs (Das *et al.*, 2003; Walker & Macko, 1999). The discrepancies in $\delta^{15}\text{N}$ values of these species compared to other pelagic marine mammals possibly reflect the higher proportion of low trophic level prey, such as the *Sardinella aurita*, in the diet of these species off the coast of Mauritania. Additionally, dolphins are opportunistic feeders and thus take advantage of the local abundance of prey (Hassani *et al.*, 1997).

The results obtained in the present study suggest the outer continental shelf and the upper slope as the areas with the greater marine mammal species diversity. However, coastal areas are home to the most vulnerable and endangered species in the Mauritanian marine ecosystems. In the first area, there could be a certain redundancy between species because their isotopic signals are very similar, indicating that they occupy a very similar ecological niche. In contrast, the reduced number of species and the greater difference in isotopic signals indicate less redundancy in the coastal areas. From this point of view, conservation of coastal species (*M. monachus* and *S. teuszii*) in Mauritania should be a priority because if these species were to disappear they could hardly be replaced in the trophic web by other marine mammals. In the case of *M. monachus*, it is likely that this species is already extinct from the ecological point of view, given the small number of individuals of this population (Forcada *et al.*, 1999). Artisanal fisheries could have an impact on *M. monachus* and *S. teuszii*, although industrial fishing operating in the deepest parts of the continental shelf are the only sources of incidental by-catch recorded to date in the



region and (*P. phocoena* and *S. frontalis* the only species known to be affected (Nieri *et al.*, 1999). Nevertheless, overfishing of the local demersal species by the intensive bottom trawling fishing in Mauritanian (Jouffre & Inejih, 2005), may have limited the prey availability for the *M. monachus*, which can partially explain the reproductive problems of this species (Pastor, 2010). Therefore, the highest priority of conservation efforts should be to reduce the impact of artisanal and industrial bottom trawling fishing on the less abundant and most vulnerable species. Nonetheless, it will also be important to regularly estimate and control the populations and catches of fishes in the whole study area because it shelters a great diversity of marine mammals. There are inevitable consequences of fishing down the food web: increased ecosystem instability, unsustainable fisheries, and an inability for the ecosystem to support healthy abundant populations of apex predators (Trites *et al.*, 2006). Excessive catches on one species may lead to the collapse of an important predator or prey in the system, which may cause changes in the growth and survival patterns of other species in the food web (Walters & Kitchell, 2001). Some groups of apex predators, such as marine mammals, may be affected by fisheries even when the prey and species do not overlap, driving species at the end of the food chain to the brick of extinction (Trites *et al.*, 2006). Conservation efforts should be made locally to protect these species because this area suffers a great fishing pressure (Hofstede & Dickey-Collas, 2006; Christensen *et al.*, 2004), and animals are constantly exposed to threats such as intensive fishing (Kenety, 2001; Christensen *et al.*, 2004), prey stock depletion (Hofstede & Dickey-Collas, 2006; Christensen *et al.*, 2004), pollution and human disturbance (Colman *et al.*, 2005). Conservation efforts are unequivocally related to the local fishing policies, such as: effective monitoring, control and surveillance of fisheries in the region (Atta-Mills *et al.*, 2004); stronger political interest to implement sustainable fisheries in North West Africa, since African leaders appear to be disregarding over-fishing as a priority issue that needs to be addressed (NEPAD, 2001); and lobby the European Union and Asian countries, such as Japan and China, for policy reforms to minimize the impacts of European and Asian fishing fleets on their marine resources.

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CHAPTER 2



RESUMEN

Distinguir unidades poblacionales de pequeños cetáceos distribuidos de forma continua en un área extensa es difícil, pero fundamental para su conservación y gestión. El uso de marcadores químicos permite investigar la ecología de forrajeo y la variabilidad inter-específica, con el fin de detectar la estructura de la población y la segregación de nichos en el delfín común (*Delphinus* spp.). Los isótopos estables de carbono ($\delta^{13}\text{C}$) y nitrógeno ($\delta^{15}\text{N}$) se midieron en el tejido óseo de los delfines comunes capturados accidentalmente o varados a lo largo del noreste y del este subtropical del Océano Atlántico, por medio de espectrometría de masa de relación isotópica. Se determinaron y compararon posiciones tróficas, teniendo en cuenta el ecosistema local de referencia trófica para cada área de estudio. Los datos obtenidos en las áreas de estudio fueron comparados cualitativamente con los de especies de delfines comunes/poblaciones distribuidas en todo el mundo. Valores de $\delta^{13}\text{C}$ y $\delta^{15}\text{N}$ fueron mayores en el este subtropical del Atlántico como consecuencia de la cohabitación del delfín de morro corto y de morro largo en el área. Los individuos del noreste Atlántico presentaron valores de $\delta^{15}\text{N}$ inferiores, reflejando diferencias en la dieta y en la variación local de la línea base isotópica. Comparaciones con otras áreas del mundo sugieren que la especie es extremadamente adaptable y se alimenta a diferentes niveles tróficos de modo a adaptarse a variaciones locales. Los isótopos estables son una herramienta útil para investigar la estructura de la población y la segregación de nicho trófico. El comportamiento trófico de las poblaciones mundiales de delfín común se analizaron con éxito y revelaron diferencias sustanciales, reflejando probablemente tanto las estrategias de adaptación del género como diferencias en la estructura de los ecosistemas.

SCIENTIFIC JOURNAL REFERENCE

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PDF available in the APPENDIX, page 207 (Appendix 2)



2

Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope values in common dolphins (*Delphinus* spp.) worldwide, with particular emphasis on the eastern North Atlantic populations

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ABSTRACT

Distinguishing population units of small cetaceans continuously distributed in a widespread area is challenging but critical for their conservation and management. The use of chemical markers allows investigating foraging ecology and inter-specific variability, in order to detect population structure and niche segregation in the common dolphin (*Delphinus* spp.). Stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) were measured in the bone tissue of common dolphins accidentally by-caught or stranded along the North-eastern and eastern Subtropical Atlantic Ocean, by means of continuous flow isotope ratio mass spectrometry. Trophic positions were determined and compared, taking into account the local ecosystem trophic baseline for each study area. Data obtained for the study areas were qualitatively compared to common dolphin species/populations distributed worldwide. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were higher in the eastern Subtropical Atlantic as a consequence of the coexistence in the area of the common dolphin short- and long-beaked morphotypes. Individuals from the North-eastern Atlantic displayed lower $\delta^{15}\text{N}$ values, reflecting dissimilarities in diet and of variation in local isotopic baselines. Comparisons with other areas around the world, suggest the species is extremely adaptive and feeds at different trophic levels to adapt to local variations. Stable isotopes are a useful tool to investigate population structure and trophic niche segregation. Trophic behaviour of worldwide populations of common dolphins were fruitfully analysed and revealed substantial differences, likely reflective of both adaptive strategies of the genus and dissimilarities in the structure of the ecosystems.



KEY-WORDS: cetaceans, common dolphins, stable isotopes, Suess effect, trophic ecology, habitat use, population structure, trophic level.

INTRODUCTION

Distinguishing population units of small cetaceans continuously distributed in a widespread area is challenging but is critical for their conservation and management (Barros *et al.*, 2010). The almost continuous distribution, alongside the inherent difficulties involved in the study of cetaceans, *i.e.* animals that spend long periods submerged and feed underwater and that are usually located at great distances from research sites (Walker *et al.*, 1999), makes it hard to study their population structure, habitat use, trophic positions or feeding behavior.

Common dolphins (*Delphinus* spp.) are present in all oceans of the world between latitudes 40° and 60° N to about 50° S except in the western Atlantic, where they appear to be absent from much of its tropical/subtropical waters (Jefferson *et al.*, 2009). Such an extensive geographical distribution has facilitated the differentiation of a number of morphotypes whose taxonomic status remains unclear still today (Heyning & Perrin, 1994). Banks and Brownell (1969) identified in the Pacific Ocean two morphotypes that could be easily differentiated by the relative length of their rostrum, and suggested that the anatomical differences were likely due to dissimilarities in food habits. Thus, the long-beaked morphotype would be mostly of coastal or neritic distribution (Niño-Torres *et al.*, 2006; Perrin, 2009), while that short-beaked would be of both inshore and offshore distribution (Banks & Brownell, 1969; Evans, 1982; Perrin, 2009). Conversely, when the same differentiation was described for the eastern Atlantic Ocean, the long-beaked morphotype revealed a tendency for an offshore/pelagic distribution when compared with the short-beaked morphotype (Pinela *et al.*, 2010; Pinela *et al.*, 2011). Decades later, the taxonomical status of these morphotypes is still matter of debate and it is unclear whether the observed diversification is determined genetically or ecologically, or by both causes (Heyning & Perrin, 1994; Rosel *et al.*, 1994; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Natoli *et al.*, 2006; Pinela *et al.*, 2010; Pinela *et al.*, 2011; Amaral *et al.*, 2012). Natoli and colleagues (2006) proposed that the long-beaked morphotype originated independently in different regions and suggested that adaptation to local environments was driving local speciation. Thus, skull morphology would be the consequence of adaptation to prey capture and habitat use. Nevertheless, no research was ever made comparing habitat use, feeding habits, trophic positions or distribution patterns of *Delphinus* spp. species/populations worldwide.

Stable isotopes constitute a useful tool for investigating diet, habitat use and distribution patterns of animals and in cetaceans they have been widely used to infer population structure (Walker *et al.*, 1999;



Borrel *et al.*, 2006; Witteveen *et al.*, 2009; Barros *et al.*, 2010; Newsome *et al.*, 2010; Ohizumi & Miyazaki, 2010; Fernández *et al.*, 2011; Borrell *et al.*, 2013; Giménez *et al.*, 2013; Quéroil, 2013; Vighi *et al.*, 2014). Stable isotopes of carbon (measured as $\delta^{13}\text{C}$ values) and nitrogen (measured as $\delta^{15}\text{N}$ values) have been used mostly as dietary tracers (Kelly, 2000) departing from the fact that the isotopic composition of these elements present in the tissues of an animal depend on that of its food sources (DeNiro & Epstein, 1978; DeNiro & Epstein, 1981) and therefore offer an integrated measure of the prey assimilated over a comparatively protracted period of time (Michener & Schell, 1994; Hobson, 1999). Additionally, the relative abundance of the heavier isotopes of both elements increases progressively through the food web due to the faster excretion rate of the lighter isotopes (Peterson & Fry, 1987; Cabana & Rasmussen, 1996; Caut *et al.*, 2009). Hence, the $\delta^{15}\text{N}$ value has been found to increase at each trophic level by 2–5 ‰ depending on species, tissue and other factors (Minagawa & Wada, 1984; Caut *et al.*, 2009), and can therefore be used as a reliable indicator of trophic level. Increase of the $\delta^{13}\text{C}$ value is more moderate (estimated at *circa*.1‰ in each trophic level) but, because other factors besides diet may also interfere with the $\delta^{13}\text{C}$ value (*e.g.*, location and ecosystem properties related to plant type and carbon cycling) (Minagawa & Wada, 1984), it is generally not used as an indicator of trophic level (Caut *et al.*, 2009). Thus, the $\delta^{13}\text{C}$ value at the base of the food web is primarily driven by physical parameters (temperature and dissolved CO_2) and the type composition of primary producers, for which reason near-shore and benthic systems are typically more ^{13}C enriched than offshore, pelagic systems (Cardona *et al.*, 2007; McMahon *et al.*, 2013). These $\delta^{13}\text{C}$ geographical differences transferred to animal tissues are a valuable tool to infer marine mammal information, such as their origins and migration patterns (Hobson, 1999).

In this study, we measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the bone of stranded common dolphins from different areas of the North-eastern Atlantic to investigate foraging ecology and potential intra-specific differences that could be associated to cranial morphology with the ultimate objective of defining population units of relevance to management and conservation. Trophic positions were calculated for the species in the different areas, and their isotopic values compared with those from other regions worldwide to investigate trophic behaviour and niche segregation within the genus. We hypothesize that there is a great trophic variability in the common dolphin and predict niche segregation between populations worldwide, most likely reflecting differential use of habitat rather than taxonomy.



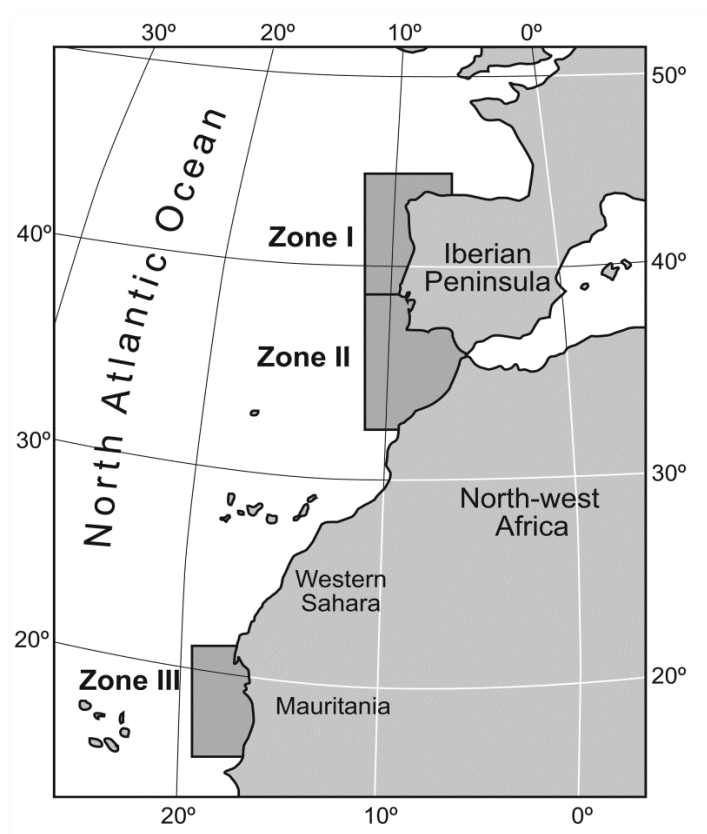
METHODOLOGY

Study site and sampling

Common dolphin's bone remains (skulls), were collected between 1975 and 2007 from the Atlantic coast of the Iberian Peninsula and between 1993 and 2008 from the North-west coast of Africa (Figure 1). For comparative purposes, the study area was divided into three areas based on the characteristics of the habitat (upwelling areas, productivity, fresh water outputs, etc.) and taking into account the natural barriers between the sampling areas. The first area, Zone I ($n=31$), extended over the northern Portuguese and Galician shelves up to Cape Finisterre ($42^{\circ}54'N$ $9^{\circ}16'W$), respectively north and south of the Douro river. The second area, Zone II ($n=38$), included the Portuguese and Andalusian shelves, from Cabo da Roca ($38^{\circ}47'N$ $9^{\circ}30'W$) to the southernmost tip of the Iberian Peninsula ($36^{\circ}0'N$ $5^{\circ}35'W$). Finally, the third area, Zone III ($n=93$), extended along the continental shelf that ranges from the southern fringe of the Western Sahara ($20^{\circ}46'N$ $17^{\circ}3'W$) to the northern coast of Senegal, including Mauritania ($16^{\circ}3'N$ $16^{\circ}30'W$).

Specimens used in this study belonged to Museum Collections held by Aquário Vasco da Gama (Portugal), the Museu Bocage (Portugal), the Parc National du Banc d'Arguin (Mauritania), the Institut Mauritanien de Recherches Océanographiques et des Pêches (Mauritania), the Centre National d'Élevage et de Recherches Vétérinaires (Mauritania), and the University of Barcelona (Spain). Most samples came from stranded animals, but some had been by-caught during fishing or old military-research activities. Specimens were identified as common dolphins on the basis of skull morphology (*e.g.*, the presence of deep palatal grooves) (Heyning & Perrin, 1994). Only adult animals were used in this study in order to avoid variability in stable isotope abundances due to ontogenic development. Physical maturity of the specimens was established as described by Pinela and co-authors (2011).

Bone was the tissue of choice because of its low turnover rate, which reflects the animal's diet throughout several years (Hobson & Clark, 1992). The bone part used for the study was a small fragment of the turbinate bones from the nasal cavity because of its easiness of sampling and because the sampling did not affect the integrity of the skull with regards to posterior morphological studies. Until the moment of the analysis, the samples were stored dry and at ambient temperature (15-25 °C).

**Figure 1**

Map of the study area, with the three study zones indicated by shaded areas (Zone I to III).

Stable isotopes analyses

Stable isotope values (expressed as delta notation, δ), in which the relative variations of stable isotope ratios are expressed in per mil (‰) deviations from predefined international standards, were calculated as (Coplen, 2011):

$$\delta X = [R_{\text{sample}}/R_{\text{standard}}] - 1$$

where X is the value of the heavy isotope of the sample (^{13}C or ^{15}N), R_{sample} is the ratio of the heavy to the light isotope of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$), and R_{standard} is the ratio of the heavy to the light isotope in the reference standards, which were the Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for carbon and the atmospheric nitrogen (air) for nitrogen.

For the pre-treatment of the samples, approximately 1 g of bone tissue was rinsed with distilled water, dried for 3 days at 60°C and powdered with mortar and pestle. Since lipids are depleted for ^{13}C as compared with proteins, and may therefore mislead the analyses by decreasing the $\delta^{13}\text{C}$ signal (DeNiro & Epstein, 1977), they were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution (Bligh & Dyer, 1959). When conducting stable isotope analysis in bone, several authors have carried out preventive demineralisation of the tissue by treating it with either a 0.5 or



1 M hydrochloric acid (HCl) solution (Bocherens *et al.*, 1997; Newsome *et al.*, 2006). However, a test using a subset of the samples revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not differ between demineralised and untreated samples ($\delta^{13}\text{C}$: $t = 0.951$, $df = 19$, $p = 0.353$; $\delta^{15}\text{N}$: $t = 0.527$, $df = 19$, $p = 0.604$; $n = 20$), so this procedure was not followed. The C/N ratio was calculated in order to evaluate if the pre-treatment of bone tissue was effective (Logan *et al.*, 2008) and to assure that isotope values obtained were in the expected range for pure bone collagen protein (DeNiro, 1985; Ambrose, 1990).

After pre-treatment, approximately 1 mg of the treated bone was weighed in tin capsules (3.3 x 5 mm) and combusted at 900 °C. Isotope analyses were carried out by means of elemental analysis-isotope ratio mass spectrometry using a Thermo Finnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser, coupled to a Delta C isotope ratio mass spectrometer via a CONFLOIII interface (Thermo Finnigan MAT, Bremen, Germany). Atropine was used as a standard for elemental composition of carbon and nitrogen. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the IAEA (International Atomic Energy Agency), namely polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$) and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios, namely $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$) and KNO_3 (IAEA NO₃, $\delta^{15}\text{N} = +4.7\text{‰}$), were used for calibration at a precision of 0.3‰. Analyses were conducted in the Centres Científics i Tecnològics of the University of Barcelona (CCiT-UB).

Trophic level determinations

$\delta^{15}\text{N}$ value provides an index for trophic position relative to the known trophic level of a primary producer, or of an organism which trophic position in the local marine ecosystem is well-known ($\delta^{15}\text{N}_{\text{base}}$ value; *i.e.*, the ecosystem baselines) (Cabana & Rasmussen, 1996). In our study, the $\delta^{15}\text{N}_{\text{base}}$ value was estimated from published isotopic values of local widespread fish prey for each of the studied zones (different prey species were used as baseline - see Table 1) whose trophic level (TL) was previously known through gut contents as reported in FishBase (Froese & Pauly, 2000) (<http://www.fishbase.org>). The trophic enrichment factor applied, specific for bone tissue ($\Delta_n = 2.03 \text{‰}$), has recently been reported in a cetacean species (fin whale, *Balaenoptera physalus*) by Borrell and colleagues (2012), and hence assumed for trophic estimations for the common dolphins from the study areas (Zone I to III).

For the consumer species, the trophic level (TL) was calculated as:

$$\text{TL} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}}) / \Delta_n$$



where λ is the trophic level of the organism used to estimate $\delta^{15}\text{N}_{\text{base}}$, Δ_n is the bone $\delta^{15}\text{N}$ fractionation value, and $\delta^{15}\text{N}_{\text{consumer}}$ is the direct measurement of $\delta^{15}\text{N}$ value for the target species (Post, 2002), *i.e.*, the common dolphin.

Table 1 Published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values, number of individuals (N), and trophic level (TI) for the species used as ecosystem baseline to determine the trophic position of common dolphins in each study area

Area	BASELINE					Reference
	Species	N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	TI ^a	
Zone I	<i>Sardina pilchardus</i> [‡]	447	-17.0	10.7	3.1	Bode <i>et al.</i> , 2007
Zone II	<i>Sardinella pilchardus</i>	5	-18.1	10.6	3.1	L. Cardona., unpublished
Zone III	<i>Sardinella aurita</i> [†]	10	-15.0	10.4	3.4	Pinela <i>et al.</i> , 2010

Common names of the baseline species: European pilchard ([‡]), Round sardinella ([†])

(^a): FishBase (Froese & Pauly, 2000).

Data analyses

Previously to data analyses, raw $\delta^{13}\text{C}$ data was adjusted to take into account the Suess effect according to year and location of sample. The Suess effect describes the ^{13}C atmospheric depletion through time, due to burning of fossil fuels since the onset of the Industrial Revolution (Schell, 2001; Körtzinger *et al.*, 2003). Körtzinger and co-authors (2003), calculated that $\delta^{13}\text{C}$ values in oceanic dissolved inorganic carbon (DIC) in the North Atlantic Ocean had decreased by a factor of $0.026 \pm 0.002\text{‰}$ per year. So, in order to minimise the Suess effect when comparing groups of samples from different periods, raw $\delta^{13}\text{C}$ data was corrected adding 0.026‰ per year, since the first year of sampling and over a 33 years' time period. Since all areas investigated in the present study are geographically situated in the North Atlantic, we used the same $\delta^{13}\text{C}$ increasing factor for the three study areas.

The assumption of normality in the distributions of the sample groups was checked with the Kolmogorov-Smirnov test of goodness of fit, and the homogeneity of variances between them was checked with the Levene's test. The variation of the results and the presence of outliers were tested graphically using boxplots, depicting the first, second (the median) and third quartiles, as well as the whiskers.



Differences in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of common dolphins between sampling years were investigated through an analysis of variance (ANOVA), for each area separately.

Differences in mean bivariate isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of common dolphins between areas were also assessed through a multivariate analysis of variance (MANOVA) because they followed a normal distribution. Univariate analysis of variance (ANOVA) was conducted separately for each isotope. If a statistical significant interaction was found among areas for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, additional pairwise comparisons were made with a generalized linear model because it admits data with non-homogeneous variances. *Post-hoc* analyses were made with least significant differences tests (LSD) based on estimated marginal means. As a method of estimation, the maximum likelihood (ML) was used in all cases. Normality distribution and identity as a link function was always used (for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values), since data followed a normal distribution. Goodness of fit (Akaike's information criterion) was used to compare different models. The confidence interval level was set at 95% for all comparisons.

The isotopic values determined in the bone of the common dolphins from the areas here studied were compared with those from other populations of common dolphins from other regions. Other tissues besides bone were considered, but tissue isotopic values were converted to those of bone by means of fractionation factors in order to avoid variability among tissues in the comparison. Trophic positions could only be determined in a qualitative manner for those worldwide populations.

Unless stated, data are presented as mean \pm standard deviation (SD) throughout the manuscript. All the statistical analyses were conducted using the SPSS ver.15 software (SPSS Inc., Chicago III).

RESULTS

The C/N ratio for all samples varied between 2.96 and 3.39 (Zone I: 3.01-3.27; Zone II: 3.12-3.39; Zone III: 2.96-3.34). These values show: 1) that the lipid extraction process in the bone samples was effective and 2) that the samples were well-preserved and the bone collagen integrity was guaranteed (DeNiro, 1985; Ambrose, 1990).

Two individuals were excluded from the dataset because they were significant outliers (Figure 2). In all common dolphin populations studied, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values followed a normal distribution ($p > 0.771$ for all areas) and were heteroscedastic ($\delta^{13}\text{C}$: $p < 0.001$, $\delta^{15}\text{N}$: $p < 0.001$).

Analysis of variance indicated that there were no significant differences between sampling years for both isotopes in Zone I ($n=28$, $\delta^{13}\text{C}$: $p=0.199$, $\delta^{15}\text{N}$: $p=0.066$), Zone II ($n=36$, $\delta^{13}\text{C}$: $p=0.331$, $\delta^{15}\text{N}$: $p=0.104$), and Zone III ($n=87$, $\delta^{13}\text{C}$: $p=0.157$, $\delta^{15}\text{N}$: $p=0.140$). Therefore, we can assume that the trophic habits of this



species haven't changed across the years and potential differences in stable isotope values are most likely due to dissimilarities between areas rather than the sampling period.

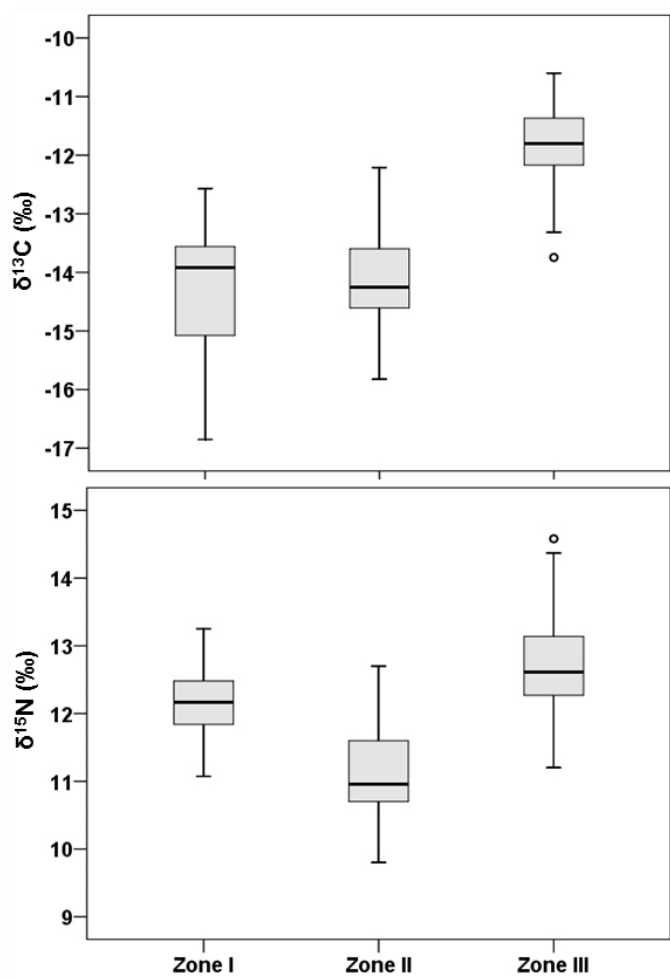
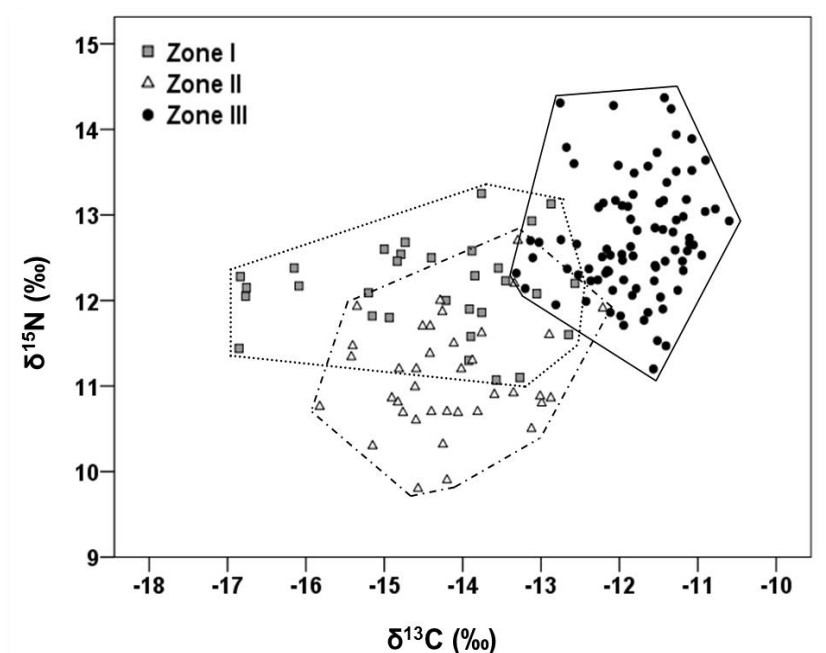


Figure 2

Boxplots illustrating the distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained from the isotopic analysis of common dolphin's bone samples from Zone I to III.

Stable isotope variability in Areas I-III

Individual bone $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the common dolphins gathered by study area are shown in Figure 3, where lines were plotted around each group to aid in visualization. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the bone of common dolphins collected from each area and other isotopic related information are presented in Table 2. Figure 2 displays a boxplot depicting an intergroup comparison between the areas analysed for each isotope, allowing the visualization of the tendency across common dolphin populations of the North-eastern and eastern Subtropical Atlantic. Maximum and minimum isotopic values varied greatly across populations: Zone I displayed the lowest $\delta^{13}\text{C}$ value (-16.85 ‰), and Zone III the highest (-10.60 ‰); while Zone II presented the lowest $\delta^{15}\text{N}$ value (9.80 ‰) and Zone III the highest (14.37 ‰).

**Figure 3**

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of the common dolphin individuals sampled in Zone I to III (see graph legend for details). Lines were plotted around each group to aid in visualization.

Multivariate analysis of variance indicated significant differences between areas for both isotopes (Wilk's $\lambda=0.226$, $F=82.192$, $p<0.001$). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values contributed to the differences between common dolphin populations ($\delta^{13}\text{C}$: $p<0.001$, $\delta^{15}\text{N}$: $p<0.001$, Table 3). The results of the *post-hoc* test for multiple comparisons between the three areas, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, show that all pairwise comparisons were highly significant ($p<0.0001$); except for dolphins from Zone I and II, which did not show differences in $\delta^{13}\text{C}$ isotope signatures ($p=0.401$). Results indicated that common dolphins from Zone III had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bone than those from animals belonging to other areas, and that $\delta^{15}\text{N}$ values in the bone of common dolphins from Zone II were significantly lower than those from Zone I (Table 2).

Trophic position of common dolphin's in Areas I-III

Table 2 presents the trophic levels (TL) calculated for each population of common dolphin taking into account the baseline isotopic values of the local marine ecosystems. The trophic position of dolphins sampled in this study were highly variable, showing a difference larger than one trophic level between Zone II (3.38 ± 0.23) and Zone III (4.52 ± 0.27). Dolphins from the northern area investigated (Zone I) displayed an intermediate trophic position (3.81 ± 0.26 , Table 2), in comparison to the other areas.



Table 2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values (mean \pm SD) for common dolphin, *Delphinus* spp., samples collected along the study area and for populations worldwide. Additional data is also presented, such as: the animals provenance (Area), common dolphin's species, number of individuals (n), sex of the individuals, the tissue used for the isotopic analyses and if the lipids were extracted or not from those tissues, common dolphin's trophic level (TL), and the reference whenever applicable.

Area	Code	Species	N	Sex	Tissue	Lipids	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{15}\text{N} \pm \text{SD}$	TL	Reference
Mediterranean Sea										
Southwestern Med.	SWM	<i>Delphinus delphis</i>	2	M	M	No	-17.8 ± 0.04	10.5 ± 0.4	ND	Borrell & Aguilar, 2005
NE Atlantic Ocean										
Zone I	ZI	<i>Delphinus delphis</i>	31	M+F	B	Yes	-14.44 ± 1.3	12.14 ± 0.5	3.8	Present study
Zone II	ZII	<i>Delphinus delphis</i>	38	M+F	B	Yes	-14.16 ± 0.8	11.12 ± 0.6	3.4	Present study
Zone III	ZIII	<i>Delphinus</i> spp.	86	M+F	B	Yes	-11.81 ± 0.6	12.72 ± 0.7	4.5	Present study
Galicia	GAL	<i>Delphinus delphis</i>	5	M+F	M	No	-17.0 ± 0.4	13.1 ± 0.8	4.2	Bode <i>et al.</i> , 2007
Bay of Biscay	BB	<i>Delphinus delphis</i>	10	M+F	M	No	-18.4 ± 0.5	10.3 ± 0.3	ND	Das <i>et al.</i> , 2000
French Channel coast	FCC	<i>Delphinus delphis</i>	8	ND	M	No	-16.5 ± 0.5	12.1 ± 0.4	ND	Das <i>et al.</i> , 2003
Irish coast	IRC	<i>Delphinus delphis</i>	14	ND	M	No	-17.1 ± 0.4	12.2 ± 1.0	ND	Das <i>et al.</i> , 2003
Azores Islands	AZO	<i>Delphinus delphis</i>	55	M+F	S	Yes	-18.1 ± 0.5	10.4 ± 0.6	ND	Qu��rouil <i>et al.</i> , 2013
Madeira Islands	MAD	<i>Delphinus delphis</i>	28	M+F	S	Yes	-17.8 ± 0.3	10.1 ± 0.3	ND	Qu��rouil <i>et al.</i> , 2013
NE Pacific Ocean										
Gulf of California	GC	<i>Delphinus capensis</i>	16	M+F	T	Yes	-13.2 ± 1.5	18.1 ± 1.9	3.8-4.1	Ni��o-Torres <i>et al.</i> , 2006
SE Pacific Ocean										
Chile	CHI	<i>Delphinus delphis</i>	15	M	T	Yes	-14.5 ± 0.3	12.4 ± 0.8	ND	Walker & Macko, 1999
SW Pacific Ocean										
Southeastern Australia	SEA	<i>Delphinus delphis</i>	3	ND	M	Yes	-19.3 ± 0.8	13.3 ± 2.1	ND	Davenport & Bax, 2002

Sex: M = male, F = female; ND = not determined; Tissue type: M = muscle, B = bone, S = skin, T = teeth.



Table 3 Summary of the results of the ANOVA to test for differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values in the bone of common dolphins from the three zones analysed (Zone I to Zone III)

	SS	df	MS	F	p	r^2
$\delta^{13}\text{C}$						
Model	236.00	2	118.22	170.74	<0.001	0.692
Intersect	23267.27	1	23267.27	33603.57	<0.001	
Zone	236.45	2	118.22	170.74	<0.001	
Error	105.25	152	0.69			
Total	26188.47	155				
$\delta^{15}\text{N}$						
Model	68.12	2	34.06	83.44	<0.001	0.523
Intersect	18446.19	1	18446.19	46154.35	<0.001	
Zone	68.12	2	34.06	83.44	<0.001	
Error	62.05	152	0.41			
Total	23253.84	155				

Stable isotope variability of common dolphin's worldwide

Stable isotope values of common dolphins from other areas were extracted from the literature (Table 2). Most studies ($n=7$) focused on the short-beaked common dolphin (*Delphinus delphis*) while only one focused on the long beaked species (*Delphinus capensis*) (Table 2). When all tissues are considered, values ranged from -19.3‰ to -12.9‰ for $\delta^{13}\text{C}$ and from 10.1‰ to 18.1‰ for $\delta^{15}\text{N}$ (Table 2). However, $\delta^{13}\text{C}$ values are clearly lower and $\delta^{15}\text{N}$ values higher in soft tissues (muscle and skin) than in hard tissues (teeth and bone) (Borrell *et al.*, 2012), because tissue-specific values depend on the physiology and composition of the tissue (Hobson & Clark, 1992). In figure 4, data from muscle and skin were converted to bone by means of fractionation factors (see Borrell and colleagues (2012), $\delta^{13}\text{C}_{\text{bone}} = \delta^{13}\text{C}_{\text{muscle}} + 1.83\text{‰}$, and $\delta^{15}\text{N}_{\text{bone}} = \delta^{15}\text{N}_{\text{muscle}} - 0.7\text{‰}$; $\delta^{13}\text{C}_{\text{bone}} = \delta^{13}\text{C}_{\text{skin}} + 1.83\text{‰}$ and $\delta^{15}\text{N}_{\text{bone}} = \delta^{15}\text{N}_{\text{skin}} - 0.8\text{‰}$). Figure 4 displays the converted isotopic values for common dolphins from all the areas. Dolphins from SWM, GAL, BB, FCC, and IRC tended to show lower $\delta^{13}\text{C}$ values than those investigated in the current study, a difference likely due to the fact that in these studies lipids were not extracted from the tissue. Figure 4 suggests that some populations have a more coastal/inshore distribution (GC and ZIII), while others occupy a more pelagic/offshore environment (SEA, AZO, MAD). When considering nitrogen isotope values, the GC population segregates



from the other populations, while the populations with lowest values appear to have a more pelagic distribution (AZO, BB, and MAD).

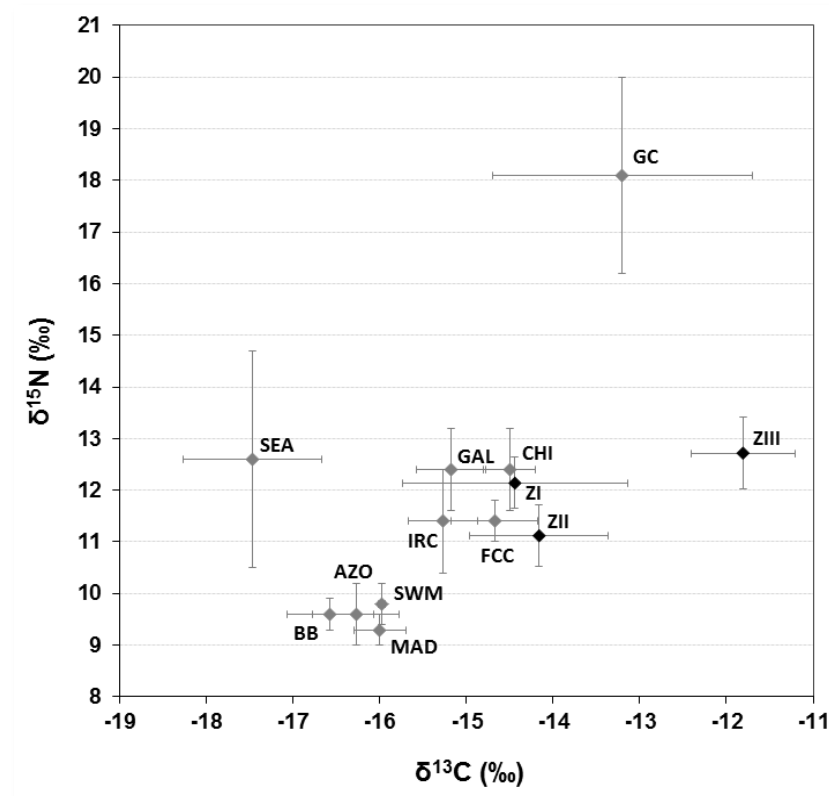


Figure 4

Comparison of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bone of common dolphins from the study area (Zone I to III, in black) and populations worldwide (in grey). Codes: SWM (South-western Mediterranean), GAL (Galicia), BB (Bay of Biscay), FCC (French Channel coast), IRC (Irish coast), AZO (Azores Islands), MAD (Madeira Islands), GC (Gulf of California), CHI (Chile), SEA (South-eastern Australia).

DISCUSSION

Marine systems are usually devoid of physical barriers, allowing top predators to be transient and to range beyond conventionally-defined ecosystem boundaries (Vander Zanden & Fetzer, 2007). The large variability found within populations suggests substantial individual-specific dissimilarities in the exploitation of food resources and, most likely, habitat use.

In our study, the variability of $\delta^{15}\text{N}$ values was particularly high in common dolphins from the north-west coast of Africa, a finding probably related to the presence of both the long-beaked and short-beaked morphotypes in this area as opposed to the northern areas, where only the short-beaked morphotype is present. Variation in rostral morphology of common dolphins, and consequently in their feeding apparatus, is associated to dissimilarities in feeding ecology and diet (Evans, 1982; Murphy *et al.*, 2006) rather than to speciation (Natoli *et al.*, 2006; Pinela *et al.*, 2011), and this would have obvious consequences on the stable isotope values.



Environmental differences in temperature, light intensity, nutrient concentrations, and species composition can cause baseline changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of primary producers (Walker *et al.*, 1999) which cascade up food chains to reach primary and secondary consumers (Michener & Schell, 1994; Burton & Koch, 1999; Kelly, 2000). Therefore, populations from different geographic locations, even when they have similar diets, can display contrasting isotopic signatures that can be used to assess habitat or resource use (Das *et al.*, 2003).

Moreover, $\delta^{15}\text{N}$ values of higher consumers relative to a previously established baseline can provide time-integrated depictions of trophic structure and are a more comprehensive technique than traditional stomach content analyses, which only offer a snapshot of animal's diet (Post, 2002; Vander Zanden & Fetzer, 2007). Although in species feeding on a wide variety of prey species diet composition is difficult to establish only through stable isotope analysis (Borrell *et al.*, 2012), inference of the trophic level at which they feed is possible by comparing ^{15}N step-wise enrichment values (Kurle & Worthy, 2001; Post, 2002).

Previously, trophic levels (TL) of common dolphins have only been determined through stomach content analysis in a couple of local studies (Table 2). Here we assess TL in several populations of common dolphins by comparing the isotopic values determined in bone of dolphins with those of a basal local prey of previously known TL (Table 1). The TLs thus obtained are consistent with those generally determined for the genus from data on stomach contents and behavioral data (Pauly *et al.*, 1998; Bode *et al.*, 2003; Niño-Torres *et al.*, 2006). Although results situated common dolphins between the third and the fifth TLs in most ecosystems (Table 2), which would imply preying on secondary consumers like sardines (Pauly *et al.*, 1998; Bode *et al.*, 2003), their position was not much higher than that of sardines, especially for the population inhabiting the southern Atlantic coast of the Iberian Peninsula. This suggests the existence of a relatively large degree of omnivory in these top predators globally.

$\delta^{13}\text{C}$ values were significantly higher in dolphins from north-west Africa than in those inhabiting the Atlantic waters around the Iberian Peninsula (Figure 4). This would in principle suggest a more in-shore distribution of the former population as opposed to the latter because $\delta^{13}\text{C}$ values of coastal primary producers (macroalgae and sea grasses) are more elevated than those of the offshore primary producers (phytoplankton) and the difference is transferred through the food web (Peterson & Fry, 1987; Vander Zanden & Rasmussen, 1999). However, in north-west Africa, *Delphinus* spp. is seldom observed inshore but rather displays a typical oceanic behaviour (Robineau & Vely, 1998; Pinela *et al.*, 2010). Besides this, other organisms inhabiting inshore waters of Mauritania display even higher $\delta^{13}\text{C}$ values than those of common dolphin's bone (Pinela *et al.*, 2010), for which reason such elevated values are considered to be a characteristic of the general ecosystem rather than a peculiarity of common dolphins. Although a



difference of this nature is not depicted in the zooplankton $\delta^{13}\text{C}$ isoscapes proposed by McMahon *et al.* for the overall Atlantic Ocean (McMahon *et al.*, 2013), a possible explanation for such high $\delta^{13}\text{C}$ values may be that the elevated temperatures and the high productivity prevailing off the north-west African coast, which is one of the largest upwelling ecosystems in the world, would lead to high $\delta^{13}\text{C}$ values as it occurs with the California current (Graham *et al.*, 2010).

$\delta^{15}\text{N}$ values were generally high, consistent with the progressive enrichment of the heavier stable isotope through food webs (Minagawa & Wada, 1984), and the fact that dolphins are top predators (Bowen, 1997). However, and similarly to $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values were significantly higher in dolphins from Mauritania than in those from the Atlantic waters around the Iberian Peninsula and once more the difference is attributed to differences in $\delta^{15}\text{N}$ baseline although this is again not apparent in the zooplankton isoscapes proposed by McMahon and colleagues (2013) for the overall Atlantic Ocean. The previous authors show that the nitrogen isotope values of zooplankton in the Mauritanian coast are approximately the same or slightly lower than those around the Iberian Peninsula; and despite that, the trophic level of sardines (used as baseline) is slightly higher in the African coast. We suggest that these differences could be associated with the feeding behaviour of the dolphins and their main prey items which would then have repercussions up the food chain.

In the Iberian Peninsula, $\delta^{13}\text{C}$ values showed no differences between Zone I and Zone II; while $\delta^{15}\text{N}$ values were lower in Zone II than Zone I (Figure 2), likely reflecting differences in diet and/or of variation in baseline (Ostrom *et al.*, 1997; Post, 2002; Newsome *et al.*, 2010), and therefore suggesting segregation.

When the above comparisons are extended to other areas not directly covered in the present study, but whose stable isotope values were transformed for comparison purposes (Figure 4), values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were extremely variable, suggesting feeding at different trophic levels and a large plasticity to adapt to local variations. The long-beaked common dolphin from the Gulf of California (GC) showed the highest $\delta^{15}\text{N}$ values followed by the north-west African population (ZIII) due probably to the presence of the long-beaked morphotype, which is present in both areas. Consistently, previous studies have shown that individuals with longer beaks tend to feed at higher trophic levels than animals with shorter beaks (Pinela *et al.*, 2010; Pinela *et al.*, 2011). The short-beaked common dolphins from Areas ZI+ZII+GAL showed the third highest $\delta^{15}\text{N}$ values of the populations analyzed, which are consistent with the published TL for the coast of Galicia.



CONCLUSIONS

Results substantiate isotopes as a useful tool to investigate population structure and segregation between demographic subunits. Trophic positions were successfully determined for the common dolphin populations of the North-eastern and eastern Subtropical Atlantic, accounting for the local marine ecosystem baseline. Trophic behaviour of several populations of common dolphins worldwide were fruitfully analysed and revealed substantial differences that are likely reflective of both adaptive strategies of the genus and dissimilarities in the structure of the ecosystems in which they live.

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CHAPTER 3



RESUMEN

En el amplio rango de distribución de los delfines comunes se han establecido varios morfotipos de adscripción taxonómica incierta, identificados por la longitud relativa de su morro. Hemos investigado la variación morfométrica del cráneo y las señales isotópicas de carbono y nitrógeno ($\delta^{13}\text{C}$ y $\delta^{15}\text{N}$) en individuos de aguas subtropicales del Océano Atlántico Norte, a fin de evaluar la población y la estructuración taxonómica. La distribución de la longitud relativa del morro de los individuos siguió una clina sin sub-agrupación. Tanto $\delta^{13}\text{C}$ como $\delta^{15}\text{N}$ mostraron gran variabilidad, lo que sugiere que los individuos utilizan el hábitat de forma heterogénea. $\delta^{15}\text{N}$ se ha correlacionado con la longitud del morro, lo que indica que los individuos de morro más largo se alimentan en un nivel trófico superior y/o habitan aguas situadas mar adentro por comparación con los de morro corto. A pesar de la correlación entre $\delta^{13}\text{C}$ y $\delta^{15}\text{N}$, la longitud del morro y $\delta^{13}\text{C}$ no mostraron ninguna correlación posiblemente porque el efecto incremental de nivel trófico de $\delta^{13}\text{C}$ se ha visto compensado por el potencial de distribución alopátrica de los morfotipos. Llegamos a la conclusión de que tanto las formas de delfín común de morro largo y de morro corto existen en Mauritania, pero, al contrario de otras áreas, la existencia de más de una especie en la región está en duda, ya que datos morfométricos del cráneo e isótopos estables parecen reflejar un uso diferencial de hábitat en lugar de taxonomía. Aunque ha sido propuesto por algunos autores, esta es la primera vez que se verificó en los delfines comunes que la diferenciación del cráneo podría estar relacionada con la segregación de nicho y no con la especiación. Esto revela que es necesario tener precaución al considerar que delfines comunes de morro largo y de morro corto de fuera del este del Pacífico Norte caen en el modelo taxonómico descrito para esa región.

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3

Common dolphin morphotypes: niche segregation or taxonomy?

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ABSTRACT

In the extensive distribution range of the common dolphin several morphotypes of uncertain taxonomic adscription, identified by the relative length of their beak, have been established. We investigated variation in skull morphometric and isotopic signatures of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in individuals from the subtropical waters of the North Atlantic Ocean, in order to assess population and taxonomic structuring. The distribution of the relative rostral length of individuals followed a cline with no sub-grouping. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ showed large variability, which suggests that individuals use the habitat heterogeneously. $\delta^{15}\text{N}$ correlated with rostrum length, indicating that longer-beaked individuals either feed at a higher trophic level and/or inhabit waters located further offshore than those shorter-beaked. Although $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were correlated, rostrum length and $\delta^{13}\text{C}$ failed to show any correlation possibly because the incremental effect of trophic level on $\delta^{13}\text{C}$ has been offset by the potential allopatric distribution of the morphotypes. We conclude that both the long-beaked and short-beaked forms of common dolphin do occur off Mauritania but, contrarily to other areas, the existence of more than one species in the region is questioned since both stable isotopes and skull morphometric appear to reflect differential use of habitat rather than taxonomy. Even though it has been proposed by some authors, this is the first time it was verified in common dolphins that skull differentiation might be related to niche segregation and not to speciation. This reveals that caution is needed when considering that long-beaked and short-beaked common dolphins from outside the eastern North Pacific fall into the taxonomic model described for that region.



KEY-WORDS: Common dolphins, skull morphometric, stable isotopes, habitat use, taxonomy.

INTRODUCTION

Common dolphins (*Delphinus spp.*) are present in all oceans of the world between latitudes 40-60 N to about 50 S with the only exception of the western Atlantic, where they are absent in tropical waters (Jefferson *et al.*, 2008, 2009). Such an extensive distribution range has facilitated the differentiation of a number of morphotypes that until today remain of unclear taxonomic adscription (White, 1999). In many areas, but not in all, two morphotypes have been distinguished: one long-beaked, mostly of coastal or neritic distribution, and one short-beaked, that occupies indistinctly inshore and offshore waters (Banks & Brownell, 1969; Evans, 1982; Perrin, 2009). Banks and Brownell (1969) differentiated the two forms through their ratio of zygomatic width to rostrum length, and suggested that the anatomical difference was likely due to dissimilarities in food habits. Later, the anatomical differentiation of the morphotypes was confirmed through additional multivariate analysis in skull measurements (Evans, 1982). In the Eastern North Pacific, Heyning and Perrin (1994) and Rosel *et al.* (1994), through morphological and genetic analysis, respectively, distinguished the two morphotypes and split the genus into two species: the short-beaked common dolphin *Delphinus delphis* (Linnaeus, 1758) and the long-beaked common dolphin *Delphinus capensis* (Gray, 1828). Currently, the genus *Delphinus* comprises two species and four subspecies: the short-beaked common dolphin *Delphinus delphis delphis*, distributed in continental shelf and pelagic waters of the Atlantic and Pacific Oceans; the Black Sea short-beaked common dolphin, *D. delphis ponticus*; Gray's common dolphin (long-beaked form), *D. capensis capensis*, distributed in nearshore tropical and temperate waters of the Pacific and South Atlantic Oceans; and the Indian long-beaked common dolphin, *D. capensis tropicalis*, which occurs in the Indian Ocean (IWC, 2009).

The two most common morphotypes (short and long-beaked) also occur off the Atlantic coast of Africa. The range of the short-beaked form is believed to extend as far south as Senegal (Heyning & Perrin, 1994; Van Waerebeek, 1997; Rice, 1998), while the long-beaked is present from the waters off Mauritania to those off South Africa (Amaha, 1994; Van Waerebeek, 1997; Rice, 1998; Samaai *et al.*, 2005). Thus, the waters off Mauritania are apparently a border region in the distribution of the two forms and indeed both morphotypes occur sympatrically in this area, as it is the case in California (Heyning & Perrin, 1994).

However, a genetic study conducted by Natoli *et al.* (2006), based on the analysis of the mtDNA D-loop region of a small number of samples, concluded that the Mauritanian population was highly differentiated from all other populations, including the long-beaked population from South Africa; and challenged the currently accepted hypothesis of a single long-beaked lineage worldwide. More locally, it raised questions about the identity of the *Delphinus* population off NW Africa. Nevertheless, Natoli *et al.*



(2006) based their study only on the genetics of six specimens from Mauritanian, thus leaving some uncertainty on the conclusiveness of their findings and about the actual structure of the population. Additionally, that study proposed that the long-beaked form originated independently in different regions, suggesting that adaptation to local environments would be driving local speciation. Therefore, skull morphology will reflect adaptation to prey capture and the habitat used by the animals. One of the most useful tools to investigate diet, as well as the habitat use, is stable isotopes.

Stable isotope analyses are based on the assumption that the isotopic composition of an animal depends on that of its food sources (DeNiro & Epstein, 1978, 1981), and offers an integrated measure of all preys assimilated over a comparatively protracted period of time (Michener & Schell, 1994; Hobson, 1999). Additionally, it assumes that the relative abundance of the heavier isotopes of some elements increases in each trophic level thus rising progressively through the food web (Peterson & Fry, 1987; Cabana & Rasmussen, 1996; Caut *et al.*, 2009); increasing its abundance from prey to predator due to the preferential excretion of the light isotope (Caut *et al.*, 2009). The nitrogen isotope $\delta^{15}\text{N}$ is enriched in each trophic level by 3 to 5 ‰ (Minagawa & Wada, 1984) and has been successfully used as an indicative of diet and trophic level. Whereas $\delta^{13}\text{C}$ is usually also enriched by each trophic level by ca. 1‰. However, few studies have used it as indicative of trophic level, since such a small enrichment may be misinterpreted and overlapped with other explanatory factors. In addition, $\delta^{13}\text{C}$ changes along ecological gradients as different plants discriminate it to a different extent against $\delta^{12}\text{C}$, and it has been most commonly used as an indicator of habitat use, *i.e.* to differentiate between pelagic versus benthic and offshore versus near shore origins (Pinela *et al.*, 2010).

In this paper we report the results of a study on the population of common dolphins from Mauritania using two independent tools: the relative abundance of nitrogen and carbon stable isotopes, and the morphometric of the skull. We conclude that, consistently with Natoli *et al.* (2006), taxonomic splitting appears not to be justified in the region.

MATERIAL AND METHODS

Sampling

Samples were collected between 1990 and 2008 along the ca700 km of Mauritanian coastline that extends from Cape Blanc Peninsula to the Senegal River, north-western Africa (Figure 1). The study site was divided in three areas: area 1, from the north to the south tip of Cape Blanc Peninsula, over passing some kilometres of the Western Sahara border; area 2, comprises the Parc National du Banc d'Arguin; and area 3, from Cape Timirist (the southern border of the Parc National du Banc d'Arguin) to the Senegal River (border line between Mauritania and Senegal).

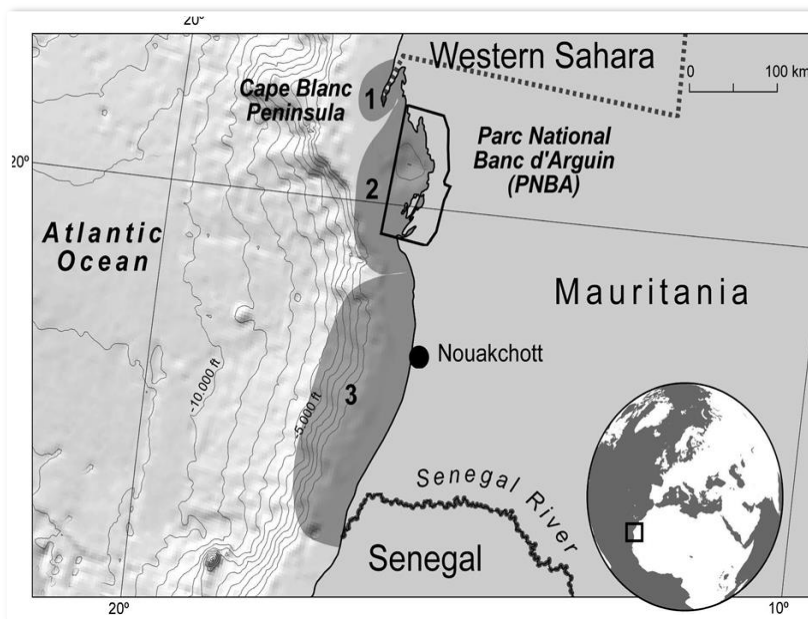


Figure 1

Map of the study site with the bathymetry shape (in feet), the over line of the continental shelf, and the three main areas of the Mauritanian coast indicated by dark grey areas with numbers (from 1 to 3).

The beaches were surveyed opportunistically or in dedicated expeditions and, when the remains of a dead dolphin were found, the skull was preserved. Sex, body length, geographical position and any indication of the cause of death were recorded whenever possible; however, most specimens were found in an advanced state of decomposition or as bone remains, so detailed biological information is lacking for large part of the sample. In total 72 specimens were examined and a sample of pterygoid bone was collected from all individuals for stable isotope analyses. The pterygoid bone was used because it was a structure present in all skulls, easy to break, and did not damage the skull for the morphometric analysis.

Stable isotopes analyses

Stable isotope abundances (expressed as delta notation, δ), in which the relative variations of stable isotope ratios of carbon and nitrogen are expressed in per mil (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where X is the value of the heavy isotope of the sample (^{13}C or ^{15}N), R_{sample} is the ratio of the heavy to the light isotope of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$), and R_{standard} is the ratio of the heavy to the light isotope in the reference standards, which were the Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for carbon and the atmospheric nitrogen (air) for nitrogen.



For the pre-treatment of the samples, approximately one gram of bone was rinsed with distilled water, dried for 3 days at 60°C and powdered with mortar and pestle. Because lipids are depleted for ^{13}C as compared with other molecules, (DeNiro & Epstein, 1977), they were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution (Bligh & Dyer, 1959). Bone was the selected tissue because, owing to its low turnover rate, it is possible to elucidate several years of dietary history (Hobson & Clark, 1992). When conducting stable isotope analysis in bone, several authors have carried out preventive demineralization of the tissue by treating it with a 0.5 and 1.0 M hydrochloric acid (HCl) solution (Bocherens *et al.*, 1997; Newsome *et al.*, 2006). However, a test using a subset of the samples ($n=20$) revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not change between demineralised and untreated samples, so this procedure was not followed. Approximately 1 mg of lipid-free bone was weighed in tin capsules (3.3×5 mm), combusted at 900 °C and analysed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan, Thermo Finnigan, Bremen, Germany). Atropine was used as a standard to check the elemental composition for carbon and nitrogen. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the IAEA (International Atomic Energy Agency), namely polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$) and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios, namely $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$) and KNO_3 (IAEA NO₃, $\delta^{15}\text{N} = +4.7\text{‰}$), were used for calibration at a precision of 0.3‰.

Morphometric measurements

Measurements were obtained from 72 common dolphin (*Delphinus* sp.) skulls and mandibles from the Mauritanian coast housed at the Headquarters of the Parc National du Banc d'Arguin (PNBA), the Institut Mauritanien de Recherches Océanographiques et des Pêches (IMROP), and the Centre National d'Élevage et de Recherches Vétérinaires (CNERV). Specimens, either deposited in scientific collections or found during beach surveys, were identified as common dolphins on the basis of skull morphology (*e.g.*, the presence of deep palatal grooves; Evans, 1994; Heyning & Perrin, 1994).

Because skull proportions change allometrically with growth, we selected from Heyning and Perrin (1994) the morphometric measurements that had been previously identified as being of taxonomical relevance or that indicate maturity status. These included the rostrum length/greatest zygomatic width ratio (RL/ZW, Figure 2) and the upper left tooth count (UTC (left), which have been reported as the most indicative measurements to discriminate between short-beaked and long-beaked forms of common dolphins (Banks & Brownell, 1969; Van Bree & Gallagher, 1978; Amaha, 1994; Evans, 1994; Heyning & Perrin, 1994; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Westgate, 2007;



Tavares *et al.*, 2010). All measurements were made by using metal vernier callipers (0.1 mm accuracy), and in order to test measurement accuracy repetitions of measurements were made on several specimens until a level of precision of ± 0.5 mm was obtained (Bell *et al.*, 2002). Additionally, the overall skull quality, overall degree of cranial fusion, and the degree of fusion of the pre-maxilla and maxilla were also annotated (Westgate, 2007). Skulls that were incomplete or sufficiently damaged to impede reliable measurement were discarded ($n=6$).

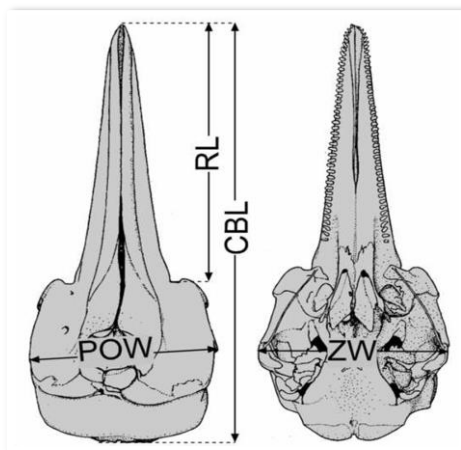


Figure 2

Common dolphin skull morphometric measurements: condylobasal length (CBL), rostral length (RL), skull post-orbital width (POW), and zygomatic width (ZW).

The final sample only included skulls that were considered to have completed their growth. Physical maturity of the specimens was accessed by examining the degree of fusion between the pre-maxillary and maxillary bones at the distal tip of the rostrum. However, Perrin and Heyning (1993) reported that this method could bring some error in the assessment of physical maturity, so additionally the condylobasal length (CBL, Figure 2), degree of fusion of the alveoli, and the degree of overall cranial fusion were used as supplementary indicators of physical maturity, due to the lack of data on the age and total body length of the specimens (Westgate, 2007). Correlations between the skull length (condylobasal length minus the rostral length, *i.e.*, CBL-RL), skull post-orbital width (POW) and rostral length (RL) were investigated to discard any potential influence of skull allometric growth on the relationship between skull morphometric and isotopic signatures (Figure 2). Nine individuals were discarded from the initial data set because their skulls did not present closed sutures, closed alveoli, and distal fusion between the pre-maxillae and maxillae at the tip of the rostrum, and thus were considered physically immature. Sexual identification was also not available for most of the specimens, and since the main objective is to detect differences at the taxonomical level we treated the dataset as a whole. Moreover, despite that on average, male common dolphins had significantly larger skulls in North Atlantic and Pacific Oceans (Heyning & Perrin, 1994; Murphy



et al., 2006; Westgate, 2007), the ratio RL/ZW were very similar between sexes in those areas (Heyning & Perrin, 1994; Westgate, 2007) and in Southern Australia (Bell *et al.*, 2002).

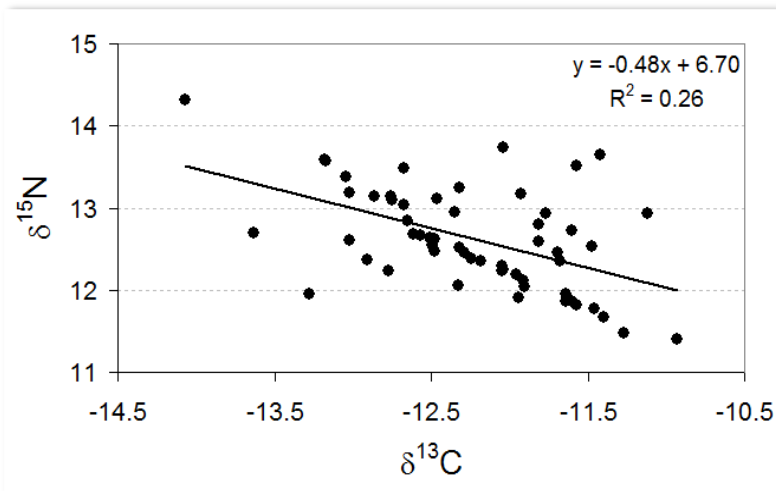
Data analyses

The normality of the data was tested through the Kolmogorov-Smirnov test and the heteroscedasticity through the Levene's test. Relationships between variables were analyzed through regression analysis. A principal component analysis (PCA) was applied to the contributions of all taxonomically (RL/ZW and UTC (left)) and ecologically ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) relevant variables in the study, in order to check whether these variables are associated with common dolphin's morphotypes. The statistical software package SPSS 15.0 was used for all analyses.

RESULTS

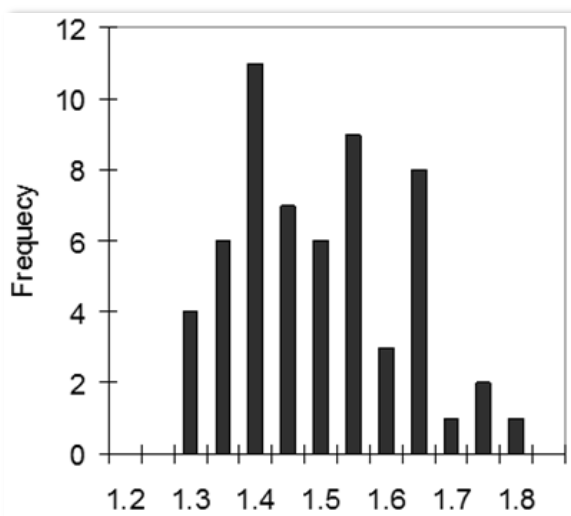
From the 72 common dolphin skulls collected along the Mauritanian coastline (Figure 1) we were able to analyse 57 individuals; due to the damage of the skulls or immaturity of the individuals the remaining skulls were removed from the dataset. The minimum CBL that was deemed as physically mature was 394 mm, because from this size forward skulls displayed the degree of fusion of the alveoli, and the degree of overall cranial fusion, proper of physical mature individuals. Individuals followed a normal distribution ($p=0.56$) and presented homogeneity of variances for all the variables analysed, both isotopic and morphometric.

The average isotopic values of the individuals sampled in this study were 11.40 ± 0.62 (‰, mean \pm S.D.) for the $\delta^{15}\text{N}$ and -12.23 ± 0.65 (‰, mean \pm S.D.) for the $\delta^{13}\text{C}$. A high intraspecific variability was observed for both stable isotopes: $\delta^{15}\text{N}$ ranged between 11.4‰ and 14.31‰, which corresponds to a difference of approximately one trophic level, while $\delta^{13}\text{C}$ ranged between -14.07‰ and -10.93‰, which indicated differences in habitat use. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were significantly and negatively correlated ($R=0.51$, $p<0.001$; Figure 3).

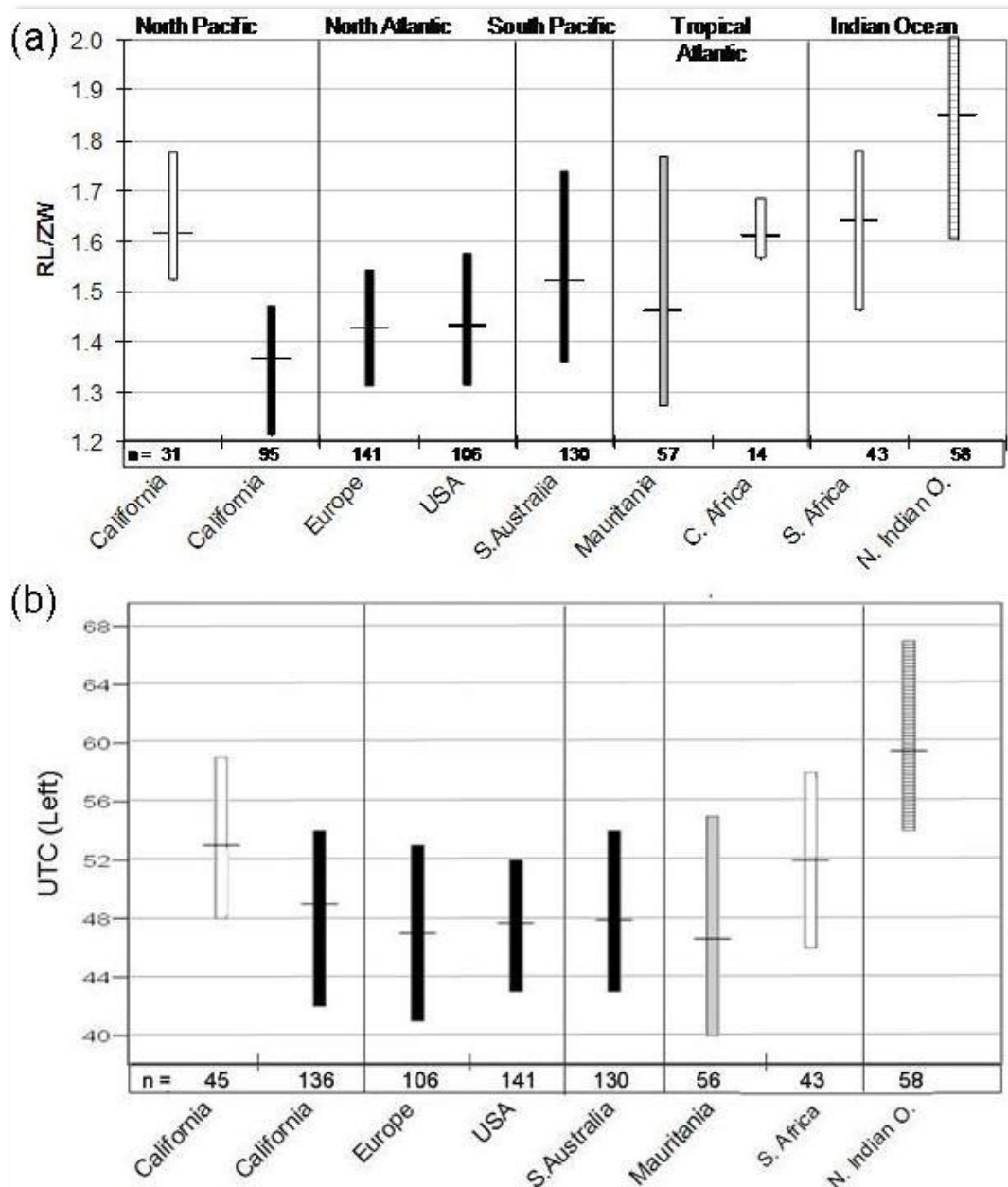
**Figure 3**

Correlation between nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) for common dolphins inhabiting the waters off Mauritania ($R^2=0.26$, $p<0.001$, $n=57$).

Investigation for possible effects of allometric growth did not produce significant results for any of the combinations tested: skull length (CBL-RL) vs. RL ($R=0.22$, $p=0.10$, $n=57$), (CBL-RL) vs. POW ($R=0.26$, $p=0.06$, $n=57$), and RL vs. POW ($R=0.05$, $p=0.73$, $n=57$). This suggests that the age of the sampled skulls is not affecting the results. The distribution of the RL/ZW ratios followed a normal distribution (Figure 4), and a separation between two putative groups, attending to their relative beak length, was not apparent. Figure 5 shows a comparison between the RL/ZW ratios and the UTC (left) observed in this study and those previously published for short- and long-beaked forms elsewhere: RL/ZW ratios (mean = 1.46, range = 1.27-1.76, Figure 5a) and UTC (mean = 46.62, range = 40-55, Figure 5b) overlap with those of *D. delphis* and *D. capensis* populations from other parts of the world.

**Figure 4**

Distribution of the RL/ZW ratios for the common dolphins sampled ($n=57$).

**Figure 5**

Comparison of the (a) RL/ZW ratios and (b) UTC (left) from *Delphinus* sp. populations worldwide: North Pacific Ocean common dolphin (California; Heyning & Perrin, 1994), North Atlantic common dolphin (Europe and USA; Westgate, 2007), South Pacific (Australia; Bell et al., 2002), Mauritania (present study), Eastern Central North Atlantic (Central Africa; Van Waerebeek, 1997) and Indian Ocean (South Africa and North Indian Ocean; Jefferson & Van Waerebeek, 2002). The bars represent the different species of common dolphin: *Delphinus delphis delphis* (black bars), *Delphinus capensis capensis* (white bars), and *Delphinus capensis tropicalis* (striped bars).



The measurement reflecting the length of the rostrum was significant and positively correlated with the $\delta^{15}\text{N}$: RL vs. $\delta^{15}\text{N}$ ($R=0.44$, $p=0.001$, $n=57$). This indicates that individuals with longer rostrum tend to feed at higher trophic levels than those of shorter rostrum. Also, a positive significant correlation ($R=0.41$, $p<0.001$, $n=57$) was found between $\delta^{15}\text{N}$ and the RL/ZW ratio (Figure 6), while no correlation was found between the $\delta^{13}\text{C}$ and RL/ZW ratios ($p=0.34$, $n=57$).

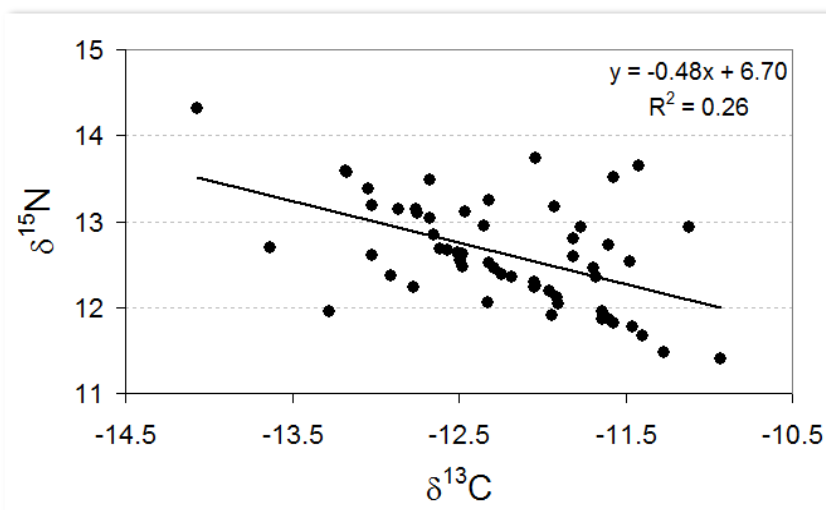
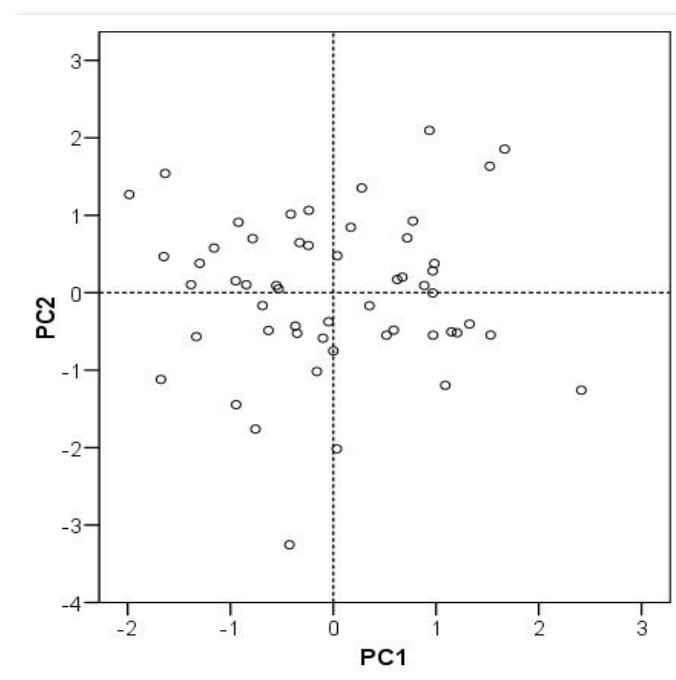


Figure 6

Correlation between nitrogen ($\delta^{15}\text{N}$) and the RL/ZW ratio in common dolphins inhabiting the waters off Mauritania ($R^2=0.174$, $p<0.001$, $n=57$).

In the PCA, we obtained two principal components, PC1 and PC2, accounting for 42.84 and 29.44% of the variance, respectively. With this small number of variables we managed to explain 72.28% of the variability in the sample. The component matrix indicated that PC1 was positively associated with $\delta^{15}\text{N}$ and RL/ZW (ordered from higher to lower coefficients), and negatively associated with $\delta^{13}\text{C}$ and UTC (left). PC2 was positively associated with $\delta^{13}\text{C}$, RL/ZW, and $\delta^{15}\text{N}$ and negatively associated with UTC (left). The graphic representation of PC1 against PC2 did not display the formation of two clear and distinct groups corresponding to short-beaked and long-beaked morphotypes (Figure 7).

**Figure 7**

Principal components (PC1-PC2) extracted from the relative contributions of the taxonomic and isotopic variables.

DISCUSSION

In the present study we report stable isotope data from common dolphin bone samples collected from 1990 to 2008. Statistical analysis were performed to detect differences of stable isotopes of carbon and nitrogen on sampling decades (1990-1999 and 2000-2008), but no differences were found (data not presented). We thus assumed that the current distribution and habitat use of individuals, as well as feeding preferences, in the study area did not change in the last decades. However, the results from the stable isotope analysis do not enable us to identify the causal factors for the differentiation of the two morphotypes on an evolutionary scale, as could report data obtained from morphometric and genetic markers. So, the three markers provide different insights to the differentiation of species, populations and ecological stocks; while bone isotopes are integrating almost all the life span of common dolphins, morphometric and genetic markers can be used to detect differences at the population and species level.

Stable isotopes of carbon and nitrogen were significantly and negatively correlated, thus reflecting variation associated to habitat use. Furthermore, the large variability found in our common dolphin sample for both isotopic indices suggests substantial differences among individuals in the exploitation of food resources and, most likely, their habitat. $\delta^{15}\text{N}$ correlated with rostrum length, indicating that longer-beaked individuals either feed at a higher trophic level than those shorter-beaked and/or they exploit mostly an offshore-pelagic habitat, according to $\delta^{15}\text{N}$ baseline in Mauritanian ecosystems. As reported, in primary



producers levels of $\delta^{15}\text{N}$ are higher (ca 5‰ $\delta^{15}\text{N}$), and those of $\delta^{13}\text{C}$ lower, in offshore habitat than in coastal habitat (Pinela *et al.*, 2010), thus fractionation through food web would depict higher levels of $\delta^{15}\text{N}$ in dolphins feeding offshore (considering the offshore habitat as open ocean pelagic habitat).

Although $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were negatively correlated, relative rostrum length (RL/ZW) and $\delta^{13}\text{C}$ failed to show any correlation, possibly because the large variability between individuals masked the effect of trophic level on $\delta^{13}\text{C}$. However, an alternative hypothesis, and according with results obtained from $\delta^{15}\text{N}$, to explain such absence of correlation may be that the incremental effect of trophic level on $\delta^{13}\text{C}$ is offset by an allopatric distribution of the morphotypes, the shorter-beaked individuals inhabiting waters closer to coast than those longer-beaked. However, this hypothesis would be inconsistent with previous observations in Chile (Sanino *et al.*, 2003) and Baja California (Niño-Torres *et al.*, 2006) that indicate that the long-beaked form is most coastal than the short-beaked form (Perrin, 2009). Indeed, in the northeastern Pacific Ocean, the region of origin of the specimens used for the differentiation between *D. delphis* and *D. capensis* (Rosel *et al.*, 1994), the long-beaked form, also known as Baja neritic, shows a well-known preference for shallower waters (inside the 180-meter isobath) than the short-beaked form (Leatherwood *et al.*, 1982). On the other hand, Bell *et al.* (2002) found similar findings to ours in *Delphinus delphis* in southern Australia. Apart from the high variability of skull morphometric parameters, they stated a tendency of dolphins with larger skulls and rostrums to be near deep water, and those with smaller skulls and rostrums to be in shallow coastal waters.

Nevertheless, the variability in relative beak lengths for the Mauritanian common dolphins was very large as compared to studied *Delphinus* populations from other parts of the world (Heyning & Perrin, 1994; Van Waerebeek, 1997; Murphy *et al.*, 2006; Westgate, 2007); especially in regions where only one of the species is present. Although our sample could not be stratified by gender, the observed variability in RL/ZW ratios and UTC is unlikely to be explained by a gender-effect since the sexual dimorphism of *Delphinus* skulls is very limited (Heyning & Perrin, 1994; Van Waerebeek *et al.*, 1994), despite the skull size differences displayed by the males from Portugal and Ireland (Murphy *et al.*, 2006). The RL/ZW ratios for the Mauritanian animals are more similar to the values from the Atlantic Ocean than to those from the Pacific Ocean but, overall, the ranges of variation are so large as to be only slightly smaller than the composite variation of the two North Pacific species, *D. delphis* and *D. capensis*, put together (Heyning & Perrin, 1994; Figure 5a). The lower fringe of RL/ZW values encompassed the values typically found in the *D. delphis* from the eastern North Atlantic (Murphy *et al.*, 2006; Westgate, 2007), while the higher fringe encompassed those found in Central West Africa, more precisely in Senegal, Gabon, Angola and Congo-Brazzaville (Van Waerebeek, 1997), and South Africa and attributed to *D. capensis* (Jefferson & Van Waerebeek, 2002). The clinal variation in rostrum size, that we observe in the Mauritanian common dolphins morphotypes, has also been observed in the Indo-Pacific (from the Middle East to China) extremely long-beaked form, *D.*



capensis tropicalis, as one moves east or west from India (Jefferson & Van Waerebeek, 2002); and in Southern Australia, as one moves from inshore/coastal to offshore/pelagic waters (Bell *et al.*, 2002). Common dolphin skulls off southern Australia overlapped in size with both the long- and short-beaked species in the eastern North Pacific (Bell *et al.*, 2002). The Australasian specimens presented a greater range of variation when compared to *D. delphis* in North Pacific and North Atlantic, especially considering that they all belong to the same species (Figure 5a). Tooth count per upper tooth row in Mauritanian *Delphinus* also presented a slightly larger variability when compared to *D. delphis* from the North Atlantic, Southern Australia or even California (Figure 5b). This result also seems to indicate that there is no stratification in our sample, because Mauritanian common dolphins presented a smaller range of UTC values than that obtained for *D. capensis* from South Africa and California, despite they slightly overlapped (Figure 5b).

Concerning the distribution of common dolphin stranding along the Mauritanian coast, most specimens were found stranded north of the tip of Cape Blanc (area 1 in Figure 1) and south of Cape Timirist (beginning of area 3 in Figure 1), even though the complete coastline was surveyed. Common dolphins thus appear to be absent or to occur at very low density in the shallow waters of the Banc d'Arguin (area 2 in Figure 1). Also, there is a clear bias toward stranding along the Grand Plage (area 3 in Figure 1). We could not detect any difference between geographical areas in relation to the length of the beak of stranded dolphins (data not presented). Unfortunately, sightings data from Mauritania are scarce and opportunistic, and simply suggest that common dolphins are rarely sighted off Mauritania close to the beach, with no indication on habitat partitioning between the short-beaked and long-beaked forms (Robineau & Vély, 1998). Currently there is practically no information on common dolphins from North-west Africa. Also, published literature does not show either obvious differences in the diet of short-beaked or long-beaked forms or species in other oceans (Ohizumi *et al.*, 1998), so further research is required both off Mauritania and elsewhere to clarify potential allopatry and trophic niche partitioning between morphotypes.

PCA analysis indicated that the combination of RL/ZW and δ -stable isotopes values explained a 72.28% of the variability in the sample. However, the principal components were not able to display a clear and distinct separation of individuals (Figure 7). The distribution of the RL/ZW ratio, UTC (left), and the isotopic indices found in Mauritanian dolphins formed a cline, and in neither case the data clustered into two distinct groups as typically occurs in studies of skull morphometric from the eastern North Pacific (Heyning & Perrin, 1994). Therefore, despite the occurrence in the area of individuals that can be ascribed to the two *Delphinus* morphotypes by their relative rostral length, neither morphometric nor the isotopic signatures support the hypothesis that the area is inhabited by more than a single species of common dolphins with intrinsic large variation in both body proportions and foraging behavior. The apparent



absence of differentiation would be consistent with the preliminary genetic research conducted by Natoli *et al.*, (2006), which suggested that the long-beaked form from Mauritania is taxonomically closer to the short-beaked form of the North Atlantic rather than to *D. capensis* from the Pacific Ocean.

We can thus conclude that both the long-beaked and short-beaked forms of common dolphin do occur off Mauritania but, contrarily to other areas, their occurrence appears to reflect differential use of habitat rather than taxonomy. Several authors suggested that geographical variation in the rostral morphology of common dolphin's, and consequently in their feeding apparatus, could indicate variations in feeding ecology, most likely due to differences in diet, even though it was never demonstrated (Evans, 1982; Amaha, 1994; Murphy *et al.*, 2006). Nevertheless, and despite that genetic analyses of a greater number of individuals have to be conducted, our data together with the genetic study conducted by Natoli *et al.* (2006) suggest that common dolphin's skull differentiation might be related to niche segregation and not to speciation. We conclude that taxonomic splitting appears not to be justified in the region. So, the existence of two putative populations or species (*D. delphis* and *D. capensis*) should be questioned until further research, particularly on genetics and on other taxonomically relevant morphometric measurements, is conducted. Moreover, the findings call for caution to assume as a general pattern that long-beaked and short-beaked common dolphins outside the eastern North Pacific fall into the taxonomic model described for that region by Heyning and Perrin (1994) and Rosel *et al.* (1994).

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CHAPTER 4



RESUMEN

El delfín común (*Delphinus* género) tiene un amplio rango de distribución que resultó en la diferenciación de un número de morfotipos, que, hasta hoy, tienen una adscripción taxonómica clara. En muchas áreas, dos morfotipos fueron distinguidos y más tarde separados en dos especies: el delfín común de morro largo, *Delphinus capensis*, y el delfín común de morro corto, *Delphinus delphis*. Con el fin de examinar la variación geográfica del género *Delphinus* en el noreste Atlántico, se realizó un análisis de morfometría geométrica 2D del cráneo. El objetivo principal de este estudio fue analizar la diferenciación entre la vista dorsal, ventral y lateral del cráneo utilizando métodos de morfometría geométrica basada en "landmarks" y análisis estadísticos multivariantes. Se analizaron un total de 120 individuos pertenecientes a las especies actualmente aceptadas para el noreste Atlántico (*D. Delphis* de la Península Ibérica y *D. capensis* de Sudáfrica) y se compararon con 41 individuos pertenecientes a una población indeterminada y poco conocida de Mauritania. Quince "landmarks" fueron digitalizados en la vista dorsal, catorce en la vista ventral, y doce en la vista lateral derecha y izquierda del cráneo. Se compararon los individuos de las tres áreas y se llevaron a cabo análisis estadísticos. Al parecer, los animales de Mauritania son más similares a los *Delphinus delphis* que a los *Delphinus capensis*, a pesar de la gran variabilidad de medidas del cráneo que presenta la población mauritana en comparación con las demás poblaciones. Sin embargo, todas las poblaciones están altamente diferenciadas, tanto para los componentes de tamaño como de forma del cráneo.

SCIENTIFIC JOURNAL REFERENCE

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Geographic variation in size and shape components of the skull of common dolphins (genus *Delphinus*) from the eastern Atlantic Ocean using landmark-based geometric morphometric methods

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ABSTRACT

The common dolphin (genus *Delphinus*) has a wide range of distribution that led to differentiation of a number of morphotypes, which, until today, have an unclear taxonomic adscription. In many areas, two morphotypes were distinguished and later separated into two species: the long-beaked common dolphin, or *Delphinus capensis*, and the short-beaked common dolphin, or the *Delphinus delphis*. In order to resolve the taxonomic uncertainties around the *Delphinus* complex from the NE Atlantic Ocean, we conducted a two-dimension geometric morphometrics analysis of the skull. The main objective of this study was to analyse the differentiation between the dorsal, ventral and lateral views of the skull using landmark-based geometric morphometric methods and their associated multivariate statistical analyses. A total of 120 individuals belonging to the current accepted species for the NE Atlantic (*D. Delphis* from the Iberian Peninsula and *D. Capensis* from South Africa) were analysed and compared to 41 individuals belonging to an undetermined and poorly known population from Mauritania. Fifteen landmarks were digitised on the dorsal view, fourteen on the ventral view, and twelve on the lateral right and left view of the skull. Individuals from the three areas were compared and statistical analyses conducted. Apparently, animals from Mauritania are more similar to the *Delphinus delphis* than to the *Delphinus capensis*, despite the great variability of skull measurements presented by the Mauritanian population compared to other populations elsewhere. Nevertheless, all populations were highly differentiated for both the size and shape components of the skull.



KEY-WORDS: cetaceans, common dolphin, *Delphinus delphis*, *Delphinus capensis*, eastern Atlantic, skull differentiation, geometric morphometric, geographic variation, population structure.

INTRODUCTION

Morphometrics is the study of shape variation and its co-variation with other variables (Bookstein, 1991; Dryden & Mardia, 1998), and traditionally, the principle of morphological similarity has been commonly used to classify populations, species or other taxonomic groups (Evin *et al.*, 2008). Though, progresses at the molecular level have shown that morphological similarity does not necessarily reflect phylogenetic proximity because many phenotypic characters are susceptible to convergence (Claude *et al.*, 2003; Stayton, 2005, Evin *et al.*, 2008). Sometimes, morphological differences between close taxonomic levels can be difficult to detect and then traditional morphometrics is inadequate to differentiate between groups (Adams *et al.*, 2004). On the contrary, modern geometric morphometrics (GM; Rohlf & Marcus, 1993) is more appropriate when investigating differences between very similar species or within them (Monteiro, 1999), as well as to study functional morphology, and ecological divergence (Claude *et al.*, 2004; McKinnon *et al.*, 2004; Woodward *et al.*, 2006). In the present study, skull size and shape differentiation between closely-related species and/or populations of common dolphins were investigated using GM methods.

Geometric morphometrics is based on the capture of shape through a set of landmarks digitised on each specimen (Marcus, 2000). In the present investigation, the skull was analysed because its complex structure makes it suitable for this kind of study (Claude *et al.*, 2003; Stayton, 2005, Evin *et al.*, 2008). Historically, there have been several morphological studies of common dolphins (*Delphinus* spp.) based on traditional morphometrics and anatomy-based variables (*e.g.* skull measurements, length and shape of the rostrum, number and size of teeth, number of vertebrae, amongst others) that have detected morphological variations (Flower, 1883; True, 1889; Banks & Brownell, 1969; Evans, 1982; Heyning & Perrin, 1994; Messenger & McGuire, 1998; Bell *et al.*, 2002; Buchholtz & Schur, 2004; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Perrin, 2009; Pinela *et al.* 2011). However, landmark-based GM methods allow: more powerful statistical analyses, the possibility to separate size and shape and the production of graphical results. Moreover, this method preserves the geometry of the shape throughout the analysis (Rohlf & Marcus, 1993; Adams *et al.*, 2004). Several studies have been published in the last years applying GM methods to investigate morphological variation between populations, species, genus, and higher taxonomic levels, of cetaceans (*e.g.* Monteiro-Filho *et al.*, 2002; Woodward *et al.*, 2006; Amaral *et al.*, 2009; Nicolosi & Loy, 2010; Loy *et al.*, 2011; Barroso *et al.*, 2012; Galatius *et al.*, 2012; Guidarelli *et al.*, 2014; Tsai & Fordyce, 2014).



Morphological variation in common dolphins has been studied in several parts of the world (Banks & Brownell, 1969; Van Bree & Gallagher, 1978; Evans, 1982; Heyning & Perrin, 1994; Evans, 1994; Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Nicolosi & Loy, 2010); but north and central African populations have received little attention and only a few studies have been conducted in the area (Van waerebeek, 1997; Pinela *et al.*, 2011). The two most common morphotypes of the genus *Delphinus* occur off the Atlantic coast of Africa: the long-beaked form is present from the waters off Mauritania to those off South Africa (Van Waerebeek, 1997; Rice, 1998; Samaai *et al.*, 2005; Ambrose *et al.*, 2013), while the range of the short-beaked form is believed to extend as far south as Senegal (Heyning & Perrin, 1994; Van Waerebeek, 1997; Rice, 1998). Thus, the waters of the eastern subtropical Atlantic are a region of overlap in the geographical distribution of the two forms, and both morphotypes occur sympatrically in this area, as is the case off the Californian coast (Heyning & Perrin, 1994; Pinela *et al.*, 2010, 2011). It has been proposed that the long-beaked form originated independently in different regions, and that adaptation to local environments might be driving local speciation (Natoli *et al.*, 2006). Therefore, it seems likely that skull morphology reflects adaptation to prey capture and the habitat used by the animals (Pinela *et al.*, 2011).

The main objective of the present study was to examine skull size and shape variability, and thus provide a quantification and effective visualization of any morphological evidence of isolation and/or differentiation, between closely-related species/populations of common dolphins along the Eastern Atlantic Ocean by means of two-dimensional geometric morphometrics. Population structure and geographic population differentiation between the dorsal, ventral and lateral skull views were investigated using landmark-based GM methods and their associated multivariate statistical analyses. Detailed objectives were to: (1) characterise and discriminate the different taxa of the *Delphinus* complex by measuring population variation in size and shape of the skull using Cartesian coordinates of anatomical landmarks and Procrustes based GM; (2) describe patterns of phenotypic variation between populations that may lead to population structuring; and (3) ascribe the potential factors responsible for the evolution of skull shape, and thus evaluate any adaptive factors that might be related to shape changes among samples, for this complex and highly mobile cetacean.

METHODOLOGY

Study site and sampling

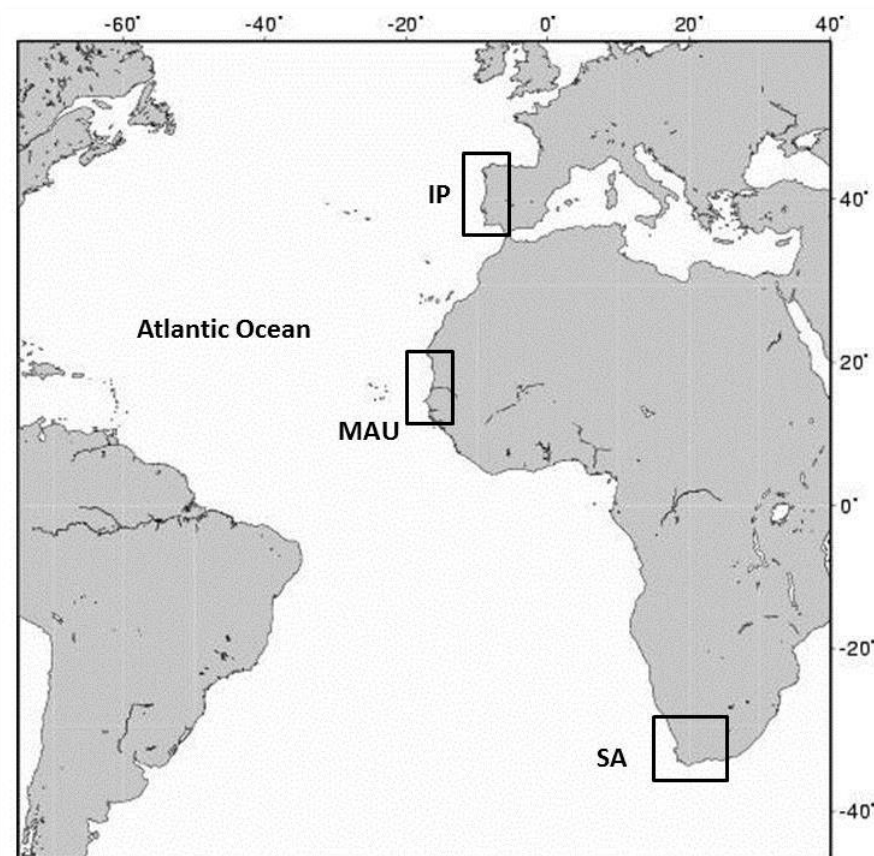
Common dolphin skulls from three geographic areas in the eastern Atlantic Ocean were analysed (for simplicity animals from each area will be designed as 'populations' henceforth): the Iberian Peninsula (Portugal and Spain, IP), the Islamic Republic of Mauritania (MAU), and South Africa (SA; Figure 1).



Common dolphin samples from the northeast Atlantic were collected between 1975 and 2007 from stranded or by-caught animals along the coast of Portugal and Spain (Galicia and Andalusia). Samples from the eastern sub-tropical Atlantic (MAU) were collected between 1992 and 2008 along the ca700 km of coastline that extends from Western Sahara to Senegal. Sex, body length, and any indication of the cause of death were recorded whenever possible, though, most specimens were found as bone remains, so detailed biological information is lacking for large part of the sample. Samples from the southeast Atlantic came from animals either stranded on the South-African coastline or taken in South African waters; for more details on the sampling see Samaai *et al.*, 2005.

Even though the common dolphin species seem to be taxonomically established for the IP and SA populations, it is yet unknown for the MAU population (Pinela *et al.*, 2011). The IP animals are known to be *Delphinus delphis* and the specimens used here belong to the collections held by the Aquário Vasco da Gama and the Museo Bocage, both located in Lisbon (Portugal) as well as to the University of Barcelona; whereas the *Delphinus capensis* specimens belong to the collection held by the Iziko Museums of Cape Town in South Africa. The *Delphinus* spp. specimens obtained from the Mauritanian coast were housed at the University of Barcelona in Spain, and at the Parc National du Banc d'Arguin (PNBA), the Institut Mauritanien de Recherches Océanographiques et des Pêches (IMROP), and the Centre National d'Élevage et de Recherches Vétérinaires (CNERV), in Mauritania. Specimens, deposited in scientific collections or found during beach surveys, were identified, or confirmed, as common dolphins on the basis of skull morphology (*e.g.*, the presence of deep palatal grooves; Evans, 1994; Heyning & Perrin, 1994).

The final sample only included skulls that were considered to have completed their growth, in order to discard ontogenic variation. Physical maturity of the specimens was established as described by Pinela *et al.* (2011). Sexual identification was also not available for most of the specimens, and since the main objective is to detect differences at the taxonomical level we treated the dataset as a whole. Furthermore, even though male common dolphins have on average significantly larger skulls, in the North Atlantic (Murphy *et al.*, 2006, Westgate, 2007), Pacific Ocean (Heyning & Perrin, 1994), and Southern Australia (Bell, 2002) the rostral length/zigomatic width (RL/ZW) ratio was very similar between sexes. Also, previous shape analyses of the common dolphin's skull registered no differences between sexes (Nicolosi & Loy, 2009, 2010; Guidarelli *et al.*, 2014), allowing us to pool males and females together. Skulls incomplete or sufficiently damaged to impede reliable measurement were also discarded from the dataset.

**Figure 1**

Map of the Atlantic Ocean basin with indication of the latitude and longitude coordinates for the study areas (highlighted by black rectangles: Iberian Peninsula - IP, Mauritania - MAU, and South Africa - SA).

Geometric Morphometrics

Geometric morphometrics (GM) uses configurations of morphological landmarks, *i.e.*, it captures the form of a structure using Cartesian coordinates of a configuration of points. These landmarks are located precisely on each specimen corresponding in a one-to-one manner from one specimen to another; establishing a simplified representation of the specimens used in statistical analyses (Rohlf & Marcus, 1993; Adams *et al.*, 2004; Zelditch *et al.*, 2004). GM aims to conduct statistical analysis of differences in form using a quantitative description that preserves the geometry of shape variation (Zelditch *et al.*, 2004; Viscosi & Cardini, 2011).

For the geometric morphometric analysis high-resolution photos of the common dolphin's skull were taken using a digital camera mounted in a tripod, adjusted to ensure that the camera's objective was always parallel to the skull. The skulls were photographed in dorsal (D), ventral (V), lateral right (LR), and lateral left (LL) views following the guidelines presented by Zelditch *et al.* (2004). The scale was obtained by placing a ruler next to the specimen. Definition and location of Landmarks were determined following Monteiro-Filho *et al.* (2002) and digitised using TpsDig 2.05 (Rohlf, 2006). Fifteen landmarks were digitised



on the dorsal view, fourteen on the ventral view, and twelve on the lateral right and lateral left views of the skull of common dolphins (Table 1 and Figure 2). For both the D and V projections of the skull, landmarks were digitized all around the skull, *i.e.* both on the right and left sides, because of the potential existence of directional asymmetry in both sides of the skull of Odontocetes associated with airway structures (*e.g.* Ness, 1967; Yurick & Gaskin, 1988; MacLeod *et al.*, 2007).

Table 1 Details on the numbering and description of landmarks for each view of the common dolphin's skull

Number	Description
Dorsal (D)	
1	rostral tip
2 and 12	anteriormost point of the notch in the maxilla
3 and 11	intersection between the frontal bone and zygomatic process
4 and 10	intersection between the parietal bone and frontal–interparietal suture
5 and 9	posteriormost point on the curve of the parietal bone
6 and 8	posteriormost point on the curve of the occipital condyle
7	posteriormost point on the edge of the supraoccipital bone
13 and 14	posteriormost point in the premaxilla bone
15	anteriormost point of the suture between the frontal and interparietal bones
Ventral (V)	
1	rostral tip
2 and 12	anteriormost point of the notch in the maxilla
3 and 11	externalmost point in the suture below the supraorbital process of the frontal bone
4 and 10	point in the suture between the frontal and alisphenoid bones
5 and 9	posteriormost point on the curve of the parietal bone
6 and 8	posteriormost point on the curve of the occipital condyle
7	posteriormost point on the edge of the supraoccipital bone
13 and 14	anteriormost point on the palatine
Lateral (LR and LL)	
1	rostral tip
2	midpoint of the nasal bone suture
3	anteriormost point of the suture between the frontal and interparietal bones
4	posteriormost point on the curve of the occipital condyle
5	ventralmost point of the paraoccipital process
6	point in the suture between the frontal and alisphenoid bones
7	posteriormost point on the edge of the supraoccipital bone
8	midpoint of the nasal bone suture
9	dorsalmost point on the pterygoid notch
10	anteriormost point of the notch in the maxilla
11	intersection between the parietal bone and frontal–interparietal suture
12	posteriormost point on the curve of the parietal bone

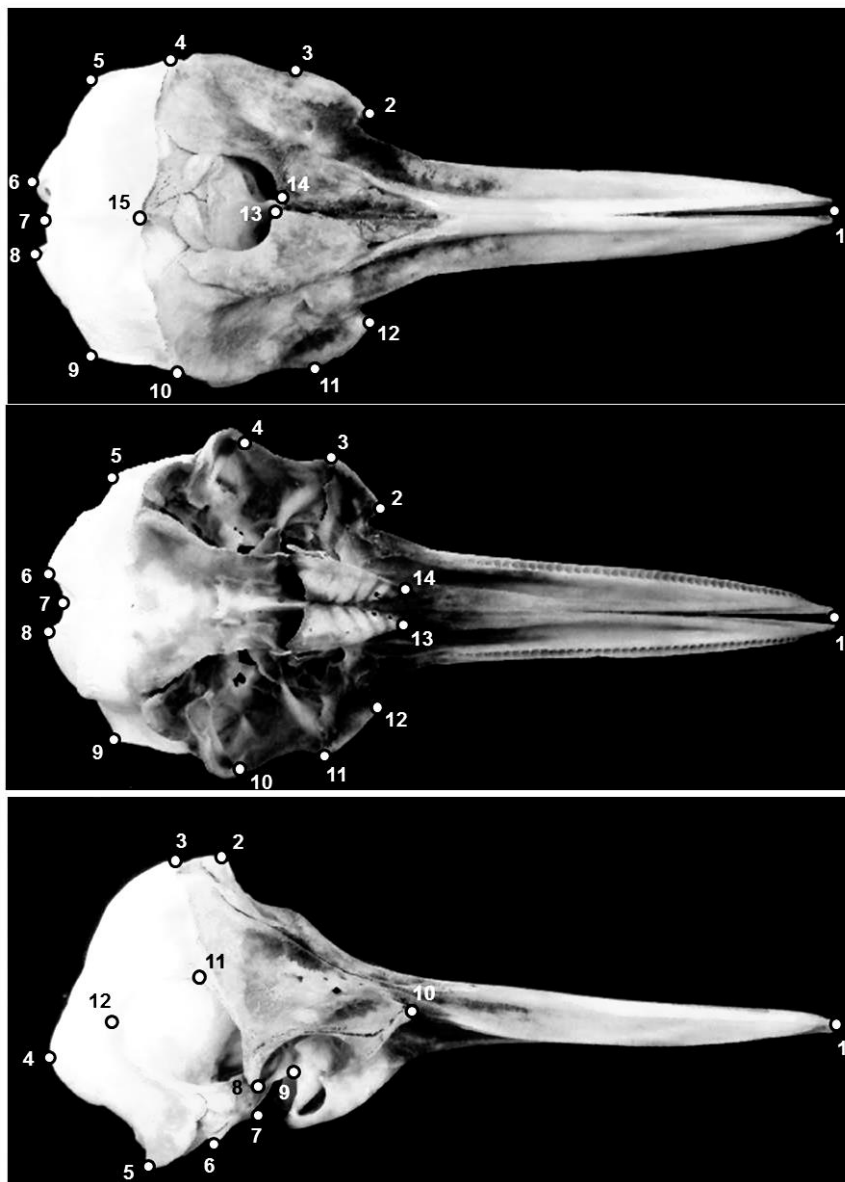


Figure 2

*Dorsal, ventral, and lateral (from up to bottom) views of a common dolphin's skull (*Delphinus* spp.) depicting the location of the landmarks and the respective numbering.*

We investigated size and shape parameters separately. Size was assessed by the centroid size (CS), which is the square root of the sum of squared distances from each landmark to the center of the skull for a given specimen (Bookstein, 1991). CS is considered to be a measure of size mathematically independent of shape and it is uncorrelated to it in the absence of allometry (Dryden & Mardia, 1998). Shape component was separated from size with a Procrustes superimposition (Rohlf & Slice, 1990) using MorphoJ (Klingenberg, 2011), which scaled (eliminating size by dividing coordinates by the centroid size), aligned and rotated landmark configurations removing any information unrelated to shape (Rohlf & Slice, 1990); and produced a new set of shape variables to be used statistically to analyse shape variation (Kendall, 1977). The Procrustes distance is the main measure of shape difference of two configurations in GM and it is



calculated as the square root of the sum of squared distances between corresponding landmarks of the two configurations after Procrustes superimposition (Klingenberg, 2011).

Outlier's or mislabelled points were detected using MorphoJ (Klingenberg, 2011), by plotting and visually inspecting landmark displacement for all configurations (Klingenberg & Monteiro, 2005) and by conducting a preliminary Principal Component Analysis (PCA) of Procrustes residuals to detect possible misidentifications (Evin *et al.*, 2008); and also an outliers analysis was conducted using SPSS version 15.0.

All the analyses dealt with the dorsal, ventral, and lateral views of the skull separately.

Statistical Analyses of morphometric data

Allometry was investigated by a multivariate regression of shape variables onto size, in this case the centroid size (CS). A correction for allometric effects on shape, whenever allometry was present, was applied to the data; hence the residuals of the multivariate regression were used (Klingenberg, 2011). Allometry was investigated for each view of the common dolphin skull (D, V, LR and LL).

To test for geographical effects on the skull size, differences in the centroid size of populations were depicted by box-plots. In order to test for significance, an analysis of variance (ANOVA) was conducted and consequential pairwise comparisons were investigated by means of Bonferroni tests. Homoscedasticity in the ANOVA for the centroid size was verified by means of a Levene's test.

To test for geographic effects on the skull shape, three different statistical analyses using the corrected shape variables (*i.e.* excluding the allometric component of the skull) were applied with MorphoJ (Klingenberg, 2011): 1) multivariate analysis of variance (MANOVA), 2) canonical variate analysis (CVA), and 3) discriminant function analysis (DFA). For illustrating shape differences between populations, landmark wireframes were depicted (Klingenberg, 2011).

CVA was used to find the shape features that maximized the separation among the three populations, being the individuals of each population assigned a priori. The graphical output consisted of two separate graphs. One showing the maximum differences in skull shape associated with the canonical variates (CV's; Rohlf *et al.*, 1996), and the other being a scatter-plot of the CV scores. The scale factor for CV shape changes was in units of Mahalanobis distance - the shape change per unit of within-group shape variation; this is the same as the units of the axes of the scatter-plots of CV scores (Klingenberg, 2011). To facilitate the visualization of shape differences, wireframe graphs were amplified by a factor of seven for the D and V, and by a factor of five for the LR and LL, views of the common dolphin's skull. The CVA analysis



included a parametric *T*-square test for the difference between group means and a permutation test (10000 permutations) using Procrustes distance and the *T*-square.

DFA implementation in MorphoJ was used to examine the separation between two populations (as in CVA the individuals of each population were assigned a priori). The main difference to CVA is that only two populations were considered in the analysis; in this case we included all possible pairs of populations. The reliability of the discrimination was assessed by leave-one-out cross-validation that indicated the percentage of correctly assigned individuals with the discriminant functions. Therefore, DFA was most useful for comparisons of specific populations; whereas CVA was more useful for general analysis of group structure. A set of wireframes graphs showing the shape differences between all combinations of two population's means was displayed. To facilitate the visualization, wireframe graphs were amplified by a factor of five for the D and V, and by a factor of three for the LR and LL views of the common dolphin's skull. Classification/misclassification tables for the discriminant scores of DFA were provided.

All GM analyses were conducted using the software MorphoJ (Klingenberg, 2011), while purely statistical analyses were conducted using the software SPSS version 15.0.

RESULTS

In total, six skulls were removed from the final dataset because they were incomplete or sufficiently damaged to impede reliable measurement (four skulls from MAU and two from SA). Additionally, nine other skulls were discarded because they did not present closed sutures, closed alveoli, and distal fusion between the pre-maxillae and maxillae at the tip of the rostrum, and thus were considered physically immature (one skull from IP, seven from MAU and one from SA). Therefore, a maximum of 157 specimens were used in this study (see Table 2 for details) and a total of 615 photographs were analysed.

Interspecific allometry was statistically significant for all views of the common dolphin's skull, despite that it explained very different percentages of the variation in shape between specimens for the three populations studied. Allometry varied greatly between the D and V views of the skull and the LR and LL views, and even though it was statistically significant for all skull's views it explained a very small percentage of the variation in shape for some of them: around 20% of the variation in shape was explained by size for the dorsal and ventral views (**D**: 20.21%, $p < 0.0001$, $n = 155$; **V**: 21.01%, $p < 0.0001$, $n = 157$); and around 8% of the variation in shape was explained by size for the lateral views (**LR**: 7.15%, $p < 0.0001$, $n =$



156; **LL**: 8.28%, $p < 0.0001$, $n = 147$). Because allometry was present, and in order to investigate the variation associated only with the shape component of the skull, we removed the size component of the skull by using the residuals of the regression (= corrected shape variables), for all analyses except when investigating differences in centroid size.

Table 2 Number of outliers and the final number of samples (N) for all views of the common dolphin's skull (D , V , LR and LL) for each study area and the total

Area	Outliers				N			
	D	V	LR	LL	D	V	LR	LL
IP	3	1	1	1	60	65	62	63
MAU	4	4	1	0	40	40	41	36
SA	1	6	3	11	55	52	53	48
Total	8	11	5	12	155	157	156	147

Differences in Size

Centroid size of the three *Delphinus* spp. populations differed significantly for all the views of the skull (**D**: $F = 43.14$, d.f. = 2, $p < 0.001$; **V**: $F = 120.71$, d.f. = 2, $p < 0.001$; **LR**: $F = 81.23$, d.f. = 2, $p < 0.003$; and **LL**: $F = 90.43$, d.f. = 2, $p < 0.001$). However, pairwise Bonferroni comparisons showed that there were no significant differences detected in centroid size between IP and MAU, meaning those populations did not appear to be different in overall skull size. In contrast, SA population showed a skull size significantly larger ($p < 0.001$) than those from the other populations.

Figure 3 displays a series of boxplot (one for each side) of the centroid size, depicting a comparison between the populations studied, allowing the visualization of the tendency across common dolphin populations. Skulls of *Delphinus* spp. from MAU showed similar size to *Delphinus delphis* (from IP), and *Delphinus capensis* (from SA) skull's size was clearly larger, presenting the highest CS, as well as the largest range of CS values, independently of the skull's view being investigated.

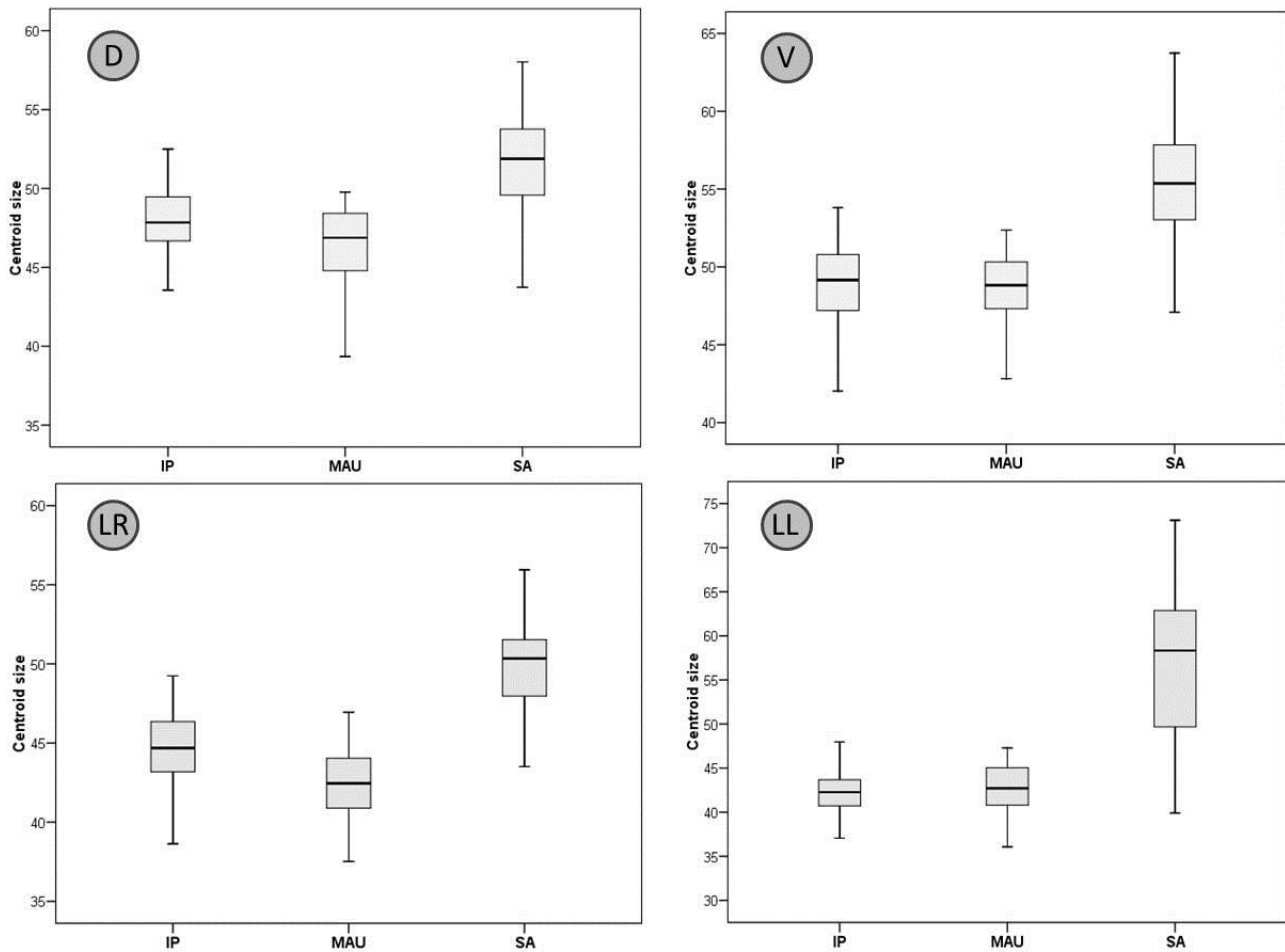


Figure 3

Size variation among populations (IP, MAU, and SA) depicted by box-plots of the centroid size for each population: median, 25-75% quartiles, minimum and maximum values are presented. D: dorsal, V: ventral, LR: lateral right, and LL: lateral left views of the skull of common dolphins from the eastern Atlantic Ocean.

Differences in Shape

Skull shape differences were found among the three populations studied for all views of the skull using MANOVA and CVA (Table 3 and Figure 4).

The multivariate analyses of variance (MANOVA) for the shape component of the skull revealed highly significant differences among populations for all the views of the skull (**D**: Wilk's $\lambda = 0.248$, $F = 10.062$, d.f. = 28, $p < 0.0001$; **V**: Wilk's $\lambda = 0.170$, $F = 14.154$, d.f. = 28, $p < 0.0001$; **LR**: Wilk's $\lambda = 0.161$, $F = 7.361$, d.f. = 52, $p < 0.0001$; and **LL**: Wilk's $\lambda = 0.104$, $F = 10.351$, d.f. = 52, $p < 0.0001$). Similarly, inter-



population pairwise comparisons (based on procrustes and mahalanobis distances), derived from CVA scores of the corrected shape variables, presented highly significant differences between all groups for all views of the skull (Table 3). Similar differences could be observed for all the views of the skull and all pairwise comparisons between the groups: IP-MAU were the two populations least morphologically distant, followed by MAU-SA, and finally IP-SA were always the two populations most morphologically distant; except for the mahalanobis distance for the D view, where MAU-SA were least distant from each other than IP-MAU (Table 3).

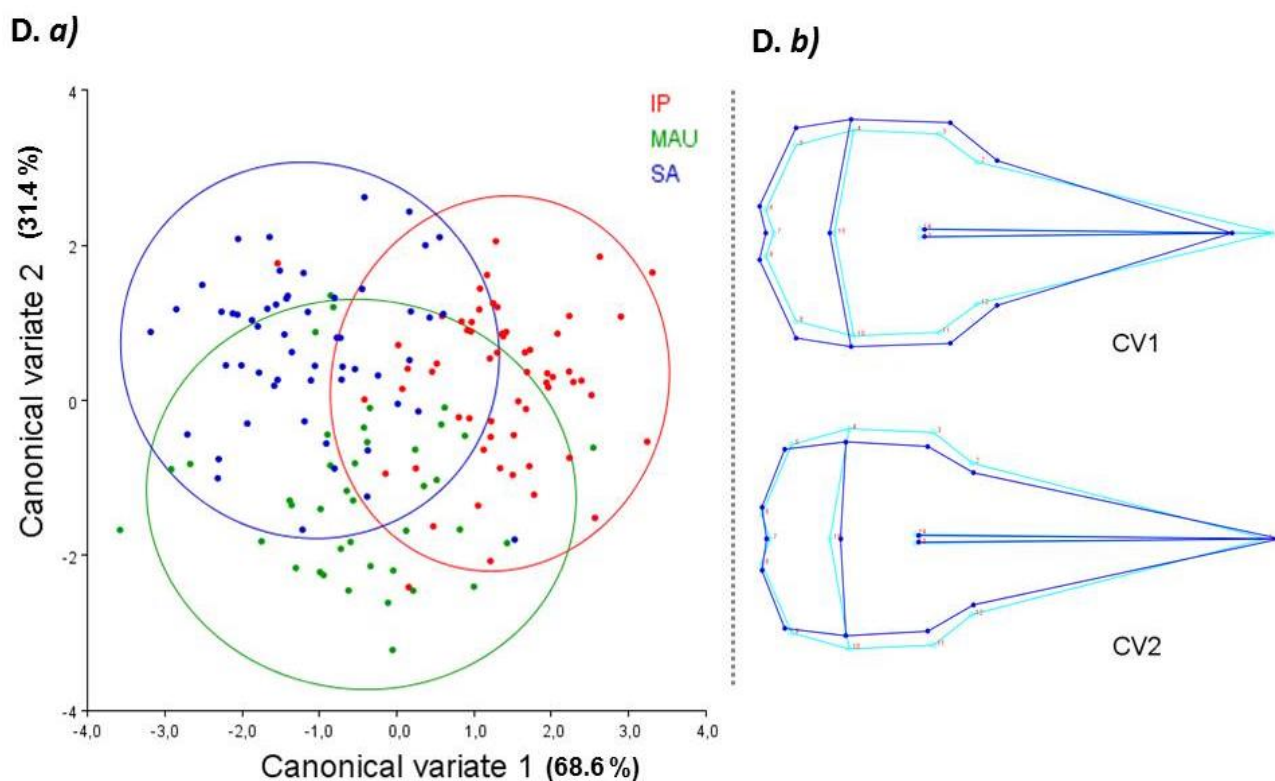
Table 3 *Procrustes distances, Mahalanobis distances, and the respective p-values for the pairwise tests between populations, derived from CVA scores of the corrected shape variables, for all views of the skull (dorsal, ventral and lateral) of common dolphins from the eastern Atlantic Ocean*

Skull's aspect	Pairwise comparison	Procrustes dist.	p-value	Mahalanobis dist.	p-value
D	IP-MAU	0.025	<0.0001	2.302	<0.0001
	IP-SA	0.043	<0.0001	2.499	<0.0001
	MAU-SA	0.027	<0.0001	1.977	<0.0001
V	IP-MAU	0.025	<0.0001	2.162	<0.0001
	IP-SA	0.043	<0.0001	3.116	<0.0001
	MAU-SA	0.030	<0.0001	2.998	<0.0001
LR	IP-MAU	0.029	<0.0001	2.392	<0.0001
	IP-SA	0.053	<0.0001	3.665	<0.0001
	MAU-SA	0.047	<0.0001	3.198	<0.0001
LL	IP-MAU	0.027	0.0017	2.626	<0.0001
	IP-SA	0.063	<0.0001	3.956	<0.0001
	MAU-SA	0.055	<0.0001	3.647	<0.0001

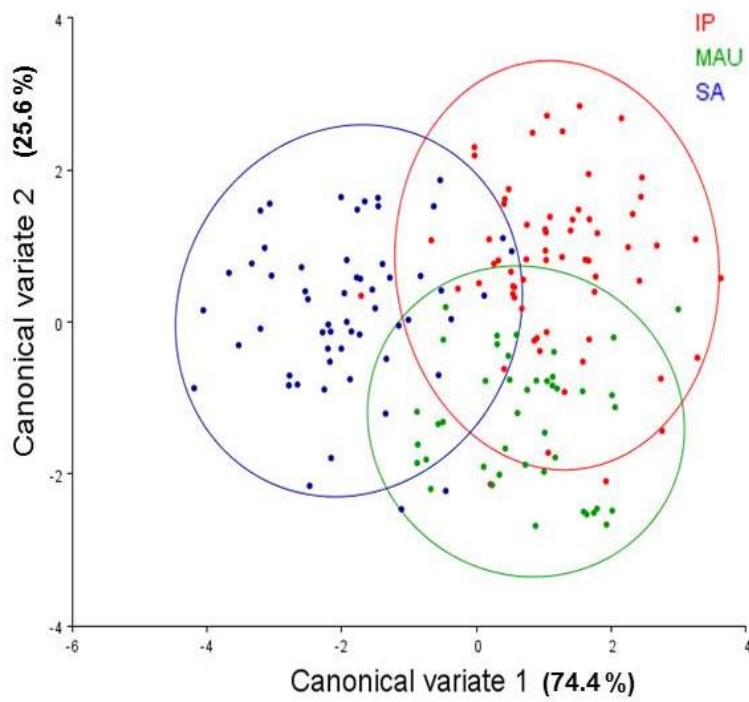
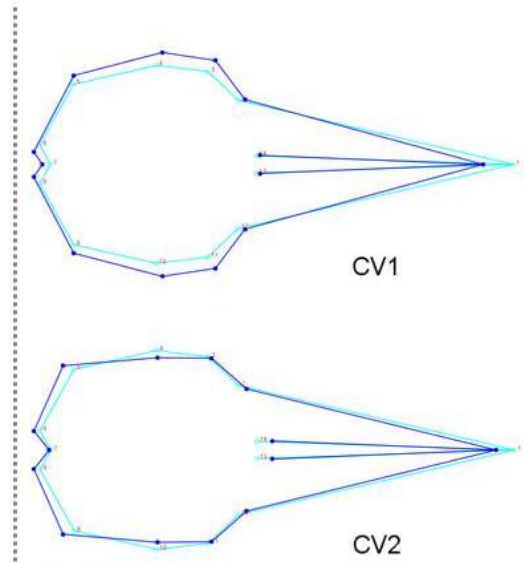
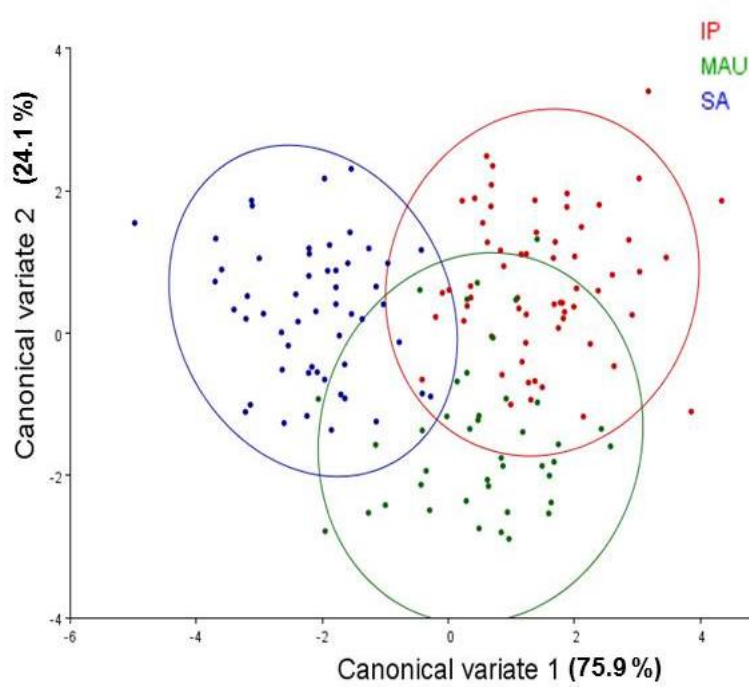
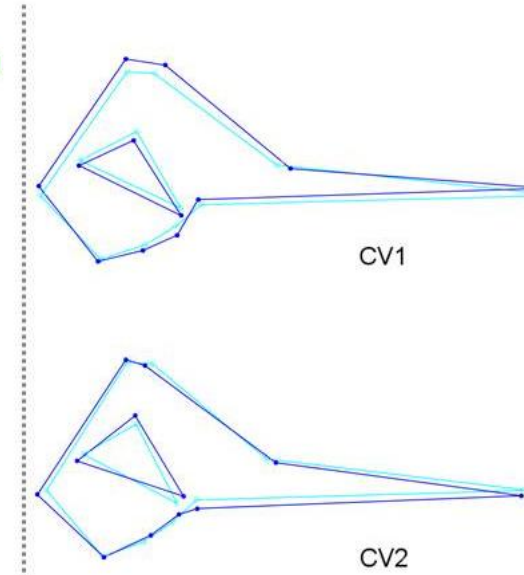
The CVA ordination, based only in the shape, presented three main clusters, for all the views of the common dolphin's skull, even though there was some degree of overlap between them: *D. delphis* (IP), *D. capensis* (SA), and *Delphinus* spp. (MAU; Figure 4a). The first CV explained between 68.6% and 75.9% of the shape variation of the skull, while the second CV explained between 24.1% and 31.6% (Figure 4a). Analyzing the four scatterplots, we verify the same tendency independently of the skull's view: individual values for



the MAU population always overlapped with both the *D. delphis* (IP) and the *D. capensis* (SA), even though the overlap tended to be slightly bigger towards the *D. delphis*; while the *D. delphis* (IP) and the *D. capensis* (SA) seemed to be more segregated from each other and moreover, strong shape differences between the extreme points were shown (Figure 4b). Main shape differences between the three populations were illustrated with the wireframe graphs attained for the extreme points of each CV axis (Figure 4b). CV1 relates to the robustness of the skull, shown by wireframes associated with differences of all landmarks around the cranial portion of the skull, and the length of the rostrum (= beak); while CV2 relates to the width of the skull, shown by wireframes associated with differences of landmarks around 3 to 5 (and 9 to 11), which captures structures of the zygomatic process.



(Figure 4)

**V. a)****V. b)****LR. a)****LR. b)****(Figure 4)**

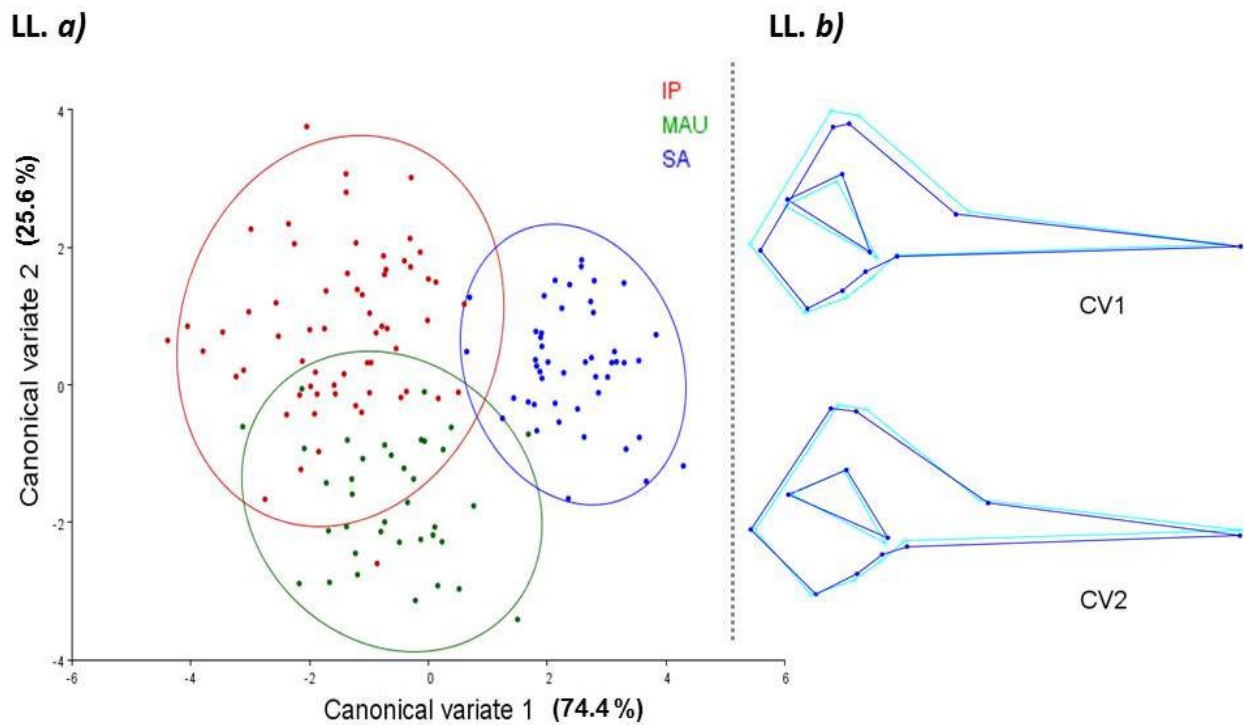


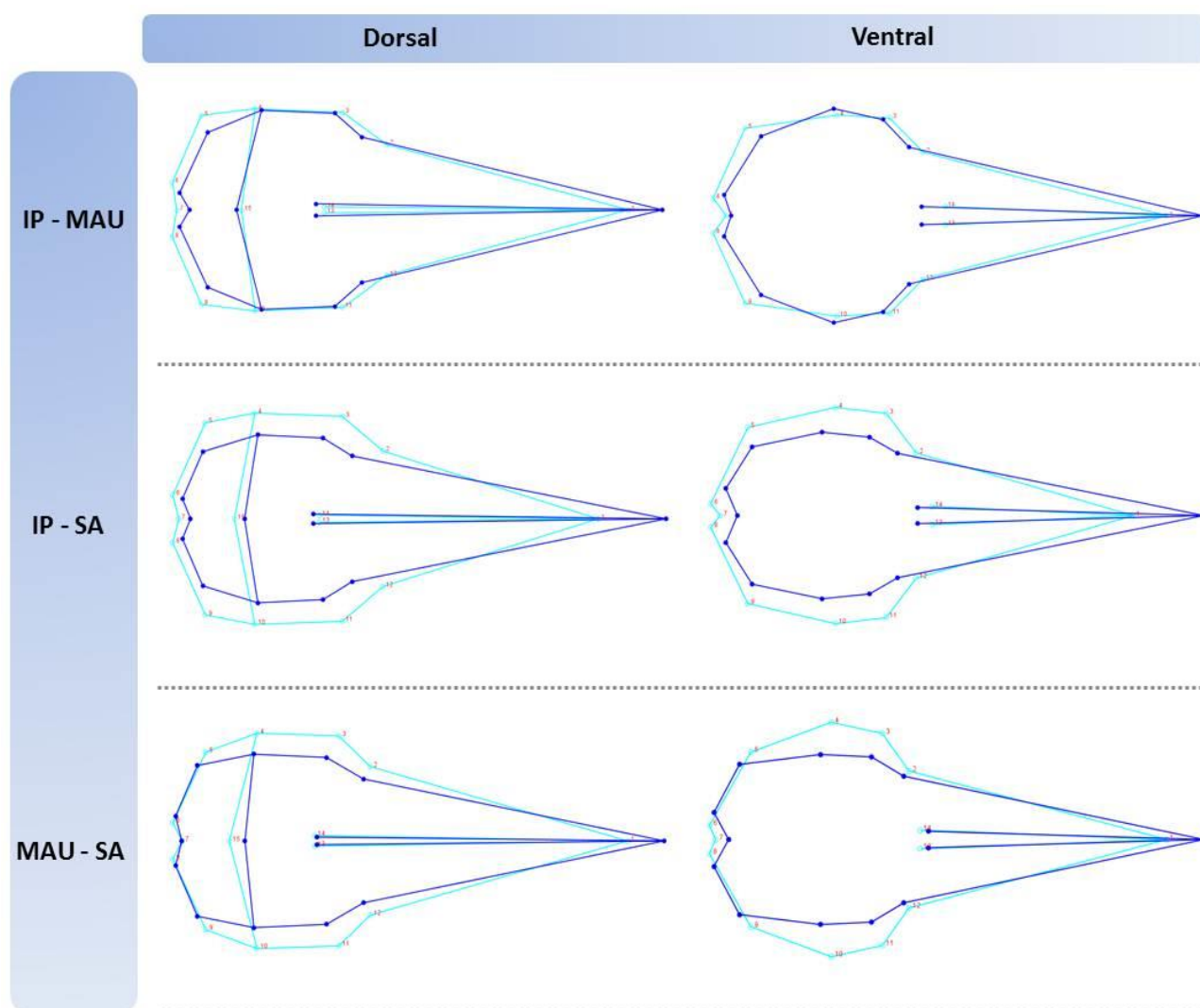
Figure 4

Scatterplots of canonical variates: (a) Scatterplot of CVA of the corrected shape variables (equal frequency ellipses, with a 0.95 probability, were drawn around each group) and (b) the schematic representation (wireframe graph) of the extreme points of the CV 1 and 2 (dark blue: positive extreme point; light blue: negative extreme point) for all views of the skull (D, V, LR and LL) of common dolphins from the eastern Atlantic Ocean; the percentage of explained variation among groups for each CV is given in parentheses.

Results from DFA analyses are depicted in Figure 5, where pairwise comparisons, between the three consensus shape configurations of common dolphin's skull, relative to IP, MAU and SA populations, are shown. We can see that the robustness of the cranium, the rostrum length, and the proportion of cranium to rostrum length are amongst the greatest variations of skull anatomy between common dolphin populations (Figure 5). Cross validated classifications assigned correctly 88.46% of all the common dolphin specimens investigated by their skull's shape. In total, 11.54% of the specimens were misclassified, although this average increased because of the misclassifications for the **D** view (19.35%) that were almost the double than those of other views (**V**: 10.19%, **LR**: 10.89%, and **LL**: 5.44%). Detailed information on the true group of the specimens and the group were specimens were allocated is shown in Table 4. Misclassified specimens included all the possible pairs of taxa, with the *Delphinus capensis* (from SA) being the taxa with the lowest number of misclassifications.



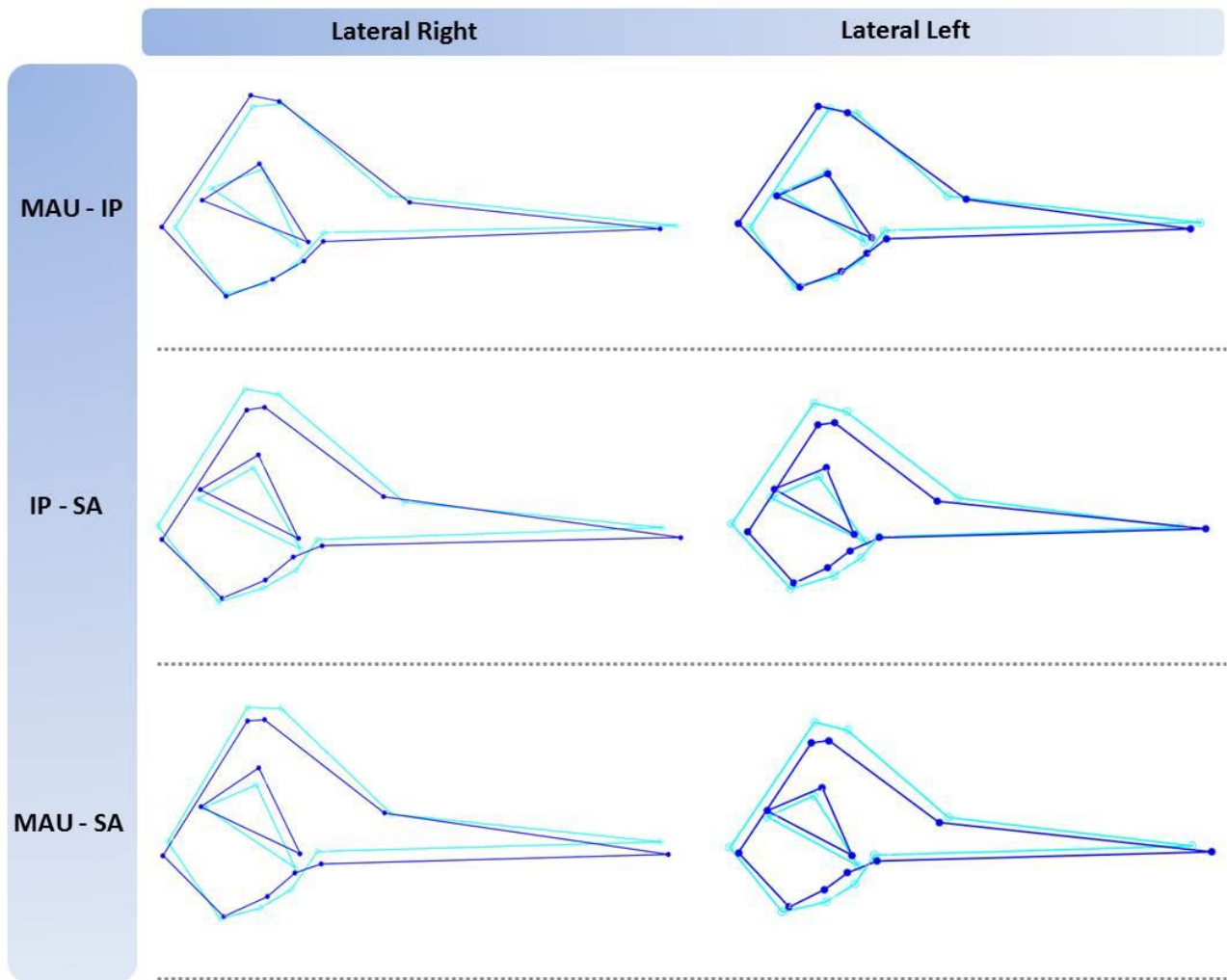
a)



(Figure 5)



b)

**Figure 5**

Pairwise comparisons of consensus configurations for the shape component of the skull: output diagrams of DFA analyses which show the magnitude and position of the shape differences between each two population means (wireframe graph; corrected shape variables were used; light blue: first population of the pairwise comparison, dark blue: second population of the pairwise comparison) for the (a) dorsal, ventral, and (b) lateral views of the skull of common dolphins from the eastern Atlantic Ocean.



Table 4 Cross validated classifications assigning specimens to a given group according to the shape component of the skull: true group of the specimens, skull view, and the group where specimens were allocated to, derived from the DFA, for all views of the skull of common dolphins from the eastern Atlantic

True Group	Skull's View	Allocated to		
		IP	MAU	SA
IP	D		8	2
	V		7	0
	LR		6	0
	LL		6	0
MAU	D	6		5
	V	3		2
	LR	6		2
	LL	3		1
SA	D	2	7	
	V	1	3	
	LR	0	3	
	LL	0	1	

DISCUSSION

The present study investigated geographic variation between populations of common dolphins (*Delphinus* spp.) from the eastern Atlantic Ocean. The main objectives were to describe the pattern of skull size and shape differentiation of the genus *Delphinus* and determine if geometric morphometrics would differentiate intra-generic populations and detect population structure. Geographical variation in morphology is used to define mammal populations (Tavares *et al.*, 2010).

The geometric morphometric results have shown significant differences in both the size and shape components of the skull of common dolphin populations from the different geographical areas analysed along the eastern Atlantic Ocean. GM multivariate statistics, along with more conventional statistical analyses, indicate that these differences follow a geographic trend in shape, and are particularly evident between the dolphins from the north and south-east Atlantic Ocean. It is no coincidence that the latter displayed the greatest skull shape variation, since they are the furthest populations, being apart by more than 4000 nautical miles. Despite some research on Cetacean species has revealed a poor relationship



amongst geographic distance and morphological/genetic differentiation (Hoelzel, 1992; 1994), there are some indications that it can occur. Thus, Loy *et al.* (2011), when analysing populations of *Stenella coeruleoalba*, found that morphological differences followed a geographical trend in shape of the skull. Besides, and more importantly, the two common dolphin populations, IP and SA, belong to two different and well-established species, the short-beaked common dolphin *D. delphis* (IP) and the long-beaked common dolphin *D. capensis* (SA). Even though it was to be expected the common dolphin (genus *Delphinus*) populations to be morphologically differentiated, this is the first study to be run through a GM approach with these populations. Only Mediterranean vs Atlantic populations of *Delphinus delphis* had been previously investigated using geometric morphometric methods to define the patterns of geographical variation (Nicolosi & Loy, 2010).

Geographic variation in morphology using geometric morphometrics has been extensively investigated in an extensive array of taxonomic groups, including terrestrial (Marcus *et al.*, 2000; Lieberman *et al.*, 2007; Evin *et al.*, 2008; Fernandes *et al.*, 2009; Milenković *et al.*, 2010) and marine mammal species, such as cetaceans (Monteiro-Filho *et al.*, 2002; Loy *et al.*, 2011; Barroso *et al.*, 2012; Galatius *et al.*, 2012; Guidarelli *et al.*, 2014; Tsai & Fordyce, 2014) and pinnipeds (Oliveira *et al.*, 2008; Ginter *et al.*, 2012). Geometric morphometric methods have only recently been used to identify shape differences between populations of the same dolphin species living in different geographical areas (Nicolosi & Loy, 2010; Loy *et al.*, 2011; Galatius *et al.*, 2012). However, when investigating geographic variation between populations or species of the common dolphin (genus *Delphinus*) ‘traditional’ measurement-based morphometrics have been the most used tool (Amaha, 1994; Heyning & Perrin, 1994; Van Waerebeek, 1997; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Santos *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011).

Due to the characteristic directional asymmetry of marine mammal skulls only the symmetric component was used for the analyses because it represents shape variation among individuals in what might be considered a left-right average, and which is of most interest in the majority of studies (Klingenberg *et al.*, 2002); whereas asymmetry is usually the focus of more specialized studies of asymmetry and developmental integration (Klingenberg *et al.*, 2002), and thus was not explored in the present study.

The results of our morphometric analyses show significant differences in the skull size of the three populations of common dolphins examined. Overall differences in size are largely concordant with previous morphological analyses (Amaha, 1994; Heyning & Perrin, 1994; Bell *et al.*, 2002; Murphy *et al.*, 2006). Several authors suggest that common dolphins in the eastern North Atlantic may have evolved a larger size than that of *D. delphis* animals from other areas (Amaha, 1994; Murphy *et al.*, 2006). Similarly to our



results, *D. capensis*, from both the coast of California and South Africa, presented a larger overall skull size than *D. delphis*; the head of the long-beaked form was absolutely longer but relatively narrower than that of the short-beaked form (Heyning & Perrin, 1994).

The population from SA is the most different with regard to cranial shape, with a skull characterized by the longest and narrowest rostrum and the narrowest (in relative terms) braincase. The population from IP is characterized by a shorter rostrum and a more robust rostrum and cranium, presenting a larger (in relative terms) braincase. The dolphins from MAU are characterized by a shape intermediate to the IP and the SA samples, though they are more similar in shape to the IP dolphins, despite being statistically different. These differences are also found to involve different features within the MAU common dolphin population (Pinela *et al.*, 2011). In delphinids, moderate to strong patterns of morphological variation over sometimes short geographical distances seem to be typical (Perrin, 1984). Common dolphin specimens from Australia did also not fit neatly with the known descriptions of either *D. capensis* or *D. delphis*; despite that they were considered morphologically closer to the *D. delphis* from California and assigned to *Delphinus delphis* species (Jefferson & Van Waerebeek, 2002).

Traditionally, the characters used to separate *D. delphis* from *D. capensis* are rostral dimensions (especially rostral length/zygomatic width, RL/ZW ratios) and upper tooth counts (Heyning & Perrin, 1994). RL/ZW ratios for the Mauritanian animals are more similar to those of animals from the Atlantic Ocean than to those from the Pacific Ocean, but overall the range of variation is so large as to be only slightly smaller than the composite variation in RL/ZW ratios of the two North Pacific species, *D. delphis* and *D. capensis*, put together (Pinela *et al.*, 2011). The lower RL/ZW ratios of Mauritanian common dolphins were similar to those found typically in *D. delphis* from the eastern North Atlantic (Murphy *et al.*, 2006; Westgate, 2007), while the higher ratios were similar to those found in Central West Africa (Van Waerebeek, 1997), and South Africa, which have been attributed to *D. capensis* (Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005). Research investigating intra-population morphological variability in the eastern Sub-tropical Atlantic Ocean is needed in order to infer on the taxonomy of common dolphins from this area.

Characteristically, the rostral elements of the skull are more influenced by selective pressures due to their association with feeding (Perrin, 1975). Thus, this part of the *Delphinus* skull complex seems to be relatively plastic, and consequently changes in the rostral apparatus of the skull could reflect changes in feeding ecology. Current results also show that the rostral apparatus is the part of the skull that exhibits more variability between the populations studied. Though, marine species are usually continuously distributed, marine ecosystems are characterized by local differences in habitat key features (*e.g.* temperature, salinity, bathymetry, food items, etc.) that hint to local niche specializations and morphological differentiation (Natoli *et al.*, 2006; Pinela *et al.*, 2011, 2015). Nevertheless, it is difficult to



say if differences in feeding ecology are responsible for the differences found in the rostral elements or if other motives are in place, such as intraspecific aggression during mating and sperm competition between males (Murphy *et al.*, 2005; Westgate, 2007; Westgate & Read, 2007).

Many published articles underline the importance of morphometric analysis to support the genetic, ecological and ethological results as a powerful tool to describe and understand the mechanism of morphological differentiation and population structure (Adams *et al.*, 2004; Cardini *et al.*, 2009; Natoli *et al.*, 2008). These results show the need to include other species and populations of common dolphins in order to better elucidate the degree and the pattern of geographic morphological variation, as well as the adaptive traits involved and the degree and pattern of asymmetry of the skull.

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CHAPTER 5



RESUMEN

Los delfines comunes (género *Delphinus*) tienen una amplia distribución geográfica y, a menudo, muestran una considerable estructuración genética y morfológica a escala regional, lo que facilita la diferenciación de morfotipos que aún están taxonómicamente en debate. Con el fin de resolver las incertidumbres taxonómicas de *Delphinus* del Atlántico oriental subtropical, se realizó un análisis de la morfometría geométrica de la vista dorsal del cráneo. El objetivo principal de este estudio fue proporcionar una cuantificación y visualización efectiva de la variación morfológica del cráneo entre morfotipos de delfines comunes. Se analizaron un total de 113 individuos de delfines comunes pertenecientes a las especies actualmente aceptadas para el noreste Atlántico (*D. Delphis* de la Península Ibérica y *D. capensis* de Sudáfrica) y se compararon con 38 individuos pertenecientes a una población indeterminada y poco conocida de Mauritania. Los morfotipos de delfines comunes mauritanos se dividieron en dos submuestras relacionadas con la relación morfométrica longitud rostral/anchura cigomática. Los grupos presentan diferencias morfológicas significativas en el tamaño del cráneo, excepto entre el morfotipo de morro largo de Mauritania y el *D. Delphis*. Las poblaciones de *Delphinus* del Atlántico oriental Subtropical son morfológicamente distintas del *D. delphis* y *D. capensis*, y también hay una clara separación entre el morfotipo de morro corto y largo. Sin embargo, el análisis de conglomerado jerárquico apoyó una similitud morfológica más cercana entre el morfotipo de morro corto y el *D. delphis*, y el morfotipo de morro largo y el *D. capensis*. Para concluir, los resultados indicaron que los componentes craneales analizados son altamente informativos y que potencialmente indican una evolución adaptativa reciente de las poblaciones y especies.

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5

Taxonomic status of the common dolphin (genus *Delphinus*) in the eastern Sub-tropical Atlantic Ocean

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ABSTRACT

The common dolphins (genus *Delphinus*) have an extensive geographical distribution and often show considerable genetic and morphological structuring on a regional scale, thus facilitating the differentiation of morphotypes that are still taxonomically under debate. In order to resolve the taxonomic uncertainties around the *Delphinus* complex from the eastern Sub-tropical Atlantic, we conducted a two-dimension geometric morphometrics analysis of the dorsal view of the skull. The main objective of this study was to provide a quantification and effective visualization of morphological variation of the skull between common dolphin morphotypes. A total of 113 common dolphin individuals belonging to the current accepted species for the NE Atlantic (*D. Delphis* from the Iberian Peninsula and *D. Capensis* from South Africa) were analysed and compared to 38 individuals belonging to an undetermined and poorly known population from Mauritania. The Mauritanian common dolphin morphotypes were divided into two sub-samples bestowing to the morphometric ratio rostral length/zygomatic width. Groups presented significant morphological differences in the size of the skull, except between the Mauritanian long-beaked morphotype and the *D. Delphis*. *Delphinus* populations from eastern Sub-topical Atlantic are morphologically distinct from *D. delphis* and *D. capensis*, and there was also a clear segregation between common dolphin short- and long-beaked morphotypes. However, hierarchical cluster analysis supported a closer morphological similarity between the Mauritanian short-beaked morphotype and *D. delphis*, and the Mauritanian long-beaked morphotype and *D. capensis*. To conclude, results indicated that the cranial components analysed were highly informative and that potentially indicated recent adaptive evolution of the populations and species.



KEY-WORDS: cetaceans, common dolphin, morphotypes, eastern Sub-tropical Atlantic, skull variation, taxonomy, specific differentiation, morphology, geometric morphometric.

INTRODUCTION

Common dolphins (*Delphinus* spp.) inhabit all the three major oceans of the world and some enclosed and semi-enclosed basins, being present between latitudes 40-60° N to about 50° S, except in the western Atlantic where they are absent from tropical waters (Jefferson *et al.*, 2008, 2009). Despite the extensive geographical distribution and dispersal potential, studies have shown that instead of having large panmictic populations, they often show considerable genetic and morphological structuring on a regional scale (Hoelzel, 2002); thus facilitating the differentiation of morphotypes (Van Waerebeek, 1997; White, 1999; Bell *et al.*, 2002; Jefferson *et al.*, 2009; Pinela *et al.*, 2011) that are still taxonomically under debate nowadays (Heyning & Perrin, 1994; Rosel *et al.*, 1994; Natoli *et al.*, 2006; Pinela *et al.*, 2011).

The taxonomic classification of organisms, and understanding the diversity of biological life, were historically based on descriptions of morphological forms (Adams *et al.*, 2004). The taxonomical classification of the common dolphin has been a challenge for many decades. Mainly, two morphotypes have been distinguished in several areas around the world, the long-beaked common dolphin usually of coastal or neritic distribution and the short-beaked common dolphin usually inhabiting both inshore and offshore waters (Banks & Brownell, 1969; Van Waerebeek *et al.*, 1994; Van Waerebeek, 1997; Evans, 1982; Perrin, 2009; Tavares *et al.*, 2010; Pinela *et al.*, 2011). These forms of common dolphin were differentiated for the first time by Banks and Brownell (1969), who used skull measurements such as the zygomatic width and the length of the rostrum to conclude that the anatomical differences were likely due to dissimilarities in feeding. Later, this morphological variation between morphotypes was confirmed by Evans (1982) through additional multivariate analyses of skull measurements, and the genus *Delphinus* was eventually split into two species in the Eastern North Pacific, based both on morphological and genetic analyses (Heyning & Perrin, 1994; Rosel *et al.*, 1994). Currently, the common dolphin, genus *Delphinus*, comprises of two species and four subspecies: the Atlantic and Pacific Oceans short-beaked common dolphin *Delphinus delphis delphis*; the Black Sea short-beaked common dolphin *D. delphis ponticus*; the Pacific and South Atlantic Oceans long-beaked common dolphin *D. capensis capensis*; and the Indian Ocean long-beaked common dolphin *D. capensis tropicalis* (IWC, 2009; Perrin, 2009).

Most areas of distribution of the common dolphin usually coincide with moderate to strong upwelling (Jefferson *et al.*, 2009), as the upwelling waters of the eastern sub-tropical Atlantic where both morphotypes live sympatrically (Pinela *et al.*, 2010, 2011), contrarily to the warm tropical waters of the



western Atlantic ocean they appear to avoid (Jefferson *et al.*, 2009). Therefore, the waters off Mauritania are a region of overlap in the geographical distribution of the two forms, with the short-beaked being distributed as far south as Senegal (Heyning & Perrin, 1994; Van Waerebeek, 1997; Rice, 1998), while the long-beaked is believed to be present from here to South Africa (Van Waerebeek, 1997; Rice, 1998; Samaai *et al.*, 2005; Ambrose *et al.*, 2013). However, Natoli *et al.* (2006) concluded, by examining a few specimens from north-west Africa, that the long-beaked morphotype was highly differentiated from other populations; thus questioning the hypothesis of a single long-beaked lineage worldwide, and suggesting that adaptation to local environments could be driving local speciation. When analysing niche segregation between morphotypes in the area Pinela *et al.* (2011) concluded that skull morphology reflects adaptation to prey capture and the habitat used by the animals. Locally, it raised questions about the identity of the common dolphin population(s), and the actual taxonomy of the morphotypes in the eastern Sub-tropical Atlantic. Morphological variation in common dolphins has been studied in several areas around the world, especially the north-east Pacific Ocean populations and species, (Banks & Brownell, 1969; Van Bree & Gallagher, 1978; Evans, 1982; Heyning & Perrin, 1994; Evans, 1994; Van Waerebeek *et al.*, 1994; Van waerebeek, 1997; Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Nicolosi & Loy, 2010), but north-west African populations have received little attention and their taxonomic status is still undetermined (Pinela *et al.*, 2011).

The base of morphometrics is to study the variation of shape and its co-variation with other variables (Bookstein, 1991; Dryden & Mardia, 1998); while 'modern' geometric morphometrics (GM; Rohlf & Marcus, 1993) is a method that is landmark-based, in which the shape and size are captured through a set of landmarks digitised on each specimen (Marcus, 2000) and the geometry of the shape is preserved throughout the analysis (Adams *et al.*, 2004). Morphological variation has been mostly used to classify populations, species or higher-level taxonomic groups (Evin *et al.*, 2008), and it is very useful when investigating systematic/taxonomic problems, and/or when dealing with closely related or cryptic species (Klingenberg *et al.*, 2003; Cardini & O'Higgins, 2004; Pizzo *et al.*, 2006; Evin *et al.*, 2008), or even when analysing populations of the same species (Milenkovic *et al.*, 2010; D'Anatro & Lessa, 2006).

Resource use and phylogeny are often correlated with morphological variation (Claude *et al.*, 2004). Variation is the source of evolutionary change, and the description of these patterns is fundamental for identifying evolutionary units in nature (D'Anatro & Lessa, 2006). Moreover, because biological shapes are often complex and evolve depending on several internal constraints, they must be assessed using integrative methods like GM (Claude *et al.*, 2004). Several studies have been published in the last decade applying GM methods to investigate morphological variation between populations (or higher taxonomic levels) of marine mammals (Monteiro-Filho *et al.*, 2002; Woodward *et al.*, 2006; Oliveira *et al.*, 2008; Loy *et*



al., 2011; Barroso *et al.*, 2012; Galatius *et al.*, 2012; Ginter *et al.*, 2012; Guidarelli *et al.*, 2014; Tsai & Fordyce, 2014) and it is currently a more popular method than traditional morphometrics.

The main objective of this study was to detect morphological variation within the common dolphin population from the eastern Sub-tropical Atlantic and to infer on their taxonomy by investigating the dorsal aspect of the skull using landmark-based GM methods and their associated multivariate statistical analyses. Additionally, we compared this population with taxonomically established species for the same Ocean: a well-known population of *Delphinus delphis* from the Iberian Peninsula and another of *Delphinus capensis* from South Africa. This study provides a quantification and effective visualization of morphological variation of the skull of common dolphins from the eastern sub-tropical Atlantic Ocean, allowing insight into the evolutionary and ecological processes of phenotypic diversification.

METHODOLOGY

Study site and sampling

Samples were collected between 1992 and 2008 along the ca700 km of Mauritanian coastline that extends from Cape Blanc (Western Sahara) to the Senegal River, north-western Africa (henceforth 'MAU', $n=38$) (Figure 1). The beaches were surveyed opportunistically or in dedicated expeditions and, when the remains of a dead dolphin were found, the skull was confirmed as common dolphin on the basis of its morphology (*e.g.*, the presence of deep palatal grooves; Evans, 1994; Heyning & Perrin, 1994), being preserved and deposited in a biological collection. Sex, body length and geographic position were recorded whenever possible; however, most specimens were found in an advanced state of decomposition or as bone remains, so detailed biological information is lacking for large part of the sample.

MAU specimens analysed in this study were housed at the University of Barcelona in Spain, and at the Parc National du Banc d'Arguin (PNBA), the Institut Mauritanien de Recherches Océanographiques et des Pêches (IMROP), and the Centre National d'Elevage et de Recherches Vétérinaires (CNERV), in Mauritania. The Mauritanian dolphins were divided into two sub-samples bestowing to the morphometric ratio rostral length/zygomatic width (RL/ZW) following Heyning and Perrin (1994): specimens with a RL/ZW ≤ 1.47 were classified as short-beaked morphotype (henceforth 'SB-MAU', $n=20$), while those with a RL/ZW > 1.52 were classified as long-beaked morphotype (henceforth 'LB-MAU', $n=18$).

The two subsamples of MAU common dolphins (SB-MAU and LB-MAU) were compared to each other and also to other common dolphin populations from the eastern Atlantic Ocean: the short-beaked common dolphin *Delphinus delphis* from the Iberian Peninsula in the north (henceforth 'SB', $n=59$), and the



long-beaked common dolphin *Delphinus capensis* from South Africa in the south (henceforth 'LB', $n=54$). Even though the specimens used in this study from the north- and south-eastern Atlantic are taxonomically established (Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007) the same doesn't apply to the eastern sub-tropical Atlantic, represented here as MAU population (Pinela *et al.*, 2011). SB common dolphin specimens used herein belong to the collections held by Aquário Vasco da Gama and Museo Bocage (Portugal), as well as to the University of Barcelona (Spain); whereas, LB specimens belong to the collection held by Iziko Museums of Cape Town (South Africa).

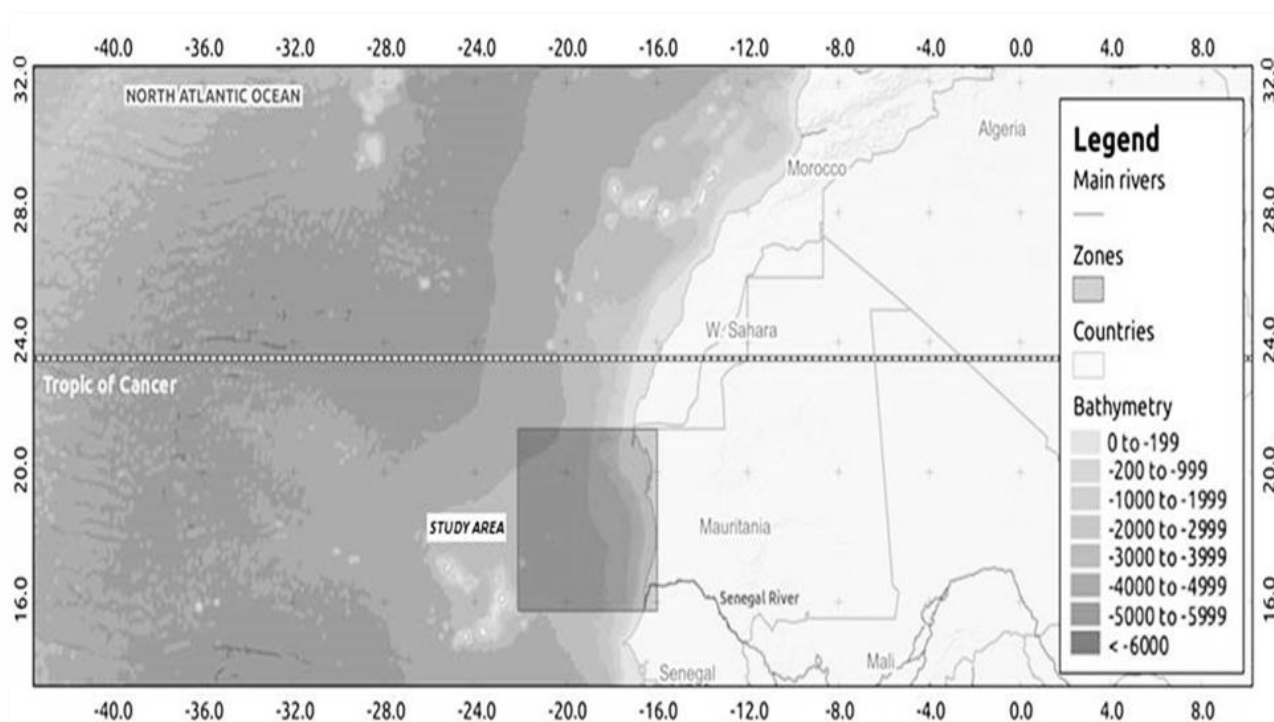


Figure 1

Map of the study area with indication of the latitude and longitude coordinates, the overline of the continental shelf and the bathymetry.

Only adult animals were used in this study to avoid morphometric variability of the skull shape due to ontogenic development, and physical maturity of the specimens was established as described by Pinela *et al.* (2011). Since there was no data on the sex of the animals, and because the main objective is to analyse variation at the taxonomical level, the dataset was examined as a whole. Also, the rostral

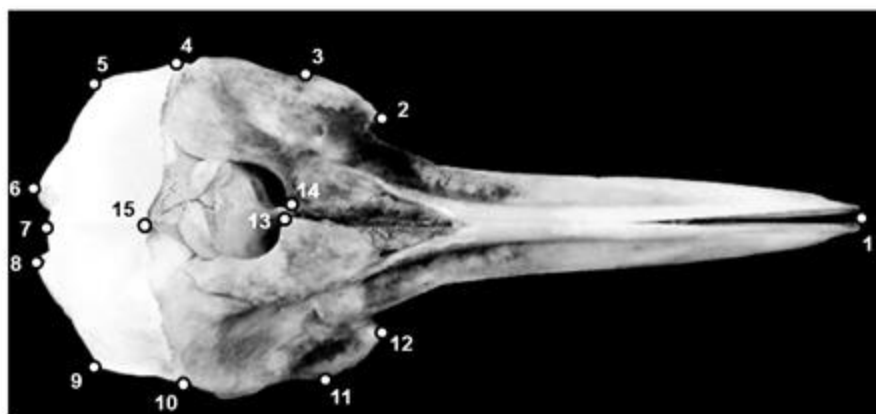


length/zigomatic width (RL/ZW) ratio was very similar between sexes despite the fact that male common dolphins have on average significantly larger skulls (North Atlantic Ocean: Murphy *et al.*, 2006 and Westgate, 2007; Pacific Ocean: Heyning & Perrin, 1994; Southern Australia: Bell, 2002). Additionally, previous shape analyses using geometric morphometrics of the skull of common dolphins from the North Atlantic Ocean and Mediterranean concluded that there were no differences between sexes (Nicolosi & Loy 2009, 2010). Skulls incomplete or sufficiently damaged to impede reliable measurement were also discarded from the dataset.

Geometric Morphometrics

Geometric morphometrics (GM) uses configurations of morphological landmarks establishing a simplified representation of the specimens, with the objective of statistically analyzing differences in form by using a quantitative description that preserves the geometry of shape variation (Rohlf & Marcus, 1993; Marcus, 2000; Adams *et al.*, 2004; Zelditch *et al.*, 2004;).

Following the guidelines presented by Zelditch *et al.* (2004), high-resolution photos of the dorsal and ventral views of the skull of common dolphin were taken using a digital camera mounted in a tripod and adjusted to ensure that the camera's objective was always parallel to the skull; the respective scale was placed next to the specimen (ruler). Only the results for the dorsal aspect of the skull are presented here due to the great similarity of results obtained for the ventral aspect of the skull (data not presented). Definition and location of fifteen landmarks were digitised on the dorsal view of the skull using TpsDig 2.05 (Rohlf, 2006) and following Monteiro-Filho *et al.* (2002). Landmarks were digitized both on the right and left sides of the skull (Figure 2), because of the directional asymmetry of the skull of Odontocetes associated with the airway structures (*e.g.* Ness, 1967; Yurick & Gaskin, 1988; MacLeod *et al.*, 2007). Details on the description and numbering of landmarks for the dorsal aspect of the common dolphin's skull are the following: rostral tip (1); anteriormost point of the notch in the maxilla (2 and 12); intersection between the frontal bone and zygomatic process (3 and 11); intersection between the parietal bone and frontal–interparietal suture (4 and 10); posteriormost point on the curve of the parietal bone (5 and 9); posteriormost point on the curve of the occipital condyle (6 and 8); posteriormost point on the edge of the supraoccipital bone (7); posteriormost point in the premaxilla bone (13 and 14); anteriormost point of the suture between the frontal and interparietal bones (15).

**Figure 2**

*Dorsal aspect of the skull of a common dolphin (*Delphinus* spp.) depicting the location of the landmarks and the respective numbering (see description in the main text).*

Size was assessed by the centroid size (CS; Bookstein, 1991), which is considered to be a measure of size mathematically independent of shape, thus uncorrelated in the absence of allometry (Dryden & Mardia, 1998). To separate size and shape, a generalized Procrustes analysis components of skull variation (Rohlf & Slice, 1990) was conducted. Thus, landmarks were superimposed using a Procrustes fit in MorphoJ (Klingenberg, 2011), which aligns, scales, and rotates landmark configurations removing any information unrelated to shape, and it produced a new set of shape variables to be used to statistically analyse shape variation (Kendall, 1977; Rohlf & Slice, 1990).

Outlier's or misplacement of landmarks were investigated using MorphoJ (Klingenberg, 2011), and according to the methods described in Pinela *et al.* (2015).

Statistical Analyses of morphometric data

Allometry was accessed by a multivariate regression of shape variables onto the centroid size (CS), and if allometry was detected a correction for allometric effects on shape was applied to the data. Consequently, the residuals of the multivariate regression were used as the corrected shape variables for the subsequent data and statistical analyses.

To test for dissimilarities on the size component of the skull, differences in the centroid size between groups were depicted by box-plots, and the statistical significance was verified with a t-test and an analysis of variance (ANOVA), while pairwise comparisons were done using Bonferroni tests. Homoscedasticity in the t-test and the ANOVA for the centroid size were verified by means of a Levene's test.

To test for differences on the shape of the skull, and therefore access the probability of shape differentiation between common dolphin's groups, a multivariate analysis of variance (MANOVA), a



canonical variate analysis (CVA), and a discriminant function analysis (DFA) were conducted using MorphoJ (Klingenberg, 2011). In these statistical analyses group membership was assumed to be known a priori.

CVA was used to find the shape features that best distinguish among the four groups of specimens (SB, LB, MAU-SB and MAU-LB). The graphical output consisted of two separate graphs. One that was a scatter-plot of the canonical variate (CV) scores (three bi-plots combining the three CV's), and another that consisted in wireframe graphs (amplified by a factor of five) showing the maximum differences in skull shape associated with each canonical variable (Rohlf *et al.*, 1996). The statistical significance of pairwise differences in mean shapes was assessed with permutation tests using both Mahalanobis and Procrustes distances (10 000 permutations per test).

DFA was used to examine the separation between two groups of individuals (all groups were compared in pairs). The reliability of the discrimination was assessed by leave-one-out cross-validation that indicated the percentage of correctly assigned individuals with the discriminant functions. Six wireframe graphs showing skull shape differences between each two group means were displayed, in which the connecting lines between landmarks of both skulls were superimposed. To facilitate the visualization, wireframe graphs were amplified by a factor of three. Classification/misclassification tables for the discriminant scores of DFA were also given.

Skull variation between sample groups for the MAU population, taking into account the overall form of the skull (size and shape not separated), and when analysing individual shape variables, was conducted using a Principal Component Analysis (PCA).

Cluster analyses were also conducted by using Mahalanobis distances to explore the morphometric relationships between the groups. Two cluster methods were used: UPGMA (Unweighted Pair Group Method with Arithmetic Mean), which is a hierarchical clustering method; and N-J (neighbour-joining; Saitou & Nei, 1987), which is an alternative method for hierarchical cluster analysis. Even though this method was originally developed for phylogenetic analysis, researchers believe it may be superior to UPGMA also for ecological and morphological data (Hammer & Harper, 2006). In contrast with UPGMA, two branches from the same internal node do not need to have equal branch lengths (Hammer & Harper, 2006). Both methods generate unrooted trees and the percentage of replicates where each node is supported is given on both the dendrogram and the phylogram (node numbering represents bootstrap percentages from 10000 replicates).

All GM analyses were conducted using the software MorphoJ (Klingenberg, 2011); purely statistical analyses were conducted using the software SPSS version 15.0; while the dendrogram, phylogram and bootstrap values were obtained using the software PAST (Hammer *et al.*, 2001).



RESULTS

In total, 154 specimens were used in this study, and consequently 154 photographs of the dorsal view of the skull of common dolphins were analysed. However, 3 specimens from Mauritania were discarded because they presented a RL/ZW ratio between the threshold values (Pinela *et al.*, 2011).

Variability within the genus Delphinus in the eastern Sub-tropical Atlantic

Cranial morphological variability within MAU population, *i.e.* the north-west African population of common dolphins (*Delphinus* spp.), was firstly investigated. Pooled within-group regression revealed that allometry was present and approximately 2% of the variation in shape was explained by size, even though it was not statistically significant between SB-MAU and LB-MAU morphotypes (2.16%, $p = 0.529$, $n = 38$). Centroid size differed significantly between common dolphin MAU morphotypes (t-test: $t = -4.003$, d.f. = 37, $p < 0.0001$). The SB-MAU specimens were smaller in size than the LB-MAU specimens, as well as all the other specimens analyzed in this study (Figure 3).

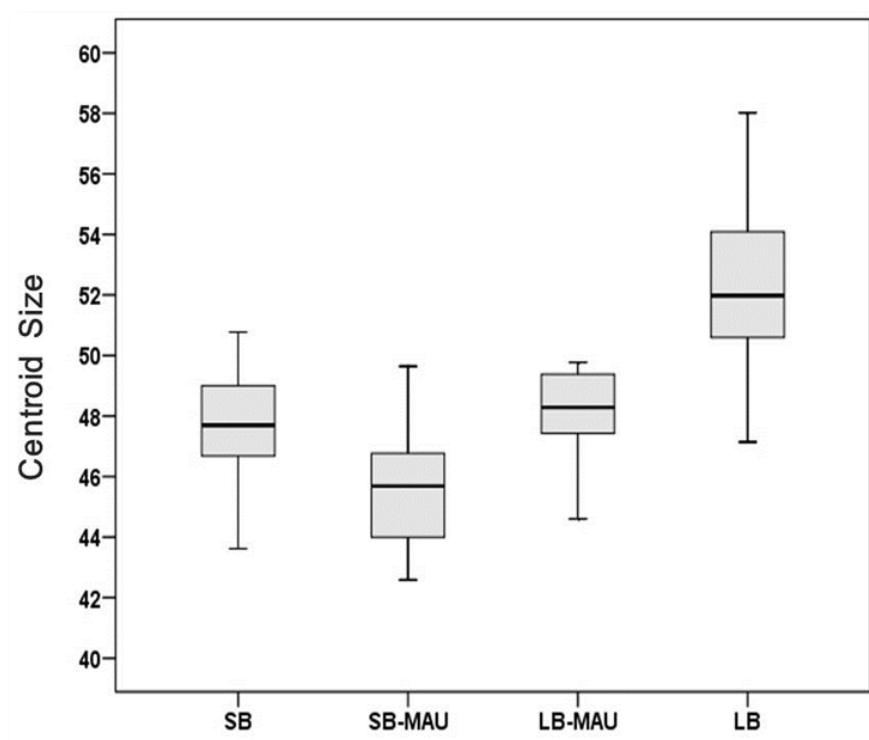


Figure 3

Size variation of the dorsal view of the common dolphin's skull from the eastern Sub-tropical Atlantic: box-plots of the centroid size for each group (= morphotype): median, 25-75% quartiles, minimum and maximum values are presented.

The multivariate analyses of variance (MANOVA) for the shape component of the skull revealed highly significant differences between the two eastern sub-tropical Atlantic populations of common dolphin



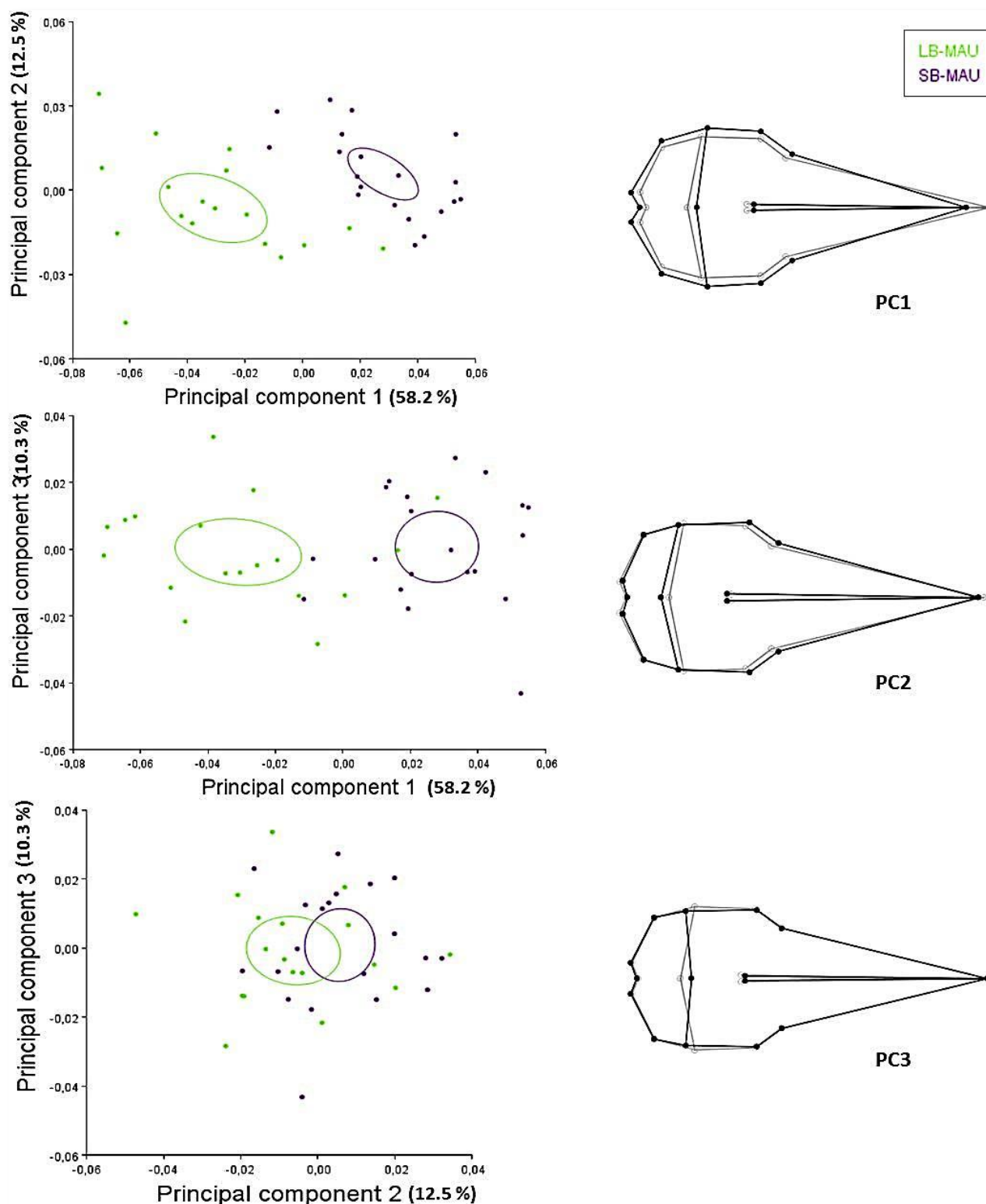
(Wilk's $\lambda = 0.030$, $F = 14.792$, d.f. = 26, $p < 0.0001$). Moreover, results from CVA analyses of permutation tests using both Mahalanobis and Procrustes distances showed that skull shape differed significantly between common dolphin MAU morphotypes; LB-MAU and SB-MAU were practically as distant from each other as *Delphinus capensis* from *Delphinus delphis* (LB-SB; Table 1).

Table 1 *Inter-population pairwise distances: morphometric distances among groups for the skull's (a) shape and (b) form (size + shape). Procrustes distances, Mahalanobis distances, and the respective p-values between groups, derived from CVA scores for the dorsal aspect of the skull of common dolphins*

Pairwise comparison	Procrustes dist.	p-value	Mahalanobis dist.	p-value
a)				
LB—SB	0.048	<0.0001	3.291	<0.0001
LB--SB-MAU	0.051	<0.0001	3.472	<0.0001
LB--LB-MAU	0.018	0.0019	1.905	<0.0001
LB-MAU--SB-MAU	0.051	<0.0001	3.013	<0.0001
SB--SB-MAU	0.019	0.0025	2.549	<0.0001
SB--LB-MAU	0.052	<0.0001	3.317	<0.0001
b)				
LB—SB	0.058	<0.0001	3.516	<0.0001
LB--SB-MAU	0.069	<0.0001	3.925	<0.0001
LB--LB-MAU	0.021	0.0011	1.987	<0.0001
LB-MAU--SB-MAU	0.060	<0.0001	3.165	<0.0001
SB--SB-MAU	0.021	0.0015	2.456	<0.0001
SB--LB-MAU	0.050	<0.0001	3.149	<0.0001

DFA results also indicated cranial differentiation between morphotypes, based on inter-group comparison of the means: Procrustes distance was 0.075 ($p < 0.0001$), and Mahalanobis distance was 9.672 ($p = 0.001$) between LB-MAU and SB-MAU populations.

The PCA also showed a clear segregation between SB-MAU and LB-MAU, except when contrasting PC2 against PC3 (Figure 4). The first three principal components accounted for 86.5% of the variance within the sample: the PC1 accounted for 68.7% of the variance, whereas PC2 and PC3 only accounted for 9.2% and 8.5%, respectively (Figure 4). The confidence ellipses for the means (probability: 0.90) are also shown in the scatterplot.

**Figure 4**

PCA for the common dolphin population from the eastern Sub-tropical Atlantic: scatterplots of principle components (frequency ellipses, with a 0.95 probability, were drawn around each group), and diagram representing the skulls morphological variation of the extreme points along each axis (wireframe graph; black: positive extreme point, light grey: negative extreme point) for the SB-MAU and LB-MAU morphotypes the percentage of explained variation among groups for each PC is given in parentheses.



Variability between common dolphin morphotypes

Cranial morphological variability between the morphotypes from our target area and the short- and long-beaked species of common dolphin (*Delphinus delphis* and *Delphinus capensis*, respectively) was also investigated. Statistically significant allometry was detected when analyzing the whole dataset, despite that it only explained around 8% of the variation in shape between populations: 7.88%, $p < 0.0001$, $n = 148$. We removed the size component of the skull by using the residuals of the regression (=corrected shape variables), for all analyses except when investigating differences in centroid size.

Centroid size differed significantly between groups (ANOVA: $F = 37.87$, d.f. = 3, $p < 0.0001$). All pairwise Bonferroni comparisons between groups were significant ($p < 0.022$), except for the SB and the LB-Mau population ($p = 1.000$), meaning those populations did not differ in overall skull size. The overall skull's size was clearly larger for the LB, which presented the highest CS, as well as the largest range of CS values (Figure 3).

The multivariate analyses of variance (MANOVA) for the shape component of the skull revealed highly significant differences among the four groups analyzed (Wilk's $\lambda = 0.054$, $F = 7.212$, d.f. = 81, $p < 0.0001$). Similarly, inter-population pairwise comparisons (based on Procrustes and Mahalanobis distances), derived from CVA scores of the corrected shape variables, presented highly significant differences between all groups for both the skull's shape and form (size + shape; Table 1). A very similar pattern was observed for all pairwise comparisons: LB—LB-MAU and SB—SB-MAU were the least morphologically distant, whereas LB—SB-MAU, together with the remaining pairwise comparisons, were the most morphologically distant (Table 1). Table 1 shows that most distances increase slightly when considering the overall form of the skull, *i.e.* when taking into account the size along with the shape.

The CVA ordination, based only in the shape of the skull, presented four main clusters representing each group, even though there was some degree of overlap between them. Furthermore, the first two CV's explained most of the shape variance (92.8%) and subtly separated the populations, even though there was an overlap between some of the specimens; while the third CV only explained 7.2% (Figure 5). The scatterplots, in Figure 5, show that values for the LB-MAU individuals usually overlapped with *D. capensis* (LB) and the values for the SB-MAU individuals usually overlapped with *D. delphis* (SB). Wireframe diagrams, in Figure 5, show strong shape differences between the extreme points of each CV axis; CV1 relates to the robustness of the skull, shown by wireframes associated with differences of all landmarks around the cranial portion of the skull, and the length of the rostrum (= beak), while CV2 relates to the width of the skull, shown by wireframes associated with differences of landmarks around 3 to 5 (and 9 to 11), which captures structures of the zygomatic process, and CV3 relates to the overall length of the skull and the braincase.

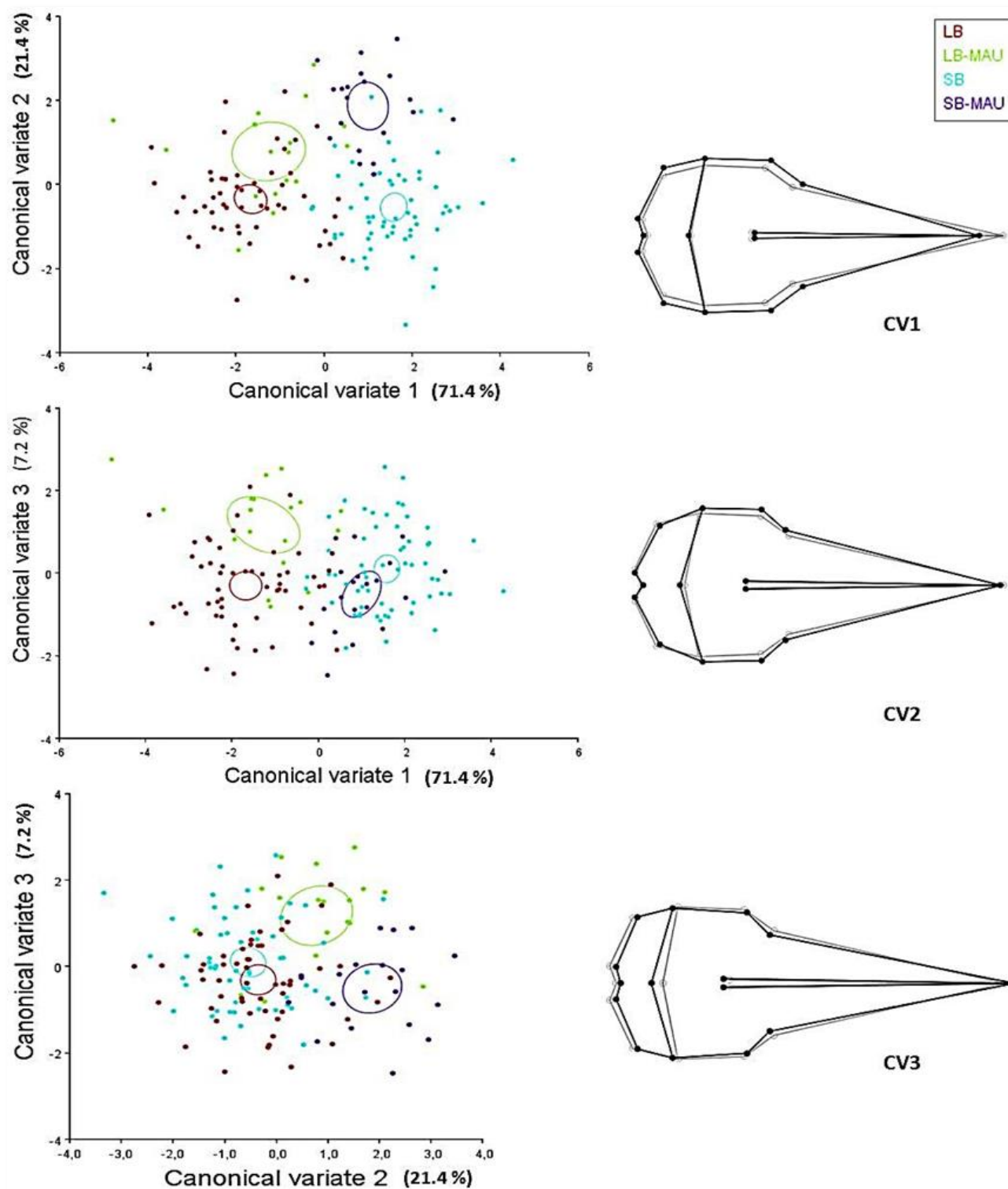


Figure 5

Scatterplots of canonical variates between common dolphin morphotypes: scatterplot of CVA of the corrected shape variables (mean frequency ellipses, with a 0.95 probability, were drawn around each group), and the schematic representation (wireframe graph) of the extreme points of the CV1, CV2, and CV3 (black: positive extreme point of the axis; light grey: negative extreme point of the axis) for the dorsal aspect of the skull of common dolphins; the percentage of variation explained among groups for each CV is given in parentheses.

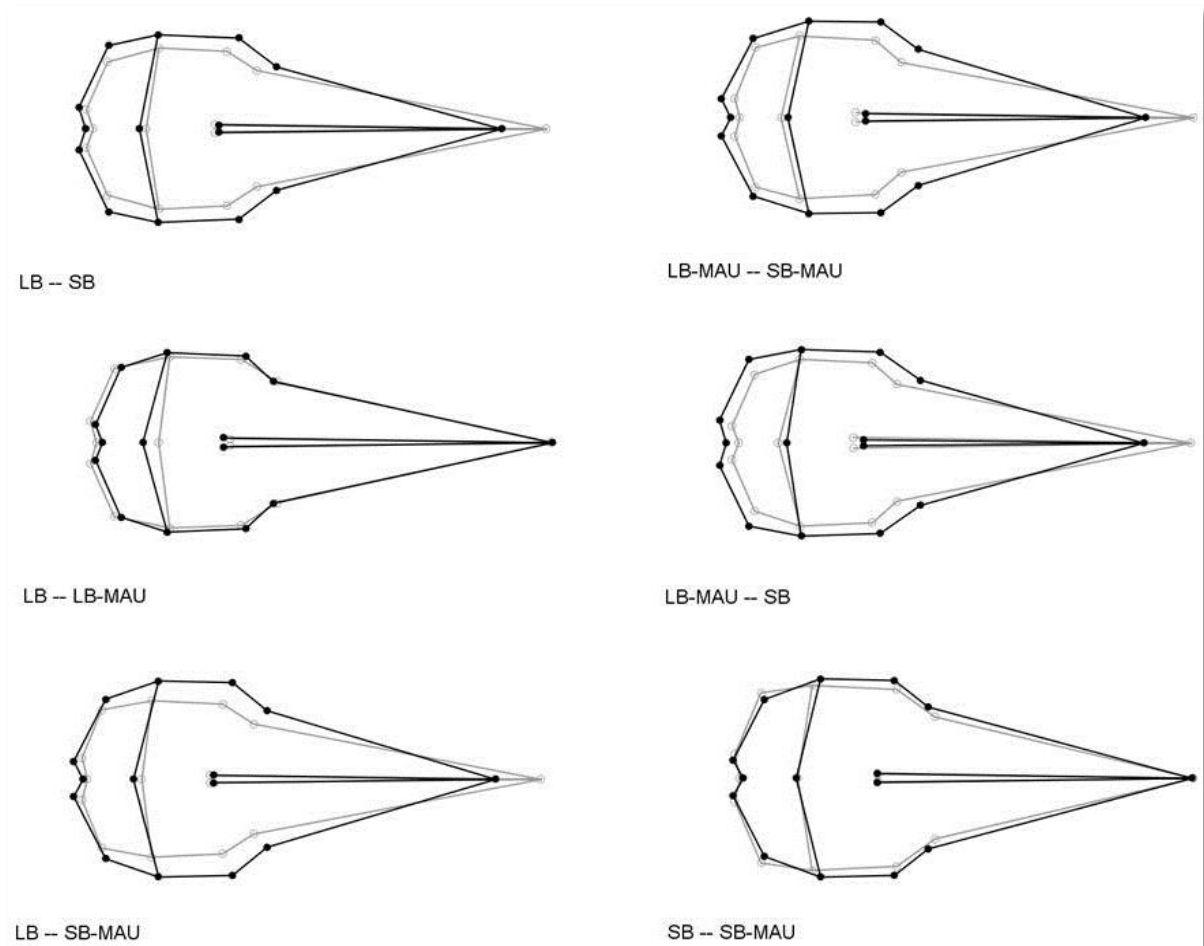


Output diagrams of DFA analyses are shown in Figure 6, which displays the pairwise comparisons between the consensus shape configurations for the skull of each common dolphin's population. The robustness of the cranium, the rostrum length, and the proportion of cranium to rostrum length are amongst the greatest variations of skull anatomy between common dolphin populations (Figure 6). Cross validated classifications assigned correctly 86.09% of all the common dolphin specimens investigated by their skull's shape (13.91% of the specimens were misclassified). Even though, misclassified specimens included all the possible pairs of groups, the specimens from the eastern sub-tropical Atlantic (LB-MAU and SB-MAU) were the groups with the lowest number of misclassifications (Table 2); and the cross-validated classifications assigning specimens to a given group only place one LB-MAU individual as belonging in fact to the SB-MAU morphotype. Contrarily, the *Delphinus delphis* (SB) and the *Delphinus capensis* (LB) exhibited the highest number of misclassifications.

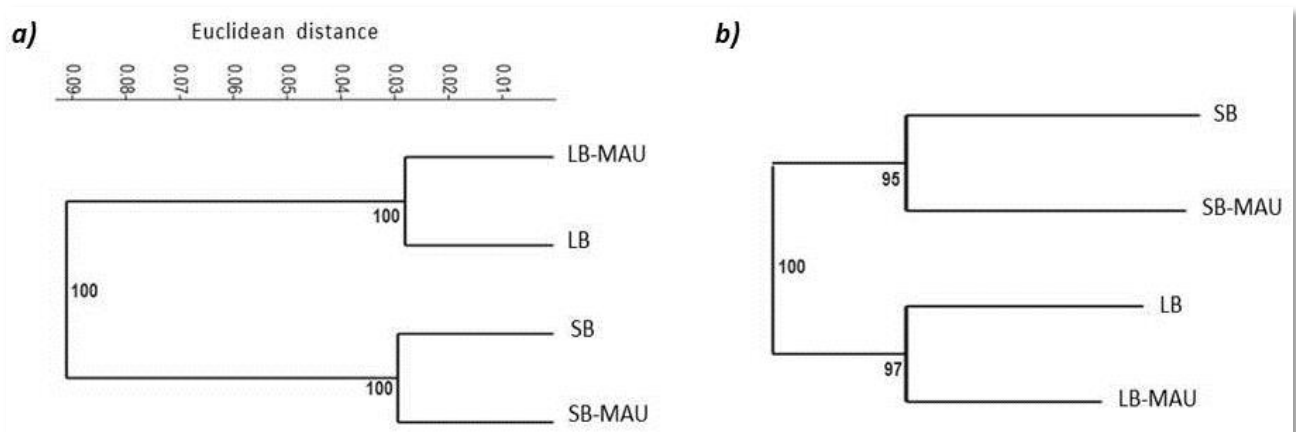
Both hierarchical cluster methods used, UPGMA and N-J, produced from mahalanobis distances (Figure 7), supported a closer morphological similarity between SB-MAU and *D. delphis* and between LB-MAU and *D. capensis*; despite the fact that previous analysis indicated that *Delphinus* spp. from MAU appears to be morphologically distinct from both the short-beak common dolphin *D. delphis* and the long-beaked common dolphin *D. capensis*.

Table 2 Cross validated classifications assigning common dolphin specimens to a given group according to the shape of the skull: the true group of the specimens, and the group where specimens were allocated to, derived from the DFA, for the dorsal aspect of the skull of common dolphins

True Group	Allocated to			
	SB	SB-MAU	LB-MAU	LB
SB		7	1	1
SB-MAU	2		0	0
LB-MAU	0	1		2
LB	1	1	5	

**Figure 6**

Inter-population pairwise comparisons of consensus configurations for the shape component of the skull: output diagrams of DFA analyses which show the magnitude and position of the shape differences between two group means (wireframe graph; corrected shape variables were used; light grey: first group of the pairwise comparison, black: second group of the pairwise comparison) for the dorsal aspect of the skull.

**Figure 7**

Morphometric relationships between common dolphin morphotypes: cluster analyses: (a) UPGMA dendrogram; (b) N-J phylogram. Node numbering represents bootstrap percentages from 10000



DISCUSSION

The present study investigated the taxonomic status of common dolphins (*Delphinus* spp.) from the eastern Sub-tropical North Atlantic Ocean (represented by MAU population). The main objective was to detect morphological variation in the size and shape components of the skull between the two recognized morphotypes of common dolphins inhabiting those waters and to explore the morphometric relationships between MAU sub-populations and well-established neighboring species, *D. delphis* and *D. capensis*. The taxonomic status of common dolphins has been investigated using morphometrics of the skull in several areas around the world (Heyning & Perrin, 1994; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010), but this is the first attempt at resolving the taxonomic uncertainties around the *Delphinus* complex in this area using geometric morphometric methods. Despite the simplicity, since only landmarks digitized on the dorsal view of the skull were analysed, it revealed to be highly informative about cranial components, potentially indicative of the recent adaptive evolution of the populations and species.

Rostral length/greatest zygomatic width ratio (RL/ZW) is the most widely used morphometric analysis to differentiate between forms of common dolphin. This ratio was first documented by Banks and Brownell in 1969 and later confirmed by several authors (Van Bree & Purves, 1972; Evans, 1982; Ross, 1984; Heyning & Perrin, 1994). Heyning and Perrin (1994) plotted rostrum length on zygomatic width using only mature animals and determined that the differences were not just size related but represented a true shape difference in the skulls of the two forms of common dolphin, the short- and long-beaked. These authors reported specific ranges for the RL/ZW ratio for the short-beaked common dolphin, *D. delphis* (1.23-1.47), and for the long-beaked common dolphin, *D. capensis* (1.52-1.77), from the coast of southern California. The majority of subsequent studies focusing on species identification using cranial morphology in common dolphins followed Heyning and Perrin (1994) guidelines (Van Waerebeek, 1997; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011).

Pinela *et al.* (2011) reported that the RL/ZW ratios for the Mauritanian animals are more similar to those of animals from the Atlantic Ocean than to those from the Pacific Ocean, but overall the range of variation is so large as to be only slightly smaller than the composite variation in RL/ZW ratios of the two North Pacific species, *D. delphis* and *D. capensis*, put together (Heyning & Perrin, 1994). The lower RL/ZW ratios were similar to those found typically in *D. delphis* from the eastern North Atlantic (Murphy *et al.*, 2006; Westgate, 2007), while the higher ratios were similar to those found in Central West Africa (Van Waerebeek, 1997), and South Africa, which have been attributed to *D. capensis* (Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005). Van Waerebeek (1997) separated common dolphins off central-west Africa (Senegal, Gabon, Angola and Congo-Brazzaville) based on key characteristics that separated the



two species (*D. delphis* and *D. capensis*) in the eastern north Pacific, which include cranial size, rostrum length relative to zygomatic width and tooth counts, with the purpose of assigning these specimens to species. Van Waerebeek (1997) assigned only two specimens to the *D. delphis*, one from Gabon and another from Angola, while the remaining 16 specimens were assigned to the *D. capensis*; concluding that the features analyzed fit very well with the distinctive cranial characteristics defined for *D. delphis* and *D. capensis* from the eastern Pacific (Heyning and Perrin, 1994; Van Waerebeek et al., 1994).

In relation to overall's skull size, the LB-MAU population presented no significant differences with the *D. delphis* (SB) from the north Atlantic coast of the Iberian Peninsula, but differed significantly from the SB-MAU population and the *D. capensis* (LB) from South Africa. Similarly, Murphy *et al.* (2006) reported that *D. delphis* specimens from the eastern North Atlantic were larger in size than the short-beaked common dolphins, *D. delphis*, from the southern Californian coast; and that several morphological characters such as minimum and maximum values for total body length, condylobasal, and rostrum lengths were also greater (Heyning & Perrin, 1994). Surprisingly, not only were eastern North Atlantic *D. delphis* larger in total body length than *D. delphis* off the southern Californian coast, their values overlapped with the majority of long-beaked common dolphins, *D. capensis*, from the same region (Murphy *et al.*, 2006). Nevertheless, despite the fact that common dolphins from the eastern North Atlantic were more similar in size to *D. capensis* than to *D. delphis* from the southern Californian coast, they had not developed rostra as long as those of the long-beaked form from the same region; and alveolar count data was within the range defined for *D. delphis* from the southern Californian coast (Murphy *et al.*, 2006).

In the present study, SB-MAU displayed the smallest overall skull size of all groups investigated. It has been proposed that in areas where the *D. delphis* lives sympatrically with the *D. capensis*, such as off the coast of California, the overall body and skull size may have decreased most likely due to character displacement (Perrin 1984). Then, it is not surprising that given the high potential for character displacement in sympatric species (Losos, 2000) *D. delphis* from California are smaller than other short-beaked common dolphin populations that live in allopatry (Westgate et al., 2007). The same could be happening off the NW African coast, where both putative forms also occur sympatrically (Van Waerebeek, 1997; Pinela *et al.*, 2011). Murphy *et al.* (2006) suggested that common dolphins in the eastern North Atlantic may have evolved a much larger size than that of *D. delphis* animals from other areas, such as the eastern North Pacific Ocean, the Black Sea, southern Australia, New Zealand, and the western North Atlantic. In the current study, the sort-beaked morphotype from NW Africa (SB-MAU) showed the smallest skull size, while *D. capensis* (LB) from South Africa presented the largest and also the greatest range values. Pinela *et al.* (2011) showed that even though the range of upper tooth count values overlapped slightly, the long-beaked morphotype from NW Africa showed a smaller range of upper tooth count values, indicative of a smaller skull, than that obtained for *D. capensis* from South Africa and California.



All groups presented morphological differences in the overall shape of the skull (Figure 6), and there was a clear segregation between the eastern Sub-tropical Atlantic populations of common dolphin (SB-MAU and LB-MAU), despite the fact that Pinela *et al.* (2011) found that individual rostral lengths of MAU common dolphins followed a cline with no subgrouping. This characteristic had also been reported in the Indo-Pacific common dolphins, *D. c. tropicalis*, with its extremely long rostrum, as one moves east or west from India (Jefferson & Van Waerebeek, 2002); and in southern Australia common dolphin *D. delphis*, as one moves from inshore/coastal to offshore/pelagic waters (Bell *et al.*, 2002). Delphinus sub-populations from NW Africa (MAU) are morphologically distinct, in terms of shape and form, from both *D. delphis* from the Atlantic coast of the Iberian Peninsula and *D. capensis* from the Atlantic coast of South Africa (despite the similarity in RL/ZW ratios range values, Pinela *et al.*, 2011). However, hierarchical cluster methods supported a closer morphological similarity between SB-MAU and *D. delphis*, and LB-MAU and *D. capensis*.

There are several studies that reports an overlap of skull size ranges within the species ranges reported for the Californian common dolphins (Van Bree & Purves, 1972; Van Waerebeek, 1997; Jefferson & Van Waerebeek, 2002; Bell *et al.*, 2002; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011), which led to the proposal of intermediate forms between *D. delphis* and *D. capensis*. Other similar studies report the same in other areas such as: Murphy *et al.* (2006), who reported that the range RL/ZW ratio was 1.31–1.57 in mature specimens of the *D. delphis* for the eastern north Atlantic Ocean (despite that 95% were less than 1.52, and consequently fall within the ratio range defined for *D. delphis*); Westgate (2007), who reported that an appropriate ratio for *D. delphis* from the whole north Atlantic would be 1.31–1.54 for males and 1.31–1.57 for females; Tavares *et al.* (2010), who reported three animals that presented intermediate RL/ZW between the two recognized species for the southwestern Atlantic; and Pinela *et al.* (2011), who also reported intermediate ratios for common dolphins from the eastern Subtropical Atlantic. Additionally, Samaai *et al.* (2005) also concluded that three common dolphin specimens from South Africa could belong to the short-beaked morphotype and not to the long-beaked morphotype as previously assumed. Similarly, in the present study, three specimens from the eastern Sub-tropical Atlantic were also removed from the dataset because they presented intermediate RL/ZW between the two recognized species.

In most areas outside southern California it has been practically impossible or extremely difficult to separate the genus *Delphinus* into two species based solely on morphological characters (Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011). The existence of intermediate RL/ZW measurements that fall between the ranges of the two recognized species of *Delphinus*, demonstrates a lack of affinity with Heyning and Perrin's (1994) proposition, and so we need to reconsider them in terms of their utility for species discrimination (Westgate, 2007). Nevertheless, it has been proposed in areas where intermediate forms of short- and long-beaked



morphotypes co-exist that local speciation events could be acting at the present to originate new species of *Delphinus* (Tavares *et al.*, 2010). Also, it is possible that more than two species of *Delphinus* spp. may exist worldwide, likely making the present taxonomy incorrect (Perrin *et al.*, 2009). Another hypothesis that has been suggested is that maybe the genus *Delphinus* is a 'ring species', *i.e.* a connected series of neighboring populations, subspecies connected by clines, maybe sexually or genetically isolated from each other, though there is a potential gene flow between them (W.F. Perrin, *personal communication*, In Tavares *et al.*, 2010).

Globally, there is a tremendous amount of morphological variability within the genus *Delphinus*, especially in elements associated with the rostrum, suggesting that common dolphin's skull differentiation could be related to niche segregation and not to speciation (Pinela *et al.*, 2011). Future endeavors to define common dolphin species using morphometrics of the skull should attempt to take this variation into consideration (Westgate, 2007).

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DISCUSSION & CONCLUSIONS





DISCUSSION

The present thesis investigates the taxonomy, morphology, habitat use, and distribution of the common dolphin (genus *Delphinus*) in the eastern Sub-tropical Atlantic Ocean (NW Africa), thus adding to the knowledge about the genus in this poorly studied area and contributing to the general understanding of the biology and taxonomy of the species worldwide.

MARINE TROPHIC WEB

We investigated the marine trophic web of the eastern Sub-tropical Atlantic Ocean, including the relationship between apex predators (marine mammal species, including common dolphins) and representatives from other trophic levels using stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) following procedures previously established (Hobson & Welch, 1992).

As is a common finding, values of $\delta^{13}\text{C}$ increased from pelagic to benthic species, and $\delta^{15}\text{N}$ increased with trophic level. However, results revealed a large variability in $\delta^{15}\text{N}$ values among macrophyte species from the same region and also between macrophytes and phytoplankton, with differences often larger than the average increase expected per trophic level (Minagawa & Wada, 1984; Caut *et al.*, 2009). As a consequence, variability in the $\delta^{15}\text{N}$ values of marine mammal bone could be reflecting not only differences in trophic level but also shifts among foraging grounds.

Differently from $\delta^{15}\text{N}$, the $\delta^{13}\text{C}$ of primary producers from Mauritania varied more consistently among trophic groups, with phytoplankton less enriched in ^{13}C than macroalgae, and macroalgae less enriched in ^{13}C than seagrasses. This allowed us to interpret the enrichment in ^{13}C of the bone of marine mammals as evidence of more coastal foraging habitats. The complexity of the oceanographic and biochemical processes in marine ecosystems makes the isotopic dynamics of marine food webs harder to interpret than that of terrestrial ecosystems (Michener & Schell, 1994). Understanding of the factors that influence the isotopic signals of organisms and isotopic landscapes optimizes the effectiveness of this methodology in the study of marine environments (West *et al.*, 2006).

The results obtained indicate that the marine mammal species studied inhabit mostly the outer continental shelf and the upper slope, with the Atlantic humpback dolphin (*Sousa teuszii*) and the Mediterranean monk seal (*Monachus monachus*) occupying the more coastal areas. Given that the latter species is highly



endangered, these results point to the need of management efforts to reduce the impact of artisanal and industrial bottom trawling fishing. This includes the regular monitoring in the area of the fish and cephalopod populations that constitute their diet because excessive catches on certain species may lead to cascading effects on the system, eventually affecting other species in the food web (Walters & Kitchell, 2001).

DISTRIBUTION AND HABITAT USE

Along the Mauritanian coast, the long-beaked and short-beaked morphotypes of common dolphins did not shown differences in their area of occupation. The sightings data from Mauritania are scarce and opportunistic, but do not produce evidences of distribution or segregation (Robineau & Vély, 1998). This was supported by the isotopic results obtained in Chapter 1 that show that in Mauritania common dolphins are seldom distributed inshore.

In the eastern Sub-tropical Atlantic Ocean, common dolphins (*Delphinus* spp.) presented the lowest $\delta^{15}\text{N}$ values of all marine mammals, which is in accordance to what was observed in other marine trophic webs (Das *et al.*, 2003; Walker & Macko, 1999). According to our results presented in Chapter 1, the discrepancies in $\delta^{15}\text{N}$ values compared to other pelagic marine mammals possibly reflect the higher proportion of low trophic level prey, such as *Sardinella aurita*, in their diet. The large variability in stable isotope values found within populations of common dolphins in Mauritania as well as in other areas, as discussed in Chapter 2, suggests substantial individual-specific dissimilarities in the exploitation of food resources.

Also, the large variability in stable isotopes described in Chapter 3 suggests substantial differences between morphotypes in terms of distribution and, most likely, in habitat. $\delta^{15}\text{N}$ correlated with rostrum length, indicating that longer-beaked individuals either feed at a higher trophic level than those shorter-beaked and/or they exploit mostly an offshore-pelagic habitat because, as reported in Chapter 1, levels of $\delta^{15}\text{N}$ in primary producers are higher (ca 5‰ $\delta^{15}\text{N}$) offshore than inshore, while those of $\delta^{13}\text{C}$ follow the opposite trend. Overall, results obtained in Chapter 2 and 3 show that individuals with longer beaks tend to feed at higher trophic levels than animals with shorter beaks, both in Mauritania and elsewhere. Thus, the long-beaked common dolphin from the Gulf of California showed the highest $\delta^{15}\text{N}$ values followed by the north-west African population, due probably to the presence of the long-beaked morphotype. Variation in rostral morphology of common dolphins, and consequently in their morphological characters related to feeding, is usually associated to dissimilarities in feeding ecology and diet (Evans, 1982; Murphy *et al.*, 2006).



$\delta^{13}\text{C}$ failed to show any correlation with rostral length, a result that can be attributed to two potential causes. The first is the large variability between individuals, which may have masked the effect of trophic level on $\delta^{13}\text{C}$. The second may be that the slight incremental effect that trophic level produces on $\delta^{13}\text{C}$ is offset by an allopatric distribution of the morphotypes, the shorter-beaked individuals inhabiting waters closer to coast than those longer-beaked. However, this hypothesis would be inconsistent with previous observations in Chile (Sanino *et al.*, 2003) and Baja California (Niño-Torres *et al.*, 2006) that indicate that the long-beaked form is more coastal than the short-beaked (Perrin, 2009). Indeed, in the northeastern Pacific Ocean, the region of origin of the specimens used for the differentiation between *D. delphis* and *D. capensis* (Heyning & Perrin, 1994; Rosel *et al.*, 1994), the long-beaked form, also known as Baja neritic, shows a well-known preference for shallower waters (inside the 180-meter isobath) than the short-beaked form (Leatherwood *et al.*, 1982). On the other hand, Bell *et al.* (2002) found similar findings to ours in *Delphinus delphis* in southern Australia. Variability in skull morphometrics was very large and dolphins with larger skulls and longer rostra tended to distribute farther away from the coast than those with smaller skulls and shorter rostra.

Although in species feeding on a wide variety of prey diet composition is difficult to establish only through stable isotope analysis (Borrell *et al.*, 2012), inference of the trophic level at which they feed is possible by comparing ^{15}N step-wise enrichment values (Kurle & Worthy, 2001; Post, 2002). In Chapter 2, trophic positions were successfully determined for the common dolphin populations of the North-eastern and eastern Subtropical Atlantic, accounting for the local marine ecosystem baselines. Previously, trophic levels of common dolphins had only been determined through stomach content analysis in a couple of studies (Pauly *et al.*, 1998b; Pusineri *et al.*, 2007; Ambrose *et al.*, 2013). We examined trophic behaviour of several populations around the world, and found them to be exploiting different trophic levels and showing large plasticity to adapt to the local variations in which they live. The resultant trophic levels were consistent with data on stomach contents and behaviour (Pauly *et al.*, 1998b; Bode *et al.*, 2003; Niño-Torres *et al.*, 2006). Our results also placed common dolphins between the third and the fourth trophic levels in most ecosystems, which implies that they prey on secondary consumers like sardines (Pauly *et al.*, 1998b; Bode *et al.*, 2003). This suggests a relatively large degree of omnivory and also that they are opportunistic feeders taking advantage of local abundance of prey (Hassani *et al.*, 1997).

No significant differences were detected between sampling decades (Chapter 2), which led us to assume that the current distribution, prey preferences and habitat use of individuals is rather constant in the study area. The isotopic data presented here did not allow a precise reconstruction of the diet composition because not all potential prey could be identified.



COMMON DOLPHIN MORPHOTYPES

Heyning and Perrin (1994) proposed that more than one form of common dolphin might be present in North-west Africa, as was the case in the coast of California. Later, Van Waerebeek (1997) confirmed that two common dolphin morphotypes did indeed exist off the central West African coast; despite they did not investigate the area north of Senegal. Here, we confirm that both common dolphin morphotypes, the short- and long-beaked forms, do occur in Mauritanian waters.

As shown in Chapter 2 and 3, the variability of $\delta^{15}\text{N}$ values was particularly high in common dolphins from the north-west coast of Africa, a finding probably related to the presence of the two morphotypes in this area as opposed to areas further north, where only the short-beaked morphotype is present. In Chapter 3, common dolphin skulls were analysed using the most informative measurements: the rostral length to zygomatic width ratio (RL/ZW) and the upper tooth count (UTC). In the coast of California, an analysis like this allowed the separation of the two forms into different species: *Delphinus delphis* and *Delphinus capensis* (Heyning & Perrin, 1994). In that study, the range of variation was also very large. The morphology of the skull of common dolphins from our study area was also compared to that of the well-studied species of *D. delphis* and *D. capensis* (Chapter 4). In Chapter 5 the specimens from Mauritania were divided into two groups applying the RL/ZW ranges that had been defined in California to separate species. Through several statistical analyses, we observed that the two groups previously selected were similar one to the short-beaked morphotype and another to the long-beaked, allowing us to infer the potential occurrence of two subpopulations in the area.

MORPHOLOGICAL VARIATION

In Chapter 3 we analysed several morphometric measurements of the skull that were previously used to distinguish common dolphin morphotypes or to define species in several areas around the world. These included, as previously stated, the RL/ZW and UTC, which have been reported as the most diagnostic measurements for discriminating between short-beaked and long-beaked forms of common dolphins (Banks & Brownell, 1969; Van Bree & Gallagher, 1978; Amaha, 1994; Evans, 1994; Heyning & Perrin, 1994; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Westgate, 2007; Tavares *et al.*, 2010) and that also allowed Heyning and Perrin (1994) to separate the genus into *D. delphis* and *D. capensis* off the coast of California. We provided a review of the range values of these morphometric measurements for common dolphin populations worldwide and compared them to the Mauritanian population.



Variability in relative beak lengths for the studied population was very large when compared with other worldwide populations (Heyning & Perrin, 1994; Van Waerebeek, 1997; Murphy *et al.*, 2006; Westgate, 2007), especially in regions where only one of the species is present. The overall range of variation in RL/ZW ratios for the Mauritanian animals is so large as to be only slightly smaller than the overall variation found in the two North Pacific species, *D. delphis* and *D. capensis*, together (Heyning & Perrin, 1994). The lower RL/ZW ratios were similar to those typically found in *D. delphis* from the eastern North Atlantic (Murphy *et al.*, 2006; Westgate, 2007), while the higher ratios were similar to those found in Central West Africa (Van Waerebeek, 1997) and South Africa, which have been assigned to *D. capensis* (Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005). However, a clinal variation in relative rostrum size was detected, which had also been observed in the Indo-Pacific common dolphin *Delphinus capensis tropicalis*, which has an extremely long rostrum (Jefferson & Van Waerebeek, 2002), and in the southern Australia common dolphins *Delphinus delphis* (Bell *et al.*, 2002). The Australian specimens showed a larger range of variation in RL/ZW ratios when compared with animals belonging to the same species, *D. delphis*, from the North Pacific and North Atlantic (Heyning & Perrin, 1994; Bell *et al.*, 2002; Murphy *et al.*, 2006; Westgate, 2007). Tooth count per upper tooth row also showed slightly greater variability compared with that of *D. delphis* from the North Atlantic, southern Australia or even California.

In Chapter 4, geometric morphometric (GM) results have shown significant differences in both the size and shape components of the skull of common dolphin populations from the different geographical areas in the eastern Atlantic Ocean. These differences followed a geographical trend in shape which is particularly sharp between the two extreme locations, the Iberian Peninsula (*Delphinus delphis*) and South Africa (*Delphinus capensis*). The skull size of common dolphins from South Africa was significantly different from that of the other two populations studied and largely concordant with previous studies (Amaha, 1994; Heyning & Perrin, 1994; Bell *et al.*, 2002; Murphy *et al.*, 2006). Similarly to our results, *D. capensis*, from both the coast of California and South Africa, presented a larger overall skull size than *D. delphis*; the skull of the long-beaked form was absolutely longer but relatively narrower than in the short-beaked form, which was relatively shorter but broader (Heyning & Perrin, 1994).

D. capensis is the most different with regards to cranial shape. It has a skull characterized by the longest and narrowest rostrum and the narrowest braincase, while *D. delphis* is characterized by a shorter rostrum and a more robust rostrum and cranium with a larger braincase. Common dolphins from Mauritania are characterized by a shape intermediate to the Iberian Peninsula *D. delphis* and the South African *D. capensis*, despite being statistically differentiated from both populations. In delphinids, moderate to strong patterns of morphological variation over sometimes short geographical distances seem to be typical (Perrin, 1984). Similarly, Jefferson and Van Waerebeek (2002) concluded that common dolphins from Australia did not fit neatly with the known descriptions of either *D. capensis* or *D. delphis*; despite that



these common dolphins were considered to be morphologically close to the *D. delphis* from California and thus assigned to the *Delphinus delphis* species.

The rostral elements of the skull are thought to be influenced by selective pressures associated with feeding (Perrin, 1975). This part of the skull seems to be relatively plastic, and consequently to change reflecting variations in feeding ecology. Our results also show that the rostral apparatus is the part of the skull that exhibits larger variability between populations. Though marine species are usually continuously distributed, marine ecosystems are characterized by local differences in key features (*e.g.* temperature, salinity, bathymetry, food items, etc.) that promote niche specialization and morphological differentiation (Natoli *et al.*, 2006; Pinela *et al.*, 2011, 2015). However, with the present level of information it is difficult to say whether differences in feeding ecology are responsible for the rostral variation or other factors are in place, such as intraspecific aggression during mating or occurrence of sperm competition (Murphy *et al.*, 2005; Westgate, 2007; Westgate & Read, 2007).

Morphometric analysis are a relevant contribute to the genetic, ecological and ethological results to understand the mechanism of morphological differentiation and population structure (Adams *et al.*, 2004; Cardini *et al.*, 2009; Natoli *et al.*, 2008). Our results raise the need to include other species and populations of common dolphins in the analyses to better elucidate the geographical pattern of morphological variation, as well as the adaptive traits involved.

TAXONOMIC STATUS

In the study area we found individuals that could be ascribed to one or the other of the *Delphinus* morphotypes, but neither the morphometric nor the isotopic signatures support the hypothesis of more than a single species of common dolphins. Evidences suggest that the area is inhabited by a population with intrinsic large variation in both body proportions and foraging behavior. We can thus conclude that both the long-beaked and short-beaked forms of common dolphin do occur off Mauritania but, contrarily to the coast of California, their occurrence appears to reflect differential use of habitat, not speciation.

The taxonomic status of common dolphins has been investigated through skull morphometrics in a number of geographical regions (Heyning & Perrin, 1994; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010), but this is the first study in the eastern Subtropical Atlantic that has done so using geometric morphometric methods. The other studies followed Heyning and Perrin (1994) guidelines (Van Waerebeek, 1997; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011),



which essentially consisted in plotting rostrum length on zygomatic width using only mature animals to determine that any existing difference was not due to size but to true shape differences.

In Chapter 5, results indicated that the cranial components analysed were highly informative and that potentially indicated recent adaptive evolution of the populations and species. All taxonomic groups investigated presented significant morphological differences in the shape and form (size + shape) of the skull. Additionally, there was a clear segregation between the eastern Sub-tropical Atlantic populations of common dolphins (short- and long-beaked morphotypes). Contrarily, Van Waerebeek (1997) concluded that the features analyzed in the skull of specimens from central-west Africa fitted very well the distinctive cranial characteristics defined for *D. delphis* and *D. capensis* from the eastern Pacific (Heyning & Perrin, 1994). *Delphinus* populations from NW Africa are morphologically distinct, in terms of shape and form, from both *D. delphis* from the Atlantic coast of the Iberian Peninsula and *D. capensis* from the Atlantic coast of South Africa (despite the similarity in RL/ZW ratios range values with both species verified in Chapter 3; Pinela *et al.*, 2011). However, results of the hierarchical clusters supported a closer morphological similarity between the Mauritanian short-beaked morphotype and *D. delphis*, and the Mauritanian long-beaked morphotype and *D. capensis*.

In relation to overall's skull size, Chapter 5 also shows that the Mauritanian long-beaked population presented no significant differences with *D. delphis*, but differed significantly from the Mauritanian short-beaked population and *D. capensis*. Similarly, Murphy *et al.* (2006) reported that common dolphins from the eastern North Atlantic were more similar in size to *D. capensis* than *D. delphis* from the southern Californian coast, even though they do not show a so long rostra as those of the long-beaked form, and the alveolar count was within the range defined for *D. delphis* from the same region. In the present study, the Mauritanian short-beaked morphotype displayed the smallest overall skull size of all populations and species investigated, while the *D. capensis* presented the largest overall skull size and the largest range of values. The long-beaked morphotype from NW Africa showed a smaller range of UTC values than that obtained for *D. capensis* from South Africa and California (Pinela *et al.*, 2011), indicative of a smaller skull.

It has been proposed that in areas where *D. delphis* lives sympatrically with *D. capensis* the overall body and skull size may have decreased, most likely due to character displacement (Perrin 1984). Therefore, given the high potential for character displacement in sympatric species (Losos, 2000) it is not surprising that *D. delphis* from California is smaller than other short-beaked common dolphin populations that live in allopatry (Westgate *et al.*, 2007). The same could be happening off the NW African coast, where both putative forms also occur sympatrically (Van Waerebeek, 1997; Pinela *et al.*, 2011).

Several studies have described an overlap with the species ranges similar to that reported for the Californian common dolphins (Van Bree & Purves, 1972; Amaha, 1994; Van Waerebeek, 1997; Jefferson &



Van Waerebeek, 2002; Bell *et al.*, 2002; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011). This has led to the proposal of intermediate forms between *D. delphis* and *D. capensis*. In the present study (Chapter 3), intermediate ratios for common dolphins from the eastern Subtropical Atlantic are also reported (Pinela *et al.*, 2011) and three specimens from Mauritania had to be removed from the dataset because they presented intermediate RL/GZW between the two recognized species (Chapter 5).

In most areas outside California it has been very difficult to separate the genus *Delphinus* into two species based only on morphological characters (Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011). The existence of intermediate RL/ZW ratios between the two recognized species of *Delphinus* appears to contradict Heyning and Perrin's (1994) proposal, demanding for a revision of their species discrimination. It has been proposed that local speciation events could be acting at the present to originate new species of *Delphinus* in areas where intermediate forms of short- and long-beaked morphotypes co-exist (Tavares *et al.*, 2010). Also, it is possible that more than two species of *Delphinus* spp. may exist worldwide, likely making the present taxonomy incorrect (Perrin *et al.*, 2009). Another hypothesis may be that the genus *Delphinus* could be a 'ring species', *i.e.* a series of interconnected neighboring populations with gene flow between them but isolated in the extremes (W.F. Perrin, *personal communication*, In Tavares *et al.*, 2010).

Several authors suggested that geographical variation in the rostral morphology of common dolphin's, and consequently in their feeding apparatus, could indicate variations in trophic ecology, most likely due to differences in diet and habitat use (Evans, 1982; Amaha, 1994; Murphy *et al.*, 2006). Nevertheless, and despite the need for further genetic analyses, our data together with the genetic study conducted by Natoli *et al.* (2006) suggest that common dolphin's skull differentiation might be related to niche segregation and not to speciation. We conclude that taxonomic splitting appears not justified in the study region.

To conclude, the existence of two putative species (*D. delphis* and *D. capensis*) in the Northwest coast of Africa should be questioned until further research is conducted on genetics and other taxonomically relevant morphometric measurements. Both isotopic and morphometric data seem to indicate that there are at least two populations of common dolphin in the study area. Finally, findings call for caution when assuming that long-beaked and short-beaked common dolphins outside the eastern North Pacific fall into the taxonomic model described for that region by Heyning and Perrin (1994) and Rosel *et al.* (1994).



CONCLUSIONS

- 1** Analysis of the isotopic landscape of the Mauritanian marine trophic network allowed to distinguish between species exploiting coastal versus pelagic foraging habitats and differences in trophic levels.
- 2** Most of the marine mammal species studied in Northwest Africa distribute in the outer continental shelf and the upper slope. Common dolphins are rarely distributed inshore and display a typical oceanic behaviour.
- 3** The presence of both short- and long-beaked morphotypes of common dolphins was confirmed off the coast of Mauritania.
- 4** Stranding data did not reveal spatial segregation between short- and long-beaked morphotypes along the Mauritanian coast. In this region, the short-beaked form inhabits waters closer to coast and feeds at a lower trophic level than the long-beaked form.
- 5** With the exception of baleen whales, common dolphins showed the lowest trophic level of all marine mammals analyzed despite the fact that there was large variability between individuals in the exploitation of food resources, possibly to adapt to local environmental variations.
- 6** The rostral apparatus presented the largest variability within skull morphometrics. Variation in relative beak length in common dolphins from Northwest Africa was



larger than other *Delphinus* populations worldwide. In this area we found clinal variation in relative rostrum size and intermediate ratios, as opposed to other regions.

- 7 There were significant differences in size and shape components of the skull between common dolphin populations and species. The skull of the short-beaked morphotype from Northwest Africa was shorter but broader than that of the long-beaked one.
- 8 Despite significant differences, there is a close morphological similarity between the short-beaked morphotype of Northwest Africa and *Delphinus delphis*, and between the long-beaked morphotype of the same region and *Delphinus capensis*.
- 9 Taxonomic splitting of common dolphins in the study area into two putative species should be postponed until further research is conducted, particularly on genetics, since skull differentiation could be related to niche segregation and not to speciation.
- 10 Findings show that the taxonomic model described for the Northeast Pacific should not be generally applied to other areas where the short- and long-beaked morphotypes co-habit.

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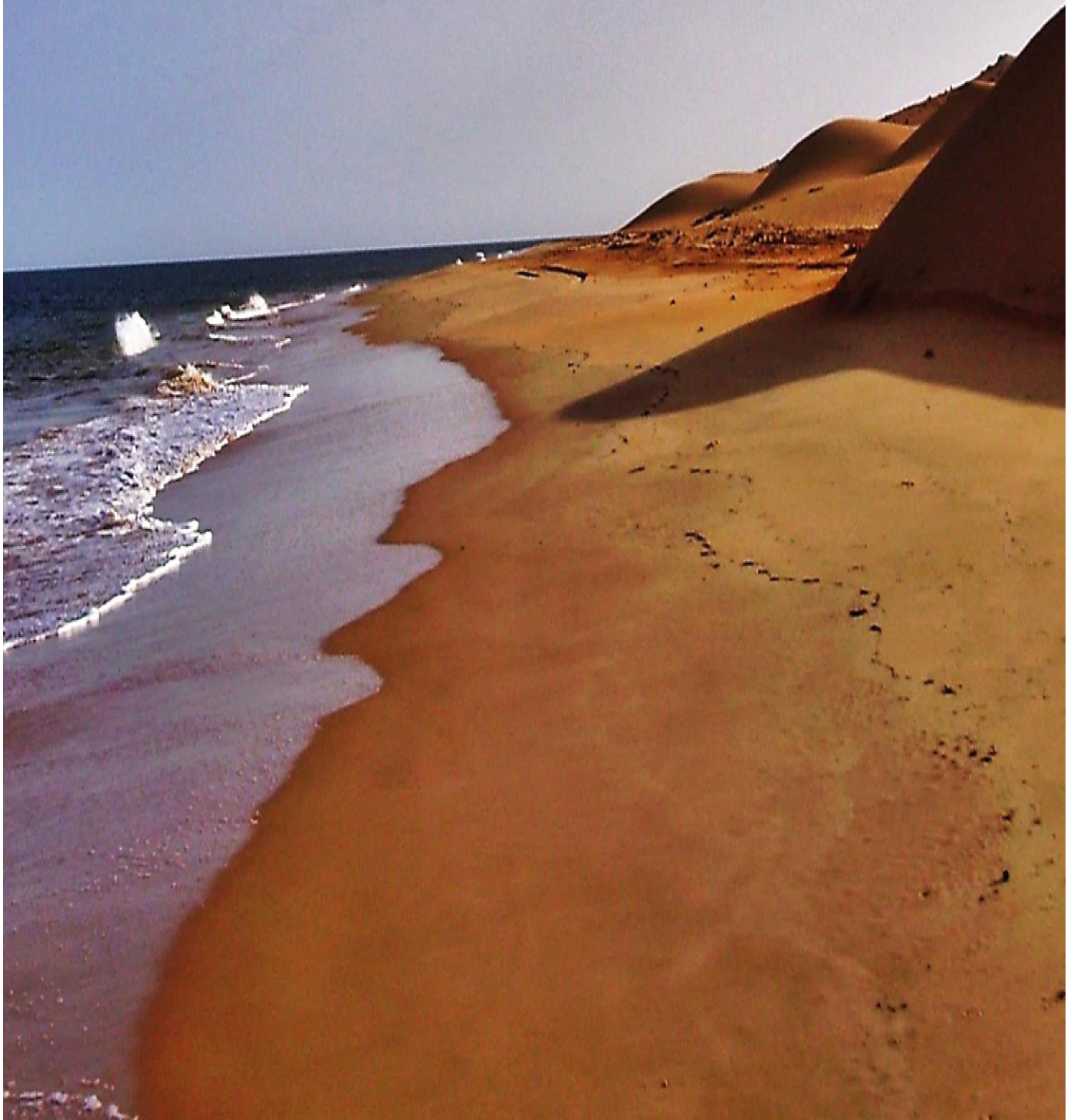


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SPANISH SUMMARY





INTRODUCCIÓN

TAXONOMÍA Y DISTRIBUCIÓN

El orden de los cetáceos es un taxón relativamente moderno, con 86 especies reconocidas actualmente (página web de la UICN). Los delfines pertenecen a la superfamilia *Delphinoidea* y a la familia *Delphinidae*, que se cree que evolucionaron a finales del Mioceno, hace cerca de 12 millones de años (Barnes *et al.*, 1985). Los delfines han evolucionado en aguas marinas y dulces, y se extendieron por el mundo teniendo únicamente como limitación la temperatura del agua, cuyos rangos difieren para cada especie. La familia *Delphinidae* es la más diversa de todas las familias de cetáceos ya que está compuesta por 35 especies que se distribuyen en todos los océanos y mares del mundo (Leduc, 2009).

El delfín común (*Delphinus* género) se encuentra en los océanos Atlántico, Pacífico e Índico, donde ocupa aguas frías, templadas y subtropicales (Evans, 1994; Heyning & Perrin, 1994). También hay poblaciones de delfines comunes que viven en cuencas cerradas, como por ejemplo el Mar Negro, y en cuencas semi-cerradas, como el Mar Mediterráneo, el Golfo de California, el Mar de Japón, el Golfo de Tailandia, el Golfo Pérsico y el Mar del Caribe (Heyning & Perrin, 1994; Jefferson *et al.*, 2008; Perrin, 2009). Esta enorme gama de distribución ha conducido a la diferenciación de un número de morfotipos que, hasta hoy, quedan por determinar taxonómicamente (Banks & Bronwell, 1969; Evans, 1982; Heyning & Perrin, 1994; White, 1999; Natoli *et al.*, 2006). Aunque en el pasado se han propuesto una serie de especies y subespecies para diferentes áreas, en general se ha considerado, hasta hace algunas décadas, que todos los tipos pertenecían a la misma especie, *Delphinus delphis* (Hershkovitz, 1966). En muchas áreas, sin embargo, dos morfotipos fueron distinguidos: una forma de morro largo o nerítica, en su mayoría de distribución costera; y una forma de morro corto, tanto de distribución pelágica como costera (Banks & Bronwell, 1969; Evans, 1982; Heyning & Perrin, 1994; Natoli *et al.*, 2006; Rosel *et al.*, 1994).

Actualmente, el género *Delphinus* se compone de dos especies y cuatro subespecies: el delfín común de morro corto, *Delphinus delphis* Linnaeus 1758, distribuido en la plataforma continental y en las aguas pelágicas de los océanos Atlántico y Pacífico; el delfín común de morro largo, *Delphinus capensis* Gray 1828, con distribución costera y en aguas tropicales y templadas de los océanos Pacífico y Atlántico Sur; el delfín común del Mar Negro, *Delphinus delphis ponticus* Barabash 1935, cuya distribución se limita al Mar Negro; y el delfín común del Indo-Pacífico, *Delphinus capensis tropicalis* Van Bree 1971, distribuido en las aguas cálidas y templadas de los océanos Pacífico e Índico (IWC, 2009; Perrin, 2009). Esta



clasificación se basa tanto en la morfología interna de los especímenes, como los caracteres craneales (por ejemplo, longitud del morro, longitud rostral/anchura cigomática, y el número de dientes) como en la morfología externa, como los patrones de longitud total del cuerpo y de coloración (Heyning & Perrin, 1994; Jefferson & Van Waerebeek, 2002).

Las diferencias entre las formas de morro corto y largo, basado en la relación entre la longitud del rostro y la anchura cigomática (RL/ZW), fueron encontradas por primera vez por Banks & Bronwell (1969), concluyendo que los especímenes con una proporción por encima de 1.55 se deberían asignar a *Delphinus bairdii* y por debajo de 1.53 a *D. delphis*. Van Bree & Purves (1972) encontraron algunos ejemplares con proporciones intermedias de RL/ZW, cuando se tomaban muestras de delfines comunes de otras cuencas oceánicas. Sin embargo, no utilizaron sólo animales maduros y los especímenes no fueron separados por sexos (Banks & Bronwell, 1969; Van Bree & Purves, 1972), lo que condujo a errores debidos a dimorfismo sexual, variación ontogenética, y variación geográfica con potenciales diferencias específicas y sub-específicas (Heyning & Perrin, 1994). Los descubrimientos de Banks & Brownell (1969) fueron posteriormente confirmados por Evans (1982) mediante el análisis multivariante de las medidas del cráneo en animales maduros.

Heyning & Perrin, en 1994, volvieron a analizar el RL/ZW de animales maduros de *Delphinus capensis* de Sudáfrica (Ross, 1984) y encontraron un rango de entre 1.59 a 1.76, mostrando que los animales sudafricanos tienen morros más largos que los del sur de California. En la actualidad, sin embargo, se cree que ambos morfotipos de delfín común habitan aguas de Sudáfrica (Best, 2007). En esta área, las medidas morfológicas varían como en otras partes del mundo, pero las evidencias genéticas no apoyan, hasta el momento, que los diferentes morfotipos correspondan a diferentes especies (Samaai *et al.*, 2005), contrariamente a lo encontrado en el Pacífico Norte oriental, donde el género se separó en dos especies: *Delphinus delphis*, o delfín común de morro corto, y *Delphinus capensis*, o delfín común de morro largo (Heyning & Perrin, 1994; Rosel *et al.*, 1994; Kingston & Rosel, 2004).

Heyning & Perrin (1994) sospecharon que las dos formas podrían ocurrir simpátricamente en la costa occidental de África, al igual que en la costa de California. Se ha demostrado que los dos morfotipos más comunes de delfín común (de morro corto y largo) ocurren en la costa atlántica central de África (Van Waerebeek, 1997). Se cree que el delfín común de morro corto se extiende desde el Atlántico norte hasta el sur de Senegal (Heyning & Perrin, 1994; Van Waerebeek, 1997; Rice, 1998), mientras que el de morro largo se encuentra presente desde Mauritania hasta Sudáfrica (Amaha, 1994; Van Waerebeek, 1997; Rice, 1998; Samaai *et al.*, 2005). Por lo tanto, las aguas de Mauritania son, aparentemente, una región de solapamiento en la distribución geográfica de los dos morfotipos como en California (Heyning & Perrin, 1994).



Más tarde, se examinaron algunos especímenes de delfín común del noroeste de África (Natoli *et al.*, 2006), y se encontraron indicios de que eran genéticamente distintos a los morfotipos del Pacífico Norte. El ADN mitocondrial, junto con el análisis del ADN nuclear, sin embargo, no apoyó la hipótesis de un único linaje de morro largo en todo el mundo (Natoli *et al.*, 2006). No se encontraron haplotipos compartidos ni mutaciones fijas entre las poblaciones de delfín común de morro largo analizadas (Sudáfrica, Pacífico y Mauritania; Natoli *et al.*, 2006). A nivel más local, se plantearon preguntas acerca de la morfología y la identidad del género *Delphinus* en el noroeste de África.

La distribución mundial del género *Delphinus* todavía no está bien establecida debido a las incertidumbres taxonómicas (Rice, 1998). Aunque la distinción entre las dos especies se hizo con especímenes del Pacífico Norte, en general se supone que esta discriminación específica podría extenderse a los dos morfotipos de delfines comunes que habitan todos los océanos (Banks & Bronwell, 1969; Evans, 1982; Heyning & Perrin, 1994; Rosel *et al.*, 1994). Jefferson *et al.* (2009) concluyeron que debe tenerse muchas precauciones en la identificación de delfines comunes de morro largo de apariencia similar, y que la aceptación acrítica de los registros puede conducir a suposiciones incorrectas acerca de los rangos de las especies implicadas.

Hubo varios estudios internacionales que reportaron una superposición con los rangos de RL/ZW de las dos especies descritas en California (Van Bree & Purves, 1972; Amaha, 1994; Van Waerebeek *et al.*, 1994; Van Waerebeek, 1997; Jefferson & Van Waerebeek, 2002; Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010), dando lugar a la posibilidad de la existencia de formas intermedias entre *D. delphis* y *D. capensis*. A pesar de que el concepto de dos especies tuvo una amplia aceptación en la comunidad científica que estudia mamíferos marinos, recientemente esta tesis ha sido re-evaluada, lo que lleva a los investigadores a reexaminar los especímenes de *Delphinus* spp. de varias partes del mundo. Se ha propuesto, en las zonas donde las formas intermedias de morfotipos de morro corto y largo coexisten, que eventos locales de especiación podrían estar actuando, en la actualidad, para originar nuevas especies de *Delphinus* (Tavares *et al.*, 2010). Según Perrin (2009), es posible que más de dos especies de *Delphinus* spp. puedan existir en todo el mundo. Si esto se demuestra ser cierto debería revisarse la taxonomía actual.

ECOLOGÍA Y USO DE HÁBITAT

La especialización de recursos, tal como el hábitat o la disponibilidad local de alimentos, puede conducir a la diferenciación intraespecífica en especies de cetáceos (Hoelzel, 1998). Como depredadores, los cetáceos son consumidores de gran parte de los niveles tróficos, jugando un papel muy importante en



los ecosistemas marinos (Bowen, 1997; Pauly *et al.*, 1998a). Su conexión con la mayoría de los niveles de la cadena trófica marina los hace más propensos a experimentar los efectos de los cambios en el medio ambiente, como por ejemplo, el aumento de las temperaturas de la superficie del mar y los cambios en la abundancia y distribución de sus presas (Bowen, 1997; Bearzi *et al.*, 2003).

Al discutir el papel ecológico y la estrategia alimenticia del delfín común, es necesario fijarnos en la selección de presas. Los estudios de ecología trófica se basan en los análisis de heces y/o de contenido estomacal, lo que dificulta establecer los tipos específicos de presas (Ambrose *et al.*, 2013). Las especies generalistas son capaces de ocupar y explotar una amplia variedad de hábitats y mostrar un alto grado de plasticidad en la dieta, aprovechándose de las presas más disponibles y cambiando según la disponibilidad o escasez de los recursos.

Los movimientos y migraciones del delfín común se asocian en general a las de sus presas potenciales, pudiendo variar estas según la zona y la temporada que se estén investigando (Young & Cockcroft, 1994; Santos *et al.*, 2004; Pusineri *et al.*, 2007; Brophy *et al.*, 2009; Ambrose *et al.*, 2013). Típicamente, se alimentan de bancos de pequeños peces epipelágicos y mesopelágicos cerca de la superficie, como sardina, caballa y anchoa (Young & Cockcroft, 1994; Santos *et al.*, 2004; Pusineri *et al.*, 2007; Cañadas & Hammond, 2008; Ambrose *et al.*, 2013; Otero & Conigliaro, 2012).

En el océano Pacífico oriental, *D. delphis* migra de acuerdo con las condiciones oceanográficas y prefiere zonas de afloramiento, evitando aguas tropicales cálidas (Ballance *et al.*, 2006, Perrin, 2009), mientras que *D. capensis* aparentemente prefiere aguas tropicales más cálidas y más costeras (Banks & Brownell, 1969). Esto parece ocurrir en todo el mundo, como por ejemplo en Chile (Sanino *et al.*, 2003) y en Baja California (Niño-Torres *et al.*, 2006), a pesar de que la distribución de *D. capensis* es bastante desconocida debido a la confusión que en el pasado ha habido entre esta especie y *D. delphis* (Rice, 1998; Sanino *et al.*, 2003). Contrariamente, en el sur de Australia, las observaciones de *D. delphis* revelaron que los delfines con cráneos más grandes y rostros más largos tendían a estar más cerca de aguas profundas, mientras que aquellos con cráneos más pequeños y más cortos se distribuyeron en aguas costeras poco profundas (Bell *et al.*, 2002).

En Sudáfrica, se cree que ambas formas de delfín común co-habitan, pero la de morro largo manteniendo una distribución más costera y el morro corto una más oceánica (Best, 2007) tal y como ocurre en la mayor parte del mundo (Heyning & Perrin, 1994; Van Waerebeek, 1997; Natoli *et al.*, 2006; Perrin, 2009). Heyning y Perrin (1994). Estas especies existen simpátricamente probablemente por estar explotando el medio ambiente de maneras sutilmente diferentes, tal como ya hemos descrito anteriormente.



Los datos de avistamientos del Atlántico este sub-tropical son escasos y oportunistas, sugiriendo simplemente que los delfines comunes raramente son avistados cerca de la playa frente a Mauritania, por lo tanto, no hay ninguna indicación de partición de hábitat entre las formas de morro corto y morro largo (Robineau & Vély, 1998). Natoli *et al.* (2006) propusieron que la forma de morro largo se originó de forma independiente en diferentes regiones, lo que sugiere que la adaptación a los entornos locales podría conducir a una especiación local. Aunque actualmente no hay prácticamente ninguna información sobre la ecología de los delfines comunes del noroeste de África, es probable que la morfología del cráneo del delfín común pueda estar reflejando la adaptación a la captura de las presas y del uso del hábitat, influyendo en su distribución a lo largo del océano Atlántico sub-tropical. Tampoco en otros océanos se tienen datos diferenciadores en la dieta de cualquiera de las formas, de morro corto o largo, o de las especies *D. delphis* y *D. capensis* (Ohizumi *et al.*, 1998). Por lo tanto, se requiere más investigación para aclarar la posibilidad de segregación de nicho trófico entre estos dos morfotipos, tanto en el noroeste de África como en otras áreas.

AMENAZAS Y CONSERVACIÓN

La explotación de los ecosistemas marinos está causando un rápido agotamiento de los depredadores terminales en todo el mundo (Pauly *et al.*, 1998a; Jackson & Sala, 2001; Myers & Worm, 2003). Los cetáceos sufren el impacto de la sobrepesca directamente, a través de muertes accidentales en la pesca (capturas accidentales), e indirectamente, a través de la degradación del hábitat y agotamiento de las presas (Bowen, 1997; Bearzi *et al.*, 2003). Las pesquerías están reemplazando a los depredadores terminales en la mayoría de las redes tróficas marinas (Pauly *et al.*, 1998a) haciendo que los ecosistemas intensamente explotados sean altamente fluctuantes (Anderson *et al.*, 2008).

Mauritania es uno de los principales países pesqueros de África y ha desarrollado la pesca industrial al lado de su pesca artesanal tradicional, convirtiéndose en uno de los principales países africanos exportadores de pescado (Ababouch, 2000) y siendo su área reconocida como prioritaria para la conservación (Atta-Mills *et al.*, 2004; ter Hofstede & Dickey-Collas, 2006). Por consiguiente, se necesita de un conocimiento preciso de su biodiversidad, para su idónea conservación. La sobrepesca, la degradación del hábitat, y los malos acuerdos pesqueros han contribuido a la disminución de las capturas pesqueras en toda la África occidental i Mauritania en particular (Atta-Mills *et al.*, 2004; ter Hofstede & Dickey-Collas, 2006 Pauly *et al.*, 1998a). Por lo tanto, sería importante estimar y controlar con regularidad las poblaciones de depredadores terminales y las capturas de peces en toda el área de estudio. Además, en Mauritania se ha reportado la captura accidental de cetáceos por la pesca industrial (Nieri *et al.*, 1999).



La evaluación del impacto de la pesca sobre el ecosistema local es de crucial importancia (Jouffre & Inejih, 2005), siendo muy necesario el conocer la ecología trófica, pobremente investigada, de los depredadores de nivel superior y sus posibles interacciones con pesquerías que operan en el noroeste de África. Esta zona sufre una gran presión de pesca (ter Hofstede & Dickey-Collas 2006; Christensen *et al.*, 2004; Colman *et al.*, 2005) lo que lleva al agotamiento de las poblaciones de presas (ter Hofstede & Dickey-Collas 2006; Christensen *et al.*, 2004). Los esfuerzos en la conservación de las poblaciones de cetáceos de esta zona deben estar inequívocamente relacionados con las políticas de pesca locales (NEPAD, 2001; Atta-Mills *et al.*, 2004).



OBJETIVOS

El objetivo general de la presente tesis es investigar la taxonomía, morfología, uso del hábitat, y distribución del delfín común (género *Delphinus*) en el este del Océano Atlántico Subtropical (noroeste de África). El objetivo principal es entender las variables que influyen sobre estos factores biológicos, contribuyendo así al conocimiento del género *Delphinus* en esta área tan poco estudiada.

Los objetivos específicos son:

- 1) estudiar la red trófica marina local y establecer la relación entre los depredadores terminales (especies de mamíferos marinos, en general, y los delfines comunes, en particular) y los representantes de otros niveles tróficos;
- 2) confirmar la presencia de los dos morfotipos de delfín común en el área de estudio (delfín común de morro corto y delfín común de morro largo);
- 3) discriminar entre los dos morfotipos e investigar si las diferencias son de importancia taxonómica;
- 4) investigar el patrón de distribución (poblaciones pelágicas vs. costeras) y la segregación de nicho trófico (el nivel trófico explotado) de los dos morfotipos;
- 5) investigar las diferencias en la morfología y uso del hábitat entre delfines comunes del NW África y de otras áreas, en particular, del Nordeste y el Sudeste del océano Atlántico.

El último objetivo es de especial relevancia porque se propuso que ambos morfotipos de delfines comunes, de morro corto y largo, ocurren simpátricamente en el este Subtropical del Atlántico Norte, surgiendo una oportunidad única para determinar la adscripción de las formas africanas del género.



OBJETIVOS ESPECÍFICOS POR CAPÍTULO:

CAPÍTULO 1.

Mediante el análisis del paisaje isotópico, estudiar el ecosistema marino de la región subtropical del Atlántico oriental, examinando la red trófica y la relación entre depredadores terminales (especies de mamíferos marinos, en general, y delfines comunes, en particular) y representantes de otros niveles tróficos.

CAPÍTULO 2.

Estudiar la ecología trófica y potenciales diferencias intraespecíficas de delfines comunes en distintas poblaciones Atlánticas mediante el análisis de isótopos estables. El último objetivo sería el de definir unidades poblacionales de interés para la gestión y conservación; no sólo en el área de estudio sino también en otras partes del mundo.

CAPÍTULO 3.

Investigar las variaciones en la morfometría del cráneo y las firmas isotópicas de carbono y nitrógeno en el delfín común de las aguas subtropicales del Atlántico oriental, con el fin de evaluar la estructura poblacional, la segregación de nicho (a través de la determinación del comportamiento trófico), y el estatus taxonómico de los delfines comunes.

CAPÍTULO 4.

Examinar las diferencias geográficas en el tamaño y forma del cráneo, y por lo tanto proporcionar una cuantificación y visualización de cualquier evidencia morfológica de aislamiento y/o diferenciación entre especies/poblaciones de delfines comunes a lo largo del Océano Atlántico oriental por medio de morfometría geométrica bidimensional.

CAPÍTULO 5.

Resolver las incertidumbres taxonómicas del género *Delphinus* a través de un análisis de morfometría geométrica bidimensional del cráneo con el objetivo de detectar la variación morfológica dentro de la población de delfines comunes de la región subtropical del Atlántico oriental, permitiendo de este modo comprender los procesos evolutivos y ecológicos de diversificación fenotípica.



PLANTEAMIENTO METODOLÓGICO

ÁREA DE ESTUDIO

El presente estudio se centra en el la región subtropical del Atlántico oriental, una de las zonas más productivas del mundo (Ferreira, 1978), donde dos corrientes importantes se juntan: la Corriente de Canarias que es fría y la corriente de Guinea que es cálida. Estas corrientes generan una zona de afloramiento casi permanente con alta productividad biológica (Wells & Bleakley, 1995; Longhurst, 1998; Shumway, 1999), que soporta una gran diversidad y abundancia de mamíferos marinos (Marchessaux & Campredon, 1988; Robineau & Vely, 1998; Nieri *et al.*, 1999). Dada la mezcla de aguas, se pueden encontrar en la zona especies boreales junto con especies tropicales y subtropicales (Robineau & Vely, 1998). Sin embargo, la información disponible acerca de los cetáceos de esta región se limita a varamientos, captura, e informes de avistamientos (Duguy, 1976; Maigret 1980 a, b, 1981; Marchessaux & Campredon, 1988; Van Waerebeek, 1997; Van Waerebeek *et al.*, 1999).

Esta tesis se centra en varios aspectos de la biología y ecología de los delfines comunes (*Delphinus* spp.), específicamente de la costa noroeste de África (Mauritania). Sin embargo, a fines comparativos, se investigaron otras poblaciones bien conocidas de delfín común. Estas poblaciones adicionales han cambiado en función de los objetivos y de los métodos aplicados. No obstante, algunas poblaciones de delfines comunes estuvieron presentes en varios de los estudios realizados: la población de delfín común de la Península Ibérica (aguas del Atlántico noreste, alrededor de España, Galicia y Andalucía, y de Portugal continental), y la población de delfín común de Sudáfrica (aguas del Atlántico sudeste). Los delfines comunes de la Península Ibérica fueron elegidos para compararlos con la población poco conocida de delfines comunes de Mauritania. Esta comparación tiene la ventaja de que los delfines Ibéricos están geográficamente próximos, pertenecen a una especie (*Delphinus delphis*) bien conocida y estudiada y demás se dispone de bastantes muestras.

La población de delfín común de Sudáfrica, se utilizó como comparación, por ser la población de *Delphinus capensis* mejor conocida de toda África. Además, en Sudáfrica cohabitan los dos morfotipos de delfines comunes, al igual que en la población del Atlántico subtropical oriental (Mauritania). Otras zonas de muestreo fueron utilizadas para fines comparativos, donde habitan especies de delfín común reconocidas por la Comisión Ballenera Internacional (CBI, 2009). Se pueden encontrar más detalles sobre



las áreas específicas y las poblaciones/especies de delfines comunes analizados directamente en los trabajos científicos.

MUESTREO

El material biológico analizado consistió en tejido óseo, obtenido de los restos óseos, y los cráneos de los especímenes de delfín común (*Delphinus* spp.) recolectados en el noroeste de África, principalmente Mauritania; así como de la costa atlántica oriental de la Península Ibérica y Sudáfrica. Los resultados de los trabajos científicos también se compararon con datos publicados anteriormente de diferentes zonas geográficas de alrededor del mundo. También, con el fin de estudiar el ecosistema marino mauritano, se recogieron y analizaron muestras de otras especies como tortugas marinas, peces, cefalópodos, plantas marinas, macroalgae, y fitoplancton. Además del material disponible en la Universidad de Barcelona, también se utilizó el material almacenado en varias instituciones gubernamentales en Mauritania. Paralelamente, se realizó trabajo de campo a lo largo de la costa de Mauritania, para recoger más material y se visitaron algunos museos para recolectar otro material adicional (detalles específicos en los trabajos científicos).

METODOLOGIAS

Varios métodos fueron utilizados en el presente estudio para investigar la biología y ecología de los delfines comunes (*Delphinus* spp.) en el noroeste de África, y más específicamente su taxonomía, morfología, distribución y posición trófica en una de las masas de agua más productivas y explotadas del mundo. Torres *et al.* (2003) señalan que la combinación de diferentes técnicas, para discernir la estructura y distribución de las poblaciones, puede mejorar la gestión de poblaciones. Así, en el presente estudio se llevaron a cabo análisis de isótopos estables de carbono y nitrógeno en hueso, junto con análisis de morfometría "tradicional" y geométrica del cráneo, para analizar diferencias intra e inter específicas de delfines comunes.

Una breve descripción de los métodos principales utilizados en el presente estudio es presenta seguidamente. Aunque, en cada trabajo científico se presenta una descripción más detallada de cada metodología, así como de los datos y los análisis estadísticos correspondientes.



ISÓTOPOS ESTABLES

Los isótopos estables de carbono ($^{13}\text{C}/^{12}\text{C}$) y nitrógeno ($^{15}\text{N}/^{14}\text{N}$) se han utilizado para estudiar la ecología trófica desde finales de 1970, principalmente como indicadores de la dieta (Kelly, 2000), ya que varios procesos bioquímicos en la naturaleza producen cambios en las proporciones isotópicas de los elementos en las redes tróficas (Deniro & Epstein, 1978, 1981). Comúnmente, los isótopos estables de carbono y nitrógeno son los más utilizados para este tipo de estudios (Michener & Schell, 1994; Kelly, 2000). En mamíferos marinos, los isótopos estables se han utilizado ampliamente para investigar la ecología trófica de las poblaciones (Hobson *et al.*, 1997; Lesage *et al.*, 2001; Drago *et al.*, 2009; Gómez-Campos *et al.*, 2011) y para inferir la estructura poblacional tanto en cetáceos (por ejemplo, Abend & Smith, 1995; Walker *et al.*, 1999; Borrell *et al.*, 2006; Borrell *et al.*, 2013; Giménez *et al.*, 2013; Vighi *et al.*, 2014) como en pinnípedos (por ejemplo, Smith *et al.*, 1996).

Las variables ambientales tales como la intensidad de la luz, la concentración de nutrientes, la salinidad y la temperatura entre otras afectan a la abundancia relativa de los isótopos pesados respecto los ligeros de los diferentes elementos que componen las biomoléculas de los productores primarios (Walker *et al.*, 1999).

Por lo tanto, poblaciones de una misma especie de diferentes ubicaciones geográficas, con distintas variables ambientales, probablemente tendrán firmas isotópicas distintas, incluso cuando tienen dietas similares (Das *et al.*, 2003; Borrell *et al.*, 2006; 2013), y se podrán diferenciar mediante mediciones de isótopos estables. Esta diferenciación es posible porque el perfil de isótopos de los consumidores refleja los de su presa de una manera predecible (Deniro & Epstein, 1978, 1981). Entonces, el análisis de isótopos estables ofrece una medida integrada de todas las presas asimiladas durante un período relativamente prolongado de tiempo (Tieszen *et al.*, 1983; Michener & Schell, 1994; Hobson, 1999). Como diferentes tejidos tienen diferentes tasas de renovación, cada tejido puede representar un período diferente de la integración de la dieta así como diferentes escalas espaciales (DeNiro & Epstein, 1978, 1981; Tieszen *et al.*, 1983; Hobson & Clark, 1992; Kurle & Worthy, 2002). En consecuencia, la elección de un tejido apropiado es de suma importancia para el análisis de isótopos estables en ecología. El hueso fue el tejido seleccionado porque es posible promediar varios años de historia dietética, debido a su baja tasa de renovación (Hobson & Clark, 1992).

Isótopos de carbono

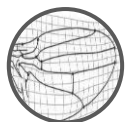
El valor de $\delta^{13}\text{C}$ en los tejidos del consumidor es similar al de la dieta y se utiliza preferentemente para indicar contribuciones relativas a la dieta de las diferentes fuentes primarias en una red trófica (Smith



et al., 1996). En el medio marino, $\delta^{13}\text{C}$ generalmente es mayor en las redes tróficas costeras o bentónicas que en las redes tróficas oceánicas o pelágicas (Deniro & Epstein, 1978; France, 1995). Por consiguiente, ayuda a discriminar entre los hábitats donde el fitoplancton es la única fuente de carbono orgánico (hábitats pelágicos y de alta mar), y aquellos en el que los macrófitos son una fuente relevante de carbono orgánico (hábitats bentónicos y costeros; Rubenstein & Hobson, 2004; Fry, 2006). Aunque el $\delta^{13}\text{C}$ cambia poco entre los niveles tróficos, que sufren poco o nada de enriquecimiento, puede ser un indicador útil de la productividad primaria en la cual se basan las redes tróficas (Deniro & Epstein, 1978).

Isótopos de nitrógeno

El valor de $\delta^{15}\text{N}$ se utiliza comúnmente como un indicador de nivel trófico (Cabana & Rasmussen, 1996) aumentando de la presa al depredador, debido a la excreción preferencial del isótopo ligero por procesos metabólicos implicados en la síntesis de proteínas, producción de urea y de ácido úrico en los consumidores (Caut *et al.*, 2009). En consecuencia, $\delta^{15}\text{N}$ muestra típicamente un incremento escalonado por nivel trófico en una red trófica (Cabana & Rasmussen, 1994), con una diferencia isotópica media de 3-5 ‰ entre los consumidores y sus presas. Este valor puede variar según el tejido analizado, la cantidad de lípidos y tipos de proteínas de las presas y otros muchos factores (Caut *et al.*, 2009, Borrell *et al.*, 2012; Deniro & Epstein, 1981; Hobson & Clark, 1992; Michener & Schell, 1994).



MORFOMETRÍA

La morfometría es el estudio de la variación de la forma y su dependencia con otras variables; utiliza cualquier medida cuantitativa de las características morfológicas y las analiza, midiendo las similitudes y diferencias morfológicas entre los grupos a comparar (Bookstein, 1991; Dryden & Mardia, 1998). Tradicionalmente, el principio de similitud morfológica se ha utilizado para clasificar las poblaciones, especies o grupos taxonómicos de nivel superior (Evin *et al.*, 2008). En los niveles taxonómicos inferiores la similitud morfológica puede ser difícil de detectar y, a veces, la morfometría basada en la medición es inadecuada para diferenciar entre los grupos (Adams *et al.*, 2004). Por el contrario, la morfometría geométrica (GM; Rohlf & Marcus, 1993) es un método especialmente apropiado para investigar la evolución de las formas entre y dentro de las especies (Monteiro, 1999; Gannon & Rácz, 2006; Loy *et al.* 2011; Galatius *et al.*, 2012), así como para investigar la morfología funcional y la divergencia ecológica (Adams & Rohlf, 2000; Claude *et al.*, 2004; McKinnon *et al.*, 2004; Woodward *et al.*, 2006).



Morfometría basada en la medición

La morfometría basada en la medición analiza sobre todo longitudes, anchuras y proporciones, y por lo tanto, todos los datos son mediciones de tamaño (Marcus, 1990). Sin embargo, a pesar de las muchas medidas que se pueden tomar, pocas son independientes porque la mayoría de estas mediciones están altamente correlacionadas (Bookstein, 1991), y puede ser muy difícil separar la información de la forma de la del tamaño (Zelditch *et al.*, 2004). La morfometría basada en la medición sigue siendo muy útil en los estudios de morfología funcional, pero los datos morfométricos ‘tradicionales’ contienen poca información sobre la distribución espacial de la forma de un organismo, y el poder estadístico para distinguir formas es bajo (Adams *et al.*, 2004).

Con el fin de distinguir entre las dos formas de delfines comunes de la costa noroeste de África, se tomaron algunas de las medidas craneales descritas en Heyning & Perrin (1994) como la longitud y la anchura del cráneo, ya que varios estudios demuestran que la longitud del cráneo relativa a su anchura (RL/ZW) es significativamente diferentes entre los dos morfotipos y éstos también difieren en el número de dientes de la mandíbula superior (UTC) (Banks & Brownell, 1969; Evans, 1982; Perrin, 1984; Heyning & Perrin, 1994).

Morfometría geométrica basada en “landmarks”

La morfometría geométrica, por el contrario, es la representación cuantitativa y el análisis de la forma morfológica mediante coordenadas geométricas en lugar de mediciones (Rohlf & Marcus, 1993). Los métodos de morfometría geométrica basada en “landmarks” se fundamenta en la definición de las coordenadas bidimensionales o tridimensionales de puntos de referencia biológicos definibles (Zelditch *et al.*, 2004). El análisis directo de estas coordenadas como variables sería inapropiado; previamente se han de eliminar los efectos de la posición, orientación y escala de los ejemplares sobre estas variables (Rohlf & Slice, 1990); de modo que todo parámetro no relacionada con la forma, sea eliminada antes del análisis estadístico (Adams *et al.*, 2004). Las variables obtenidas, exclusivamente dependientes de la forma, ya se pueden utilizar para los análisis estadísticos, y para hacer representaciones gráficas para la comparación de muestras o grupos (Adams *et al.*, 2004).

Los análisis geométricos de coordenadas solucionan muchos de los problemas de los métodos tradicionales de medición (Adams *et al.*, 2004; Zelditch *et al.*, 2004). Las ventajas de las representaciones geométricas son: 1) los resultados se presentan visualmente como gráficos de forma y no como una tabla



de números; 2) los datos se exportan fácilmente de fotografías digitales; 3) se elimina matemáticamente el tamaño del objeto de medición, centrándonos solamente en la forma (Zelditch *et al.*, 2004).

Se han encontrado patrones de variación morfológica entre todas las especies de delfínidos en distancias geográficas cortas (Mitchell, 1970; Perrin, 1984). Las fuertes variaciones geográficas y los tamaños de muestra pequeños han obstaculizado estudios de taxonomía de 'nivel-*alfa*'. Para distinguir entre diferencias intraespecíficas y interespecíficas, que sería el objetivo de la taxonomía de 'nivel-*gamma*' y 'nivel-*beta*', respectivamente, es necesario un número de muestras alto (Perrin, 1984; Heyning & Perrin, 1994; Rosel *et al.*, 1994; Natoli *et al.*, 2006). Los análisis de GM bidimensional, basada en fotografías del cráneo, se llevaron a cabo con el fin de evaluar la variación geográfica en la morfología craneal, investigar la posible discriminación entre los morfotipos de morro corto y largo y con esta información inferir sobre el estatus taxonómico de la especie en el área de estudio.



DISCUSIÓN

La presente tesis investiga la taxonomía, morfología, uso del hábitat y distribución del delfín común (género *Delphinus*) en la región subtropical del Atlántico oriental (noroeste de África), ampliando el conocimiento que se tiene sobre el género en esta área poco estudiada. Los objetivos propuestos fueron alcanzados y los resultados obtenidos permitieron una mejor comprensión de los temas estudiados.

RED TRÓFICA MARINA

La red trófica marina del Atlántico oriental subtropical (Mauritania) fue examinada y la relación entre depredadores terminales (especies de mamíferos marinos, incluyendo los delfines comunes) y representantes de otros niveles tróficos fueron investigados utilizando isótopos estables de carbono ($\delta^{13}\text{C}$) y nitrógeno ($\delta^{15}\text{N}$). En general, los valores isotópicos del ecosistema marino fueron los esperados, mostrando un incremento de los valores del $\delta^{13}\text{C}$ al pasar de especies pelágicas a especies bentónicas y un incremento de los valores del $\delta^{15}\text{N}$ con el nivel trófico.

No obstante, los valores del $\delta^{15}\text{N}$ entre las especies de macrófitos de la misma región y también entre macrófitos y fitoplancton revelaron una alta variabilidad, con diferencias a menudo mayores que el aumento medio previsto por nivel trófico (Minagawa & Wada, 1984; Caut *et al.*, 2009). Como consecuencia a esta alta variación del $\delta^{15}\text{N}$ en los productores primarios, la variabilidad en los valores del $\delta^{15}\text{N}$ del hueso de los mamíferos marinos podría estar reflejando no sólo las diferencias en el nivel trófico sino también las debidas entre zonas de alimentación.

A diferencia del $\delta^{15}\text{N}$ los valores del $\delta^{13}\text{C}$ de los productores primarios de Mauritania variaron de manera más consistente entre los grupos tróficos. El fitoplancton estaba menos enriquecido en ^{13}C que las macroalgas y las macroalgas menos enriquecidas en ^{13}C que las plantas acuáticas. Esto nos permitió interpretar el enriquecimiento en ^{13}C del hueso de los mamíferos marinos como evidencia de hábitats de alimentación más costeros.

Los resultados obtenidos sugieren que las especies de mamíferos marinos estudiadas ocupan principalmente la plataforma continental exterior y el talud superior. La foca monje del Mediterráneo (*Monachus monachus*), una especie en peligro de extinción, y el delfín jorobado del Atlántico (*Sousa teuszii*) son los mamíferos marinos de distribución más costera y por ello aparecen como unas de las más



vulnerables al impacto humano. La prioridad de los esfuerzos de conservación debe concentrarse en reducir el impacto de la pesca artesanal e industrial de arrastre para asegurar su conservación. Paralelamente, también deberían monitorizarse y controlarse las poblaciones y las capturas de peces en el área de estudio, ya que estos recursos representan el sustento de una gran diversidad de mamíferos marinos. Las capturas excesivas pueden conducir al colapso de un depredador o una presa significativos para el sistema, lo que podría causar cambios en otras especies de la red alimentaria (Walters & Kitchell, 2001).

DISTRIBUCIÓN Y USO DEL HÁBITAT

En cuanto a la distribución de delfín común a lo largo de la costa de Mauritania no hemos podido detectar ninguna diferencia entre las áreas geográficas en relación con la longitud del morro de los animales varados. Por desgracia, los datos de avistamientos de Mauritania son escasos y oportunistas, y simplemente sugieren que los delfines comunes son raramente avistados cerca de la playa (Robineau & Vély, 1998). Esto fue apoyado por los resultados isotópicos obtenidos en el capítulo 1 que revelan que los delfines comunes raramente están muy cercanos a la costa en Mauritania y tienen un comportamiento más oceánico que el de otras especies.

Los delfines comunes (*Delphinus* spp.) de Mauritania presentaron los valores más bajos de $\delta^{15}\text{N}$ en relación al resto de mamíferos marinos estudiados, exceptuando al de las ballenas (*Balaenoptera acutorostrata*), lo que está de acuerdo con lo observado en otras redes tróficas marinas (Das *et al.*, 2003; Walker & Macko, 1999). Estos bajos valores de $\delta^{15}\text{N}$, comparados con las de los otros mamíferos marinos pelágicos (capítulo 1), posiblemente reflejan la proporción más alta de presas de bajo nivel trófico en su dieta, como por ejemplo la *Sardinella aurita*. No obstante, la gran variabilidad de los valores de isótopos estables en los ejemplares de Mauritania y de otras áreas, como se evidencia en el capítulo 2, sugiere diferencias individuales sustanciales en la explotación de los recursos alimenticios.

Distintos autores (Evans, 1982; Murphy *et al.*, 2006) apuntan que la variación en la morfología rostral de delfines comunes, y por consiguiente en su aparato de alimentación, por lo general se asocia a diferencias en la ecología de la alimentación y la dieta. En nuestro estudio (capítulo 3), los valores de $\delta^{15}\text{N}$ mostraron un aumento significativo con el ratio RL/ZW (longitud del rostro/amplitud del rostro), lo que indicaría que o bien los individuos de morro más largo se alimentan a un nivel trófico más alto que los de morro más corto o que explotan un hábitat más pelágico o ambas cosas. Ya que, tal y como se informó en el capítulo 1, los productores primarios del hábitat pelágico presentaban valores de $\delta^{15}\text{N}$ más altos (aproximadamente en un 5 ‰) que los de hábitat costero, así que los delfines que se alimentan en alta mar, deben presentar valores de $\delta^{15}\text{N}$ más altos.



Consistentemente, los resultados obtenidos en el capítulo 2 han demostrado que, al igual que en Mauritania (capítulo 3), los individuos con morros más largos en otras zonas del mundo, presentan niveles superiores de $\delta^{15}\text{N}$ que los animales con morros más cortos. Además, al comparar distintas poblaciones vemos que los delfines comunes de morro largo del Golfo de California presentaron los valores más altos del $\delta^{15}\text{N}$, seguido por las poblaciones de Australia y Mauritania, debido probablemente a la presencia del morfotipo de morro largo en esta última población.

En Mauritania, no se encontró ninguna correlación entre el valor de $\delta^{13}\text{C}$ y el ratio RL/ZW, no pudiéndose verificar, mediante esta variable, las diferentes zonas de ocupación (si las hay) de los dos morfotipos. Observaciones anteriores en otras poblaciones son un poco contradictorias. En Chile (Sanino *et al.*, 2003) y Baja California (Niño-Torres *et al.*, 2006) indican que la forma de morro largo es más costera que la forma de morro corto (Perrin, 2009). Similarmente, en el noreste del Océano Pacífico (región de origen de los ejemplares utilizados para la diferenciación entre *D. delphis* y *D. capensis* (Heyning & Perrin, 1994; Rosel *et al.*, 1994)), la forma de morro largo, también conocida como Baja nerítica, muestra una preferencia por aguas menos profundas que la forma de morro corto (Leatherwood *et al.*, 1982). Por el contrario, Bell *et al.* (2002) encontraron resultados distintos en los *Delphinus delphis* en el sur de Australia. Aparte de la alta variabilidad de los parámetros morfométricos del cráneo, estos autores reportaron una tendencia de los delfines con cráneos y rostros mayores por aguas más profundas que aquellos con cráneos y rostros más pequeños.

Aunque en especies que se alimentan de una amplia variedad de presas es difícil de establecer la composición de la dieta sólo a través del análisis de isótopos estables (Borrell *et al.*, 2012), establecer su nivel trófico es más fácil (Kurle & Worthy, 2001; Post, 2002). En el capítulo 2, se determinaron los niveles tróficos de distintas poblaciones de delfines comunes del Atlántico oriental, utilizando los valores de $\delta^{15}\text{N}$ de especies basales de niveles tróficos conocidos de los ecosistemas marinos locales (Post, 2002). Anteriormente, los niveles tróficos de delfines comunes sólo se habían determinado en unos pocos estudios mediante análisis de contenido estomacal (Pauly *et al.*, 1998b; Pusineri *et al.*, 2007; Ambrose *et al.*, 2013). Viéndose la variabilidad existente entre poblaciones, se intuyó que el delfín común presentaba gran plasticidad para adaptarse a las presas locales; posiblemente revelando diferentes estrategias de adaptación a la estructura de los ecosistemas donde viven. Los niveles tróficos encontrados fueron consistentes con los obtenidos a partir de datos basados en el contenido estomacal y en datos de comportamiento (Pauly *et al.*, 1998b; Bode *et al.*, 2003; Niño-Torres *et al.*, 2006). Los resultados sitúan a los delfines comunes entre el tercero y el cuarto nivel trófico en la mayoría de los ecosistemas, lo que implicaría alimentarse de consumidores secundarios como las sardinas (Pauly *et al.*, 1998b; Bode *et al.*, 2003). Por otra parte, los delfines son animales oportunistas que se aprovechan de la abundancia local de presas para su alimentación (Hassani *et al.*, 1997).



En el capítulo 2, se investigó también si habían diferencias isotópicas entre décadas de muestreo y no se detectaron diferencias significativas, por lo que se asumió que la distribución y el uso del hábitat actual de los individuos, así como las preferencias de alimentación, en el área de estudio, no cambió en las últimas décadas y que sigue siendo aplicable hoy en día. Los datos isotópicos aquí presentados no han permitido una reconstrucción precisa de la composición de la dieta porque no se disponía de las presas potenciales.

MORFOTIPOS DE DELFÍN COMÚN

Heyning y Perrin (1994) propusieron que más de una forma de delfín común podría estar presente en el noroeste de África como ocurre en la costa de California, a pesar de que esta zona no era el foco de su estudio. Más tarde, Van Waerebeek (1997) confirmó que, efectivamente, existían dos morfotipos delfín común en la costa occidental de África. En la presente tesis confirmamos que ambos morfotipos de delfín común, de morro corto y largo, viven en simpatria en aguas de Mauritania. Como se verifica en los Capítulos 2 y 3, la variabilidad de los valores del $\delta^{15}\text{N}$ fue particularmente alta en los delfines comunes de Mauritania, un hallazgo probablemente relacionado con la presencia de ambos morfotipos en esta área en contraposición a las zonas más al norte, donde sólo el morfotipo de morro corto está presente.

En el capítulo 3, los cráneos de delfines comunes se analizaron usando diferentes medidas morfométricas, entre ellas las medidas más informativas: RL/ZW y UTC. Estas medidas corroboraron los análisis genéticos que permitieron la separación de los dos morfotipos en dos especies, *Delphinus delphis* y *Delphinus capensis*, en la costa de California (Heyning & Perrin, 1994). El rango de variación de RL/ZW y UTC fue muy elevado en los delfines de Mauritania, indicando la presencia de los dos morfotipos. Además, los resultados que comparaban la morfología del cráneo de los delfines comunes de Mauritania con especies bien estudiadas de *D. delphis* y *D. capensis* mostraron un patrón intermedio entre las dos especies (capítulo 4). En el capítulo 5, se dividieron los ejemplares de Mauritania en dos colectivos aplicando los rangos del RL/ZW definidos para cada una de las especies de delfines comunes de California. A través de varios análisis estadísticos se observó que los dos grupos previamente seleccionados se asemejaban uno al morfotipo morro corto y otro al morro largo, permitiendo sugerir la posibilidad de la existencia de dos subpoblaciones en el área de estudio.

VARIACIÓN MORFOLÓGICA

La variación morfológica en delfines comunes (género *Delphinus*) se investigó mediante morfometría tanto tradicional como geométrica. Ambos métodos revelaron una gran variabilidad en las



características morfométricas del cráneo de los delfines comunes del área de estudio, lo que indica la presencia de morfotipos de morro corto y largo, así como diferencias significativas entre ellos.

En el capítulo 3 se analizaron varias medidas morfométricas del cráneo que son de relevancia taxonómica, y que se utilizaron anteriormente para distinguir morfotipos de delfines comunes, e incluso definir las especies, en varias zonas del mundo (Banks & Brownell, 1969; Van Bree & Gallagher, 1978; Amaha, 1994; Evans, 1994; Heyning & Perrin, 1994; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Westgate, 2007; Tavares *et al.*, 2010). En este capítulo proporcionamos una revisión de los valores de RL/ZW y UTC para varias poblaciones de delfines comunes de alrededor del mundo y las comparamos con la población mauritana. La variabilidad en la longitud del morro de la población estudiada fue muy grande en comparación con otras poblaciones de *Delphinus* (Heyning & Perrin, 1994; Van Waerebeek, 1997; Murphy *et al.*, 2006; Westgate, 2007), especialmente en las regiones donde está presente sólo una de las especies. El rango de variación global del RL/ZW para los delfines comunes de Mauritania es casi tan grande como la variación conjunta de las dos especies descritas para el Pacífico Noreste, *D. delphis* y *D. capensis* (Heyning & Perrin, 1994). Los valores menores del RL/ZW fueron similares a los que se encuentran típicamente en *D. delphis* del noreste Atlántico (Murphy *et al.*, 2006; Westgate, 2007), mientras que los valores mayores del RL/ZW fueron similares a los encontrados en el centro de África occidental (Van Waerebeek, 1997), y Sudáfrica, y que se han atribuido a *D. capensis* (Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005). Sin embargo, se detectó una variación clinal en tamaño relativo del rostro, que también se había observado en el delfín común del Indo-Pacífico *Delphinus capensis tropicalis*, con un rostro extremadamente largo (Jefferson & Van Waerebeek, 2002), y en el delfín común del sur de Australia *Delphinus delphis* (Bell *et al.*, 2002). Los especímenes de Australia mostraron un mayor rango de variación del RL/ZW en comparación con los animales que pertenecen a la misma especie, *D. delphis*, del Pacífico y Atlántico Norte (Heyning & Perrin, 1994; Bell *et al.*, 2002; Murphy *et al.*, 2006; Westgate, 2007). En Mauritania, UTC también mostró mayor variabilidad en comparación con el *D. delphis* del Atlántico norte, el sur de Australia o incluso California, y a pesar de que se superponen ligeramente con *D. capensis* de Sudáfrica y California, parece que en nuestra muestra no hay estratificación.

En el Capítulo 4, los resultados de la morfometría geométrica (GM) han demostrado diferencias significativas en los componentes de tamaño y forma del cráneo de las poblaciones de delfines comunes de diferentes áreas geográficas del Océano Atlántico oriental (península Ibérica, Mauritania y Sudafrica). Los resultados indican que estas diferencias siguen una tendencia geográfica en la forma del cráneo, y que son particularmente evidentes entre las dos localidades extremas, la Península Ibérica (*Delphinus delphis*) y Sudáfrica (*Delphinus capensis*).

El tamaño del cráneo de los delfines de Sudáfrica fue significativamente mayor que el de las otras dos poblaciones de delfines comunes examinadas y concordantes con estudios previos (Amaha, 1994;



Heyning & Perrin, 19994; Bell *et al.*, 2002; Murphy *et al.*, 2006). Similarmente a nuestros resultados, *D. capensis*, tanto de la costa de California como de Sudáfrica, presentó un tamaño del cráneo más grande que *D. delphis*; el cráneo del morfotipo morro largo es absolutamente más largo y relativamente más estrecho que el cráneo del morfotipo morro corto (Heyning & Perrin, 1994).

Los delfines comunes de Mauritania se caracterizan por una forma intermedia entre el *D. delphis* de la Península Ibérica y el *D. capensis* de Sudáfrica, a pesar de que se diferencian estadísticamente de ambas poblaciones. Se verificó que estas diferencias tienen que ver con diferentes características dentro de la población de delfines comunes de Mauritania, como se ha visto en el capítulo 3 y descrito anteriormente (Pinela *et al.*, 2011). Del mismo modo, Jefferson y Van Waerebeek (2002) llegaron a la conclusión de que los delfines comunes de Australia tampoco encajan perfectamente con las descripciones conocidas de *D. delphis* o *D. capensis*, a pesar de que fueron considerados morfológicamente más cerca de los *D. delphis* de California y asignados a la especie *Delphinus delphis*.

Los elementos rostrales del cráneo están influenciados por presiones selectivas debido a su asociación con la alimentación (Perrin, 1975). Por lo tanto, esta parte del cráneo de los *Delphinus* parece ser relativamente plástica, y consecuentemente los cambios en el aparato rostral del cráneo podrían reflejar los cambios en la ecología de la alimentación. Nuestros resultados también muestran que el rostro es la parte del cráneo que exhibe más variabilidad entre las poblaciones estudiadas. Aunque las especies marinas suelen distribuirse de forma continua, los ecosistemas marinos se caracterizan por diferencias locales en las características del hábitat (por ejemplo, temperatura, salinidad, batimetría, alimentación, etc.) que hacen alusión a las especializaciones de nicho locales y a la diferenciación morfológica (Natoli *et al.*, 2006; Pinela *et al.*, 2011, 2015). Sin embargo, es difícil decir si las diferencias en la ecología trófica son responsables de las diferencias encontradas en los elementos rostrales o lo son por otros motivos, como la agresión intraespecífica durante el apareamiento y la competencia espermática (Murphy *et al.*, 2005; Westgate, 2007; Westgate & Read, 2007).

Muchos artículos publicados subrayan la importancia del análisis morfométricos como una poderosa herramienta de apoyo de los resultados genéticos, ecológicos y etológicos para describir y comprender el mecanismo de diferenciación morfológica y la estructura de la población (Adams *et al.*, 2004; Cardini *et al.*, 2009; Natoli *et al.*, 2008). Los resultados de nuestro estudio muestran la necesidad de añadir otras especies y poblaciones de delfines comunes para un estudio global, con el fin de aclarar mejor el grado y el patrón de variación morfológica a nivel geográfico, así como los rasgos adaptativos involucrados.



ESTADO TAXONÓMICO

En el área de estudio existen individuos que se pueden atribuir a los dos morfotipos *Delphinus*, pero ni los datos morfométricos ni las señales isotópicas apoyan la hipótesis de más de una sola especie de delfines comunes. Existen evidencias de que la zona está habitada por delfines con gran variación intrínseca en la proporción del cuerpo y en el comportamiento trófico. Podemos entonces suponer que los dos morfotipos coexisten en Mauritania, pero, al contrario que en otras áreas, su ocurrencia parece reflejar el uso diferencial del hábitat.

La mayoría de los estudios que investigan la identificación de especies mediante la morfología craneal en delfines comunes siguen las directrices apuntadas por Heyning y Perrin (1994) (Van Waerebeek, 1997; Bell *et al.*, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011). Heyning y Perrin (1994), utilizando sólo animales maduros, determinaron que las diferencias entre los dos morfotipos no estaban solamente relacionadas con el tamaño sino también con la forma de los cráneos.

En el capítulo 5, los resultados indicaron que los caracteres craneales analizados fueron altamente informativos, y potencialmente indicativos de la reciente evolución de adaptación de las poblaciones y especies. Todos los grupos taxonómicos investigados presentaban diferencias morfológicas significativas en la forma del cráneo. Igualmente, hubo una clara separación entre las poblaciones del Atlántico Subtropical oriental de delfín común (morfotipos de morro corto y largo). Por otro lado, Van Waerebeek (1997) llegó a la conclusión de que las características analizadas en el cráneo de especímenes del centro-oeste de África encajan muy bien con las características craneales definidas para el *D. delphis* y el *D. capensis* del Pacífico oriental (Heyning & Perrin, 1994). Las poblaciones de *Delphinus* del noroeste de África son morfológicamente distintos, en la forma del cráneo, tanto del *D. delphis* de la costa atlántica de la Península Ibérica como del *D. capensis* de la costa atlántica de Sudáfrica (a pesar de la similitud de valores del RL/ZW con ambas especies verificadas en el capítulo 3; Pinela *et al.*, 2011). No obstante, los resultados de los análisis jerárquicos de los grupos taxonómicos señalaron una similitud morfológica más cercana entre el morfotipo de morro corto de Mauritania y el *D. delphis*, y el morfotipo de morro largo de Mauritania y el *D. capensis*.

En relación con el tamaño del cráneo, en el capítulo 5, la población de morro largo de Mauritania no presentó diferencias significativas con el *D. delphis*, pero difería significativamente de la población de morro corto de Mauritania y del *D. capensis*. Del mismo modo, Murphy *et al.* (2006) han reportado que los delfines comunes del Atlántico Norte oriental eran más similares en tamaño a *D. capensis* que a *D. delphis* de la costa sur de California, a pesar de que no tenían un rostro tan largo y que el UTC estaba dentro del rango definido para *D. delphis* de la misma región. En el presente estudio, el morfotipo de morro corto de



Mauritania presentó un tamaño del cráneo en general más pequeño que el de las demás poblaciones y especies investigadas, mientras que el *D. capensis* presentó valores mayores en tamaño y rango. El morfotipo de morro largo del noroeste de África presentó un rango menor de los valores de UTC que los obtenidos por *D. capensis* de Sudáfrica y California (Pinela *et al.*, 2011), indicativo de un cráneo más pequeño como confirman los resultados obtenidos.

Se ha propuesto que en las zonas donde el *D. delphis* vive simpátricamente con el *D. capensis*, el tamaño total del cuerpo y del cráneo pueden haber disminuido, probablemente debido a un desplazamiento de los caracteres (Perrin, 1984). Entonces, no es de extrañar que, dado el alto potencial de desplazamiento de los caracteres en especies simpátricas (Losos, 2000), el *D. delphis* de California sea más pequeño que el de otras poblaciones de delfines comunes de morro corto que viven en alopatria (Westgate *et al.*, 2007). Lo mismo puede estar sucediendo en la costa noroeste de África, donde ambas formas también ocurren en simpatria (Van Waerebeek, 1997; Pinela *et al.*, 2011).

Varios estudios describen una superposición en los rangos de caracteres morfológicos reportados para las dos especies de delfines comunes de California (Van Bree & Purves, 1972; Amaha, 1994; Van Waerebeek, 1997; Jefferson & Van Waerebeek, 2002; Bell *et al.*, 2002; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011), dando lugar a propuestas de formas intermedias entre *D. delphis* y *D. capensis*. En la presente tesis, en el capítulo 3, también se reportaron proporciones intermedias para los delfines comunes del Atlántico subtropical oriental (Pinela *et al.*, 2011); y, en el capítulo 5, tres ejemplares de Mauritania tuvieron que ser retirados del conjunto de datos porque presentaban un RL/ZW intermedio entre las dos especies reconocidas.

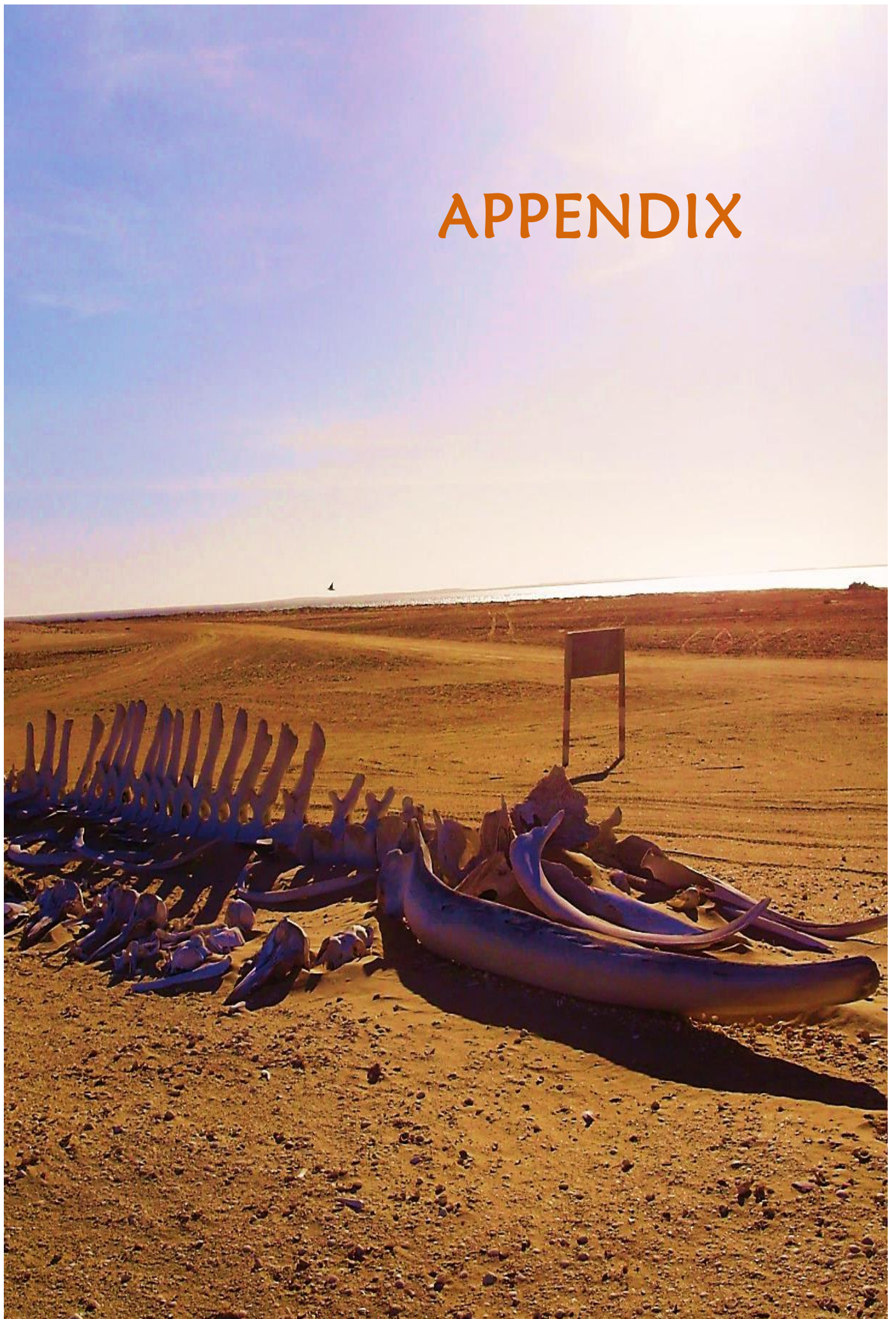
En la mayoría de las áreas fuera del sur de California ha sido muy difícil separar el género *Delphinus* en dos especies basándose solamente en caracteres morfológicos (Bell *et al.*, 2002; Samaai *et al.*, 2005; Murphy *et al.*, 2006; Westgate, 2007; Tavares *et al.*, 2010; Pinela *et al.*, 2011). La existencia de valores intermedios de RL/ZW, que caen entre o fuera de los rangos reportados para las dos especies reconocidas de *Delphinus*, demuestra una falta de afinidad con lo propuesto por Heyning y Perrin (1994), por lo que sería conveniente una revisión de los parámetros aportados por estos autores. Tavares *et al.* (2010) sugirieron que en las zonas donde coexisten formas intermedias de morfotipos de morro corto y largo, los eventos de especiación locales podrían estar actuando para originar nuevas especies de *Delphinus* en la actualidad, que posiblemente no se adaptaran a los parámetros de Heyning y Perrin (1994). Además, es posible que más de dos especies de *Delphinus* pueden existir en el mundo, haciendo que la taxonomía actual sea incorrecta (Perrin *et al.*, 2009). Otra hipótesis es que el género *Delphinus* podría ser una "especie anillo", es decir, varias poblaciones vecinas con flujo génico entre ellas pero genéticamente aisladas en los extremos (WF Perrin, comunicación personal, en Tavares *et al.*, 2010).



Varios autores han sugerido que la variación geográfica en la morfología rostral del delfín común, y por consiguiente en su aparato de alimentación, podría indicar variaciones en la dieta y en el uso del hábitat, aunque nunca se ha demostrado (Evans, 1982; Amaha, 1994; Murphy *et al.*, 2006). Con todo, y a pesar de que deberían llevarse a cabo análisis genéticos de un mayor número de individuos, nuestros datos junto con el estudio genético realizado por Natoli *et al.* (2006) sugieren que la diferenciación craneal del delfín común podría estar relacionada con la segregación de nicho y no a la especiación. Llegamos a la conclusión de que una división taxonómica no parece estar justificada en el área de estudio.

Para concluir, la existencia de dos especies putativas (*D. delphis* y *D. capensis*) en Mauritania debe ser cuestionada hasta que se realicen investigaciones sobre genética y otras mediciones morfométricas taxonómicamente relevantes. Tanto los datos isotópicos como morfométricos parecen indicar que al menos existen dos poblaciones de delfín común en el área de estudio. Finalmente, nuestros resultados impiden asumir que ambos morfotipos se ajusten al modelo taxonómico descrito en el noreste del Pacífico por Heyning y Perrin (1994) y Rosel *et al.* (1994), fuera de esa región.

APPENDIX





Stable isotope analysis reveals habitat partitioning among marine mammals off the NW African coast and unique trophic niches for two globally threatened species

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ABSTRACT: Stable isotope abundances of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in the bone of 13 species of marine mammals from the northwest coast of Africa were investigated to assess their positions in the local trophic web and their preferred habitats. Also, samples of primary producers and potential prey species from the study area were collected to characterise the local isotopic landscape. This characterisation indicated that $\delta^{13}\text{C}$ values increased from offshore to nearshore and that $\delta^{15}\text{N}$ was a good proxy for trophic level. Therefore, the most coastal species were *Monachus monachus* and *Sousa teuszii*, whereas the most pelagic were *Physeter macrocephalus* and *Balaenoptera acutorostrata*. $\delta^{15}\text{N}$ values indicated that marine mammals located at the lowest trophic level were *B. acutorostrata*, *Stenella coeruleoalba* and *Delphinus* sp., and those occupying the highest trophic level were *M. monachus* and *P. macrocephalus*. The trophic level of *Orcinus orca* was similar to that of *M. monachus*, suggesting that *O. orca* preys on fish. Conservation of coastal and threatened species (*M. monachus* and *S. teuszii*) off NW Africa should be a priority because these species, as the main apex predators, cannot be replaced by other marine mammals.

KEY WORDS: Marine mammals · Stable isotopes · Trophic ecology · Habitat use · NW Africa

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INTRODUCTION

Exploitation of marine ecosystems is causing a rapid depletion of top predators worldwide (Pauly et al. 1998a, Jackson & Sala 2001, Myers & Worm 2003) and, as a result, marine food webs are undergoing extraordinary changes in their structure and function (Jackson et al. 2001, Emslie & Patterson 2007). Fisheries are replacing apex predators in most marine food webs and exercise a formidable top-down control on these predators, but major differences in the behaviour of fisheries and that of marine apex predators (Trites et al. 2006) make intensely exploited ecosystems highly fluctuant (Anderson et al. 2008). An ecosystem-based management system has been advocated to avoid these problems, but this approach is often hindered by

a poor knowledge of the basic biological traits of most apex predators.

The waters of the NW coast of Africa are among the most productive in the world (Ferreira 1978, Feidi 1996). In these waters, 2 important currents meet—the cold Canary Current and the warm Guinea Current—generating a quasi-permanent upwelling zone that triggers a high biological productivity (Wells & Bleakley 1995, Longhurst 1998, Shumway 1999). Such productivity supports a highly diverse and abundant fauna of marine mammals, (Marchessaux & Campredon 1988, Robineau & Vely 1998, Nieri et al. 1999) which typically include, given the mix of waters, boreal species as well as tropical or subtropical species (Robineau & Vely 1998). Additionally, the region shelters the last remnant populations of some scarce or highly endangered spe-

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cies, such as the Mediterranean monk seal *Monachus monachus* (González et al. 1997), the Atlantic humpback dolphin *Sousa teuszii* (Van Waerebeek et al. 2004) and the African manatee *Trichechus senegalensis* (Padiá & Ibáñez 2005). Mauritania is one of the major African fishing nations and has developed, alongside traditional artisanal fishing, industrial fisheries, becoming one of the major fish exporting African countries (Ababouch 2000). Overfishing, overcapacity, habitat degradation and inequitable access agreements have contributed to the decline in catches throughout West Africa (Atta-Mills et al. 2004, ter Hofstede & Dickey-Collas 2006). Assessing the impact of fisheries on the local ecosystem is of great interest (Jouffre & Inejih 2005) but no studies have been conducted involving marine mammals.

Available information on the marine mammals of the region is limited to stranding and sighting reports in the case of cetaceans (Duguy 1976, Maigret 1980a,b, 1981, Marchessaux & Campredon 1988, Van Waerebeek et al. 1999), although a few demographic parameters are known for *Monachus monachus* (González et al. 1997, Gazo et al. 2000). Nevertheless, the trophic relationships among the marine apex predators of the region have never been investigated, and their possible interactions with the fisheries operating in the area are largely unknown.

The methods traditionally used in the study of the trophic ecology of marine mammals have been faecal or stomach-content analyses. These methods present limitations because they only show recently eaten prey, and results can be severely biased owing to differences in prey digestion (Hobson et al. 1996). Conversely, stable isotope analysis offers an integrated measure of all prey assimilated over a comparatively protracted period of time (Tieszen et al. 1983, Michener & Schell 1994, Hobson 1999). In marine mammals, stable isotopes of carbon and nitrogen have been extensively used to investigate the trophic ecology of populations (e.g. Hobson et al. 1997, Lesage et al. 2001, Drago et al. 2009). The relative abundance of heavy to light carbon isotopes ($\delta^{13}\text{C}$) has been used to discriminate between habitats where phytoplankton is the only source of organic carbon (pelagic and offshore habitats) and those where macrophytes are a relevant source of organic carbon (vegetated onshore benthic habitats) (Rubenstein & Hobson 2004, Fry 2006, Cardona et al. 2007). By contrast, the relative abundance of heavy to light nitrogen isotopes ($\delta^{15}\text{N}$) is commonly taken as an indicator of trophic level (Cabana & Rasmussen 1996) because this abundance increases from prey to predator owing to the preferential excretion of the light isotope (Caut et al. 2009).

In the present study, stable isotope profiles of carbon and nitrogen were used to investigate the habitat use

and trophic level of the marine mammals occurring in the waters off the northwest coast of Africa to determine their positions in the marine food web.

MATERIALS AND METHODS

Study site and sampling. The study was conducted in the Islamic Republic of Mauritania, located in the northwest region of Africa. Its coastline extends latitudinally more than 700 km from Cape Blanc to the Senegal River (Fig. 1). The northern part of the coast is composed of rocky cliffs with scattered small, tide-dependent and sandy beaches (Habitat 1, Fig. 1); the intermediate region consists of tidal mudflats inhabited by the seagrass *Halodule wrightii* and *Zostera noltii* and shallow waters that extend several kilometres offshore, supporting dense sublittoral meadows of the seagrass *Cymodocea nodosa* with scattered deep unvegetated water channels in between (Parc National du Banc d'Arguin, Habitat 2, Fig. 1); and the southern region is a nearly continuous sandy beach with scattered sublittoral patches of the seagrass *Cymodocea nodosa* (La Grand Plage, Habitat 3, Fig. 1). Despite this heterogeneity, there are no major differences among the 3 regions in the distribution of the potential prey for marine mammals, whose abundance depends mainly on the depth of the coastal waters and the variation in sea surface temperature related to upwelling (ter Hofstede & Dickey-Collas 2006).

Bone samples from 13 species of marine mammals (243 individuals; Table 1) were collected opportunistically between 1990 and 2008 along the Mauritanian coastline. Except for *Monachus monachus*, for which all samples came from natural mortalities, we have no information on the cause of death of the marine mammals used in this study. Bone was selected because, owing to its low turnover rate, it is possible to elucidate several years of dietary history (Hobson & Clark 1992). The bone collected from each skull was a small fragment of turbinate bones from the nasal cavity because this fragment is easy to crush and its sampling did not damage the skull for subsequent studies. Furthermore, samples from 7 species of potential prey (fish and cephalopods), 1 species of sea turtle, 7 species of benthic macrophytes (benthic primary producers) and phytoplankton (pelagic primary producers) were also collected to characterise the isotopic landscape off the coast of Mauritania (Table 1).

The samples of leaf and thallus collected from seagrass and macroalgae, respectively, were dried with sodium chloride; and fish dorsal white muscle, cephalopod mantle and turtle muscle were stored in a freezer at -20°C until analysis. Samples of skull and other bone tissues were stored without preservatives at

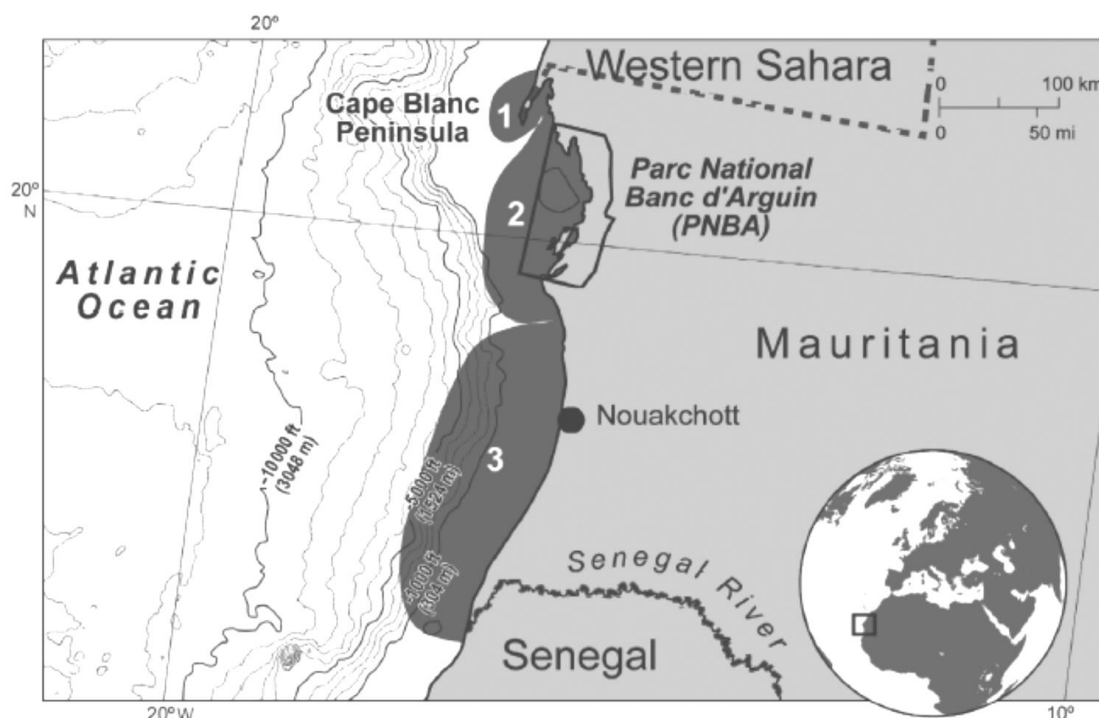


Fig. 1. Study area, with bathymetry, the overline of the continental shelf and the 3 main habitat types, indicated by dark grey areas with numbers (see 'Materials and methods' for habitat characterisation)

ambient temperature (15 to 25°C). Phytoplankton (whole organisms in collective samples) was refrigerated at 4°C, brought to the laboratory, filtered in a pre-combusted GF/C filter and processed for isotopic determination.

Stable isotope analyses. Stable isotope abundances (expressed as delta notation, δ), in which the relative variations of stable isotope ratios are expressed in per mille (‰) deviations from predefined international standards, were calculated as:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where X is the value of the heavy isotope of the sample (^{13}C or ^{15}N), R_{sample} is the ratio of the heavy to the light isotope of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) and R_{standard} is the ratio of the heavy to the light isotope in the reference standards, which were Vienna Pee Dee Belemnite calcium carbonate for carbon and atmospheric nitrogen (air) for nitrogen.

For the pre-treatment of the samples, ~1 g of tissue was rinsed with distilled water, dried for 3 d at 60°C and powdered with a mortar and pestle. Because lipids are depleted for ^{13}C compared with other molecules, and thus can mislead the analyses by decreasing the $\delta^{13}\text{C}$ signal (DeNiro & Epstein 1977), they were removed from the samples by rinsing the powdered tissue several times with a 2:1 chloroform:methanol solu-

tion (Bligh & Dyer 1959). When conducting stable isotope analysis in bone, several authors have carried out preventive demineralisation of the tissue by treating it with either a 0.5 or 1 M hydrochloric acid (HCl) solution (Bocherens et al. 1997, Newsome et al. 2006). However, a test using a subset of the samples ($n = 20$) revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not differ between demineralised and untreated samples, so this procedure was not followed. However, seagrass and macroalgae were treated with a 0.5 M HCl solution to eliminate inorganic carbonates that could bias the results (Ng et al. 2007). Phytoplankton was also treated with 0.05 M HCl to remove carbonates following the protocol of Ogawa & Ogura (1997). Some authors agree that it is possible that HCl treatment adversely affects the nitrogen isotopic signature (Bunn et al. 1995), thus primary producer samples (seagrass, macroalgae and collective phytoplankton samples) were separated into 2 subsamples used for carbon (after the HCl treatment) and for nitrogen (without the HCl treatment).

Finally, ~1 mg of bone, 0.5 mg of white muscle from fish and of mantle from cephalopods, 4 mg of homogenised seagrass and of macroalgae and 12 mg of the homogenised phytoplankton with filter were weighed in tin capsules (3.3×5 mm), combusted at 900°C and analysed in a continuous flow isotope ratio mass spec-



Table 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of each species collected along the Mauritanian coast. Data are given as means \pm SD (‰), minimum and maximum isotope values, identification codes (ID code) and number of individuals per species (N). **Bold** values represent means \pm SD (‰), and minimum and maximum isotope values for each category

Species	ID code	N	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$ (‰)		
			Mean \pm SD	Min.	Max.	Mean \pm SD	Min.	Max.
Pelagic primary producers								
Phytoplankton	PHYTO	3	-24.4 ± 3.5	-26.9	-20.3	8.9 ± 0.3	8.6	9.2
Benthic primary producers		34	-14.6 ± 0.8	-19.9	-5.3	3.8 ± 0.18	1.8	5.8
Seagrass								
<i>Cymodocea nodosa</i>	CNOD	5	-6.3 ± 1.1	-7.5	-5.3	2.6 ± 0.8	1.8	3.8
<i>Halodule wrightii</i>	HWRI	5	-11.8 ± 0.8	-13.0	-10.7	4.3 ± 1.1	2.8	5.8
<i>Zostera noltii</i>	ZNOL	5	-10.5 ± 1.3	-7.5	-5.3	2.9 ± 0.2	2.7	3.2
Macroalgae								
<i>Asparagopsis</i> sp.	ASPA	2	-19.7 ± 0.4	-19.9	-19.4	3.7 ± 0.5	3.3	4.1
<i>Dictyota</i> sp.	DICT	5	-18.8 ± 0.2	-19.1	-18.7	4.7 ± 0.4	4.3	5.3
<i>Padina</i> sp.	PADI	2	-16.1 ± 1.3	-17.0	-15.2	3.4 ± 0.9	2.7	4.0
<i>Polysiphonia</i> sp.	POLY	5	-19.6 ± 0.2	-19.9	-19.4	3.6 ± 0.4	3.2	4.1
<i>Sargassum</i> sp.	SARG	5	-17.7 ± 0.2	-17.9	-17.5	5.1 ± 0.4	4.4	5.4
Fish		43	-15.4 ± 0.91	-18.0	-14.1	13.1 ± 1.73	9.7	16.0
<i>Caranx rhonchus</i>	CRHO	11	-14.8 ± 0.29	-15.5	-14.4	14.1 ± 0.50	13.5	14.8
<i>Mullus barbatus</i>	MBAR	10	-16.2 ± 0.77	-17.5	-15.0	13.1 ± 0.83	11.6	14.2
<i>Pagellus bellotii</i>	PBEL	9	-15.7 ± 1.25	-18.0	-14.2	14.3 ± 1.07	13.0	16.0
<i>Plectorhynchus mediterraneus</i>	PMED	1	-14.1			14.2		
<i>Sardinella aurita</i>	SAUR	10	-15.0 ± 0.39	-15.7	-14.3	10.4 ± 0.66	9.7	11.0
<i>Scomberomorus tritor</i>	STRI	2	-15.7 ± 0.21	-15.8	-15.5	14.1 ± 0.32	14.0	14.4
Cephalopods								
<i>Loligo</i> sp.	LOLI	1	-15.1			14.4		
Marine turtles								
<i>Chelonia mydas</i>	CMID	4	-9.3 ± 2.2	-11.6	-6.8	7.8 ± 1.2	6.0	8.6
Marine mammals		243	-11.7 ± 1.23	-15.9	-8.1	13.4 ± 1.16	10.8	17.1
<i>Balaenoptera acutorostrata</i>	BACU	1	-15.9			11.8		
<i>Delphinus</i> sp.	DDEL	95	-12.4 ± 0.81	-15.3	-9.7	12.7 ± 0.83	10.8	15.9
<i>Globicephala macrorhynchus</i>	GMAC	9	-11.5 ± 0.53	-12.5	-10.7	14.0 ± 0.39	13.5	14.6
<i>Globicephala melas</i>	GMEL	2	-11.1 ± 0.42	-11.4	-10.8	14.1 ± 0.27	13.9	14.3
<i>Grampus griseus</i>	GGRI	1	-12.0			13.8		
<i>Monachus monachus</i>	MMON	12	-10.4 ± 0.63	-11.2	-8.9	14.5 ± 0.70	12.9	15.4
<i>Orcinus orca</i>	ORCA	3	-11.9 ± 1.39	-13.2	-10.4	14.5 ± 0.52	14.0	15.0
<i>Phocoena phocoena</i>	PPHO	42	-11.7 ± 0.94	-14.0	-9.7	13.6 ± 0.76	11.6	15.4
<i>Physeter macrocephalus</i>	PMAC	2	-13.9 ± 2.11	-15.4	-12.4	15.9 ± 0.06	15.9	16.0
<i>Sousa teuszii</i>	STEU	11	-9.7 ± 0.93	-11.5	-8.1	13.3 ± 1.33	11.7	15.3
<i>Stenella coeruleoalba</i>	SCOE	1	-12.0			11.9		
<i>Stenella frontalis</i>	SFRO	4	-12.1 ± 0.30	-12.4	-11.8	13.2 ± 1.02	12.3	14.6
<i>Tursiops truncatus</i>	TTRU	60	-11.1 ± 1.23	-15.4	-8.3	13.9 ± 1.30	11.4	17.1

trometer (Flash 1112 IRMS Delta C Series EA, Thermo Finnigan, Bremen, Germany). Atropine was used as a standard to check the elemental composition for carbon and nitrogen. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the IAEA (International Atomic Energy Agency)—namely, polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$) and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$)—were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios—namely, $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$) and KNO_3 (IAEA NO₃, $\delta^{15}\text{N} = +4.7\text{‰}$)—were used for calibration at a precision of 0.3‰.

Data analyses. The assumption of normality in the distributions of the sample groups was checked with the Kolmogorov-Smirnov test. The assumption of homogeneity of variances between sample groups was checked with Levene's test.

Differences in mean bivariated isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) among marine mammal species were assessed using a multivariate analysis of variance (MANOVA) because they displayed a normal distribution. Univariate ANOVA was conducted separately for both isotopes. If a statistical significant interaction was found between marine mammal species for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using an ANOVA, additional pairwise comparisons were made using a generalised linear model because it admits data that do not meet the assumption

of homogeneity of variance. Post hoc analyses were made by means of least significant differences (LSD) tests based on estimated marginal means. As a method of estimation, the maximum likelihood (ML) was used in all cases. Normality distributions and identity as a link function were always used (for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), because data followed a normal distribution. Goodness of fit values (Akaike's information criterion, AIC) were used to compare different models. The confidence interval level was set at 95 % for all comparisons. The post hoc pairwise comparison by generalised linear model is a more flexible and less conservative statistical tool than the post hoc pairwise comparison by general linear model because several types of distribution and models can be chosen to fit the data. The post hoc test was conducted for the species represented by more than one sample (*Stenella coeruleoalba*, *Grampus griseus* and *Balaenoptera acutorostrata* were excluded from the analysis).

Unless otherwise stated, data are presented as means \pm standard deviation (SD) throughout the man-

uscript, except when a species is represented by a single individual. All the statistical analyses were conducted using the SPSS ver.15.

RESULTS

The isotopic landscape

Macroalgae presented lower $\delta^{13}\text{C}$ values in comparison with seagrass but presented higher $\delta^{13}\text{C}$ values in comparison with phytoplankton (Fig. 2, Table 1). The $\delta^{13}\text{C}$ value of green turtles was close to that of the seagrass that they consume (Cardona et al. 2009), whereas the $\delta^{13}\text{C}$ of fish and cephalopods were between those of phytoplankton and benthic primary producers. As expected, coastal fish (*Plectorhinchus mediterraneus*, *Caranx rhonchus* and *Sardinella aurita*) displayed higher $\delta^{13}\text{C}$ values than did more offshore species (*Pagellus bellottii*, *Scomberomorus tritor* and *Mullus barbatus*), but none of the sampled species

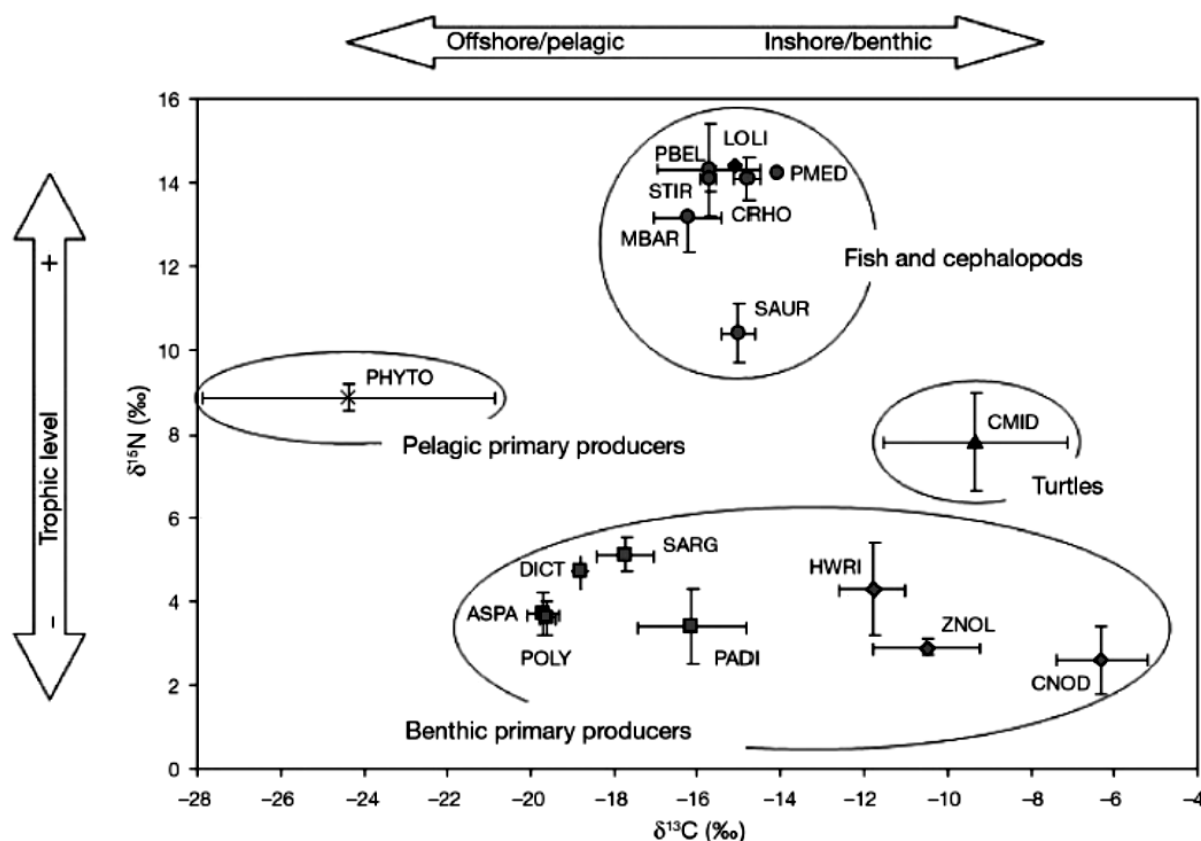


Fig. 2. Mean (\pm SD, ‰) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values for all trophic groups considered, except marine mammals: phytoplankton (x), seagrass (♦), macroalgae (■), fish (●), cephalopods (◆) and sea turtle (▲). The identification codes and number of individuals for each species are presented in Table 1; ellipses enclose groups listed in Table 1



was truly oceanic. The $\delta^{15}\text{N}$ of macrophytes was highly variable and ranged from 2.6‰ (the seagrass *Cymodocea nodosa*) to 5.4‰ (the macroalgae *Sargassum* sp.). Phytoplankton displayed higher $\delta^{15}\text{N}$ values than did any benthic macrophyte. As expected, green turtles were more enriched for $\delta^{15}\text{N}$ than were benthic primary producers, and the zooplanktophagous fish *S. aurita* was more enriched for $\delta^{15}\text{N}$ than was phytoplankton. Likewise, fish including only small invertebrates in their diets (*M. barbatus* and *S. aurita*) presented lower $\delta^{15}\text{N}$ values when compared with fish consuming large invertebrates and fish (*C. rhonchus*, *S. tritor* and *P. mediterraneus*). Such an isotopic landscape indicates that the bone of oceanic marine mammals should present lower $\delta^{13}\text{C}$ values than that of coastal marine mammals, especially those inhabiting seagrass meadows. Furthermore, $\delta^{15}\text{N}$ is a good proxy for trophic level in the considered system, although species inhabiting seagrass meadows might present lower $\delta^{15}\text{N}$ values in comparison with oceanic species at the same trophic level.

As revealed by the $\delta^{13}\text{C}$ values, 4 major groups of marine mammals exist as delineated by their distribution along the coastal–oceanic axis (Fig. 3). Carbon isotope values together with the results of the post hoc pairwise comparisons allowed us to group species according to the similarity of their $\delta^{13}\text{C}$ values. The most coastal group, characterised by values of the $\delta^{13}\text{C}$ close to those of seagrass, included *Monachus monachus* ($\delta^{13}\text{C} = -10.4\text{‰}$) and *Sousa teuszii* ($\delta^{13}\text{C} = -9.7\text{‰}$). The most oceanic group, characterised by highly negative $\delta^{13}\text{C}$ values, included *Physeter macrocephalus* ($\delta^{13}\text{C} = -13.9\text{‰}$). *Balaenoptera acutorostrata* ($\delta^{13}\text{C} = -15.9\text{‰}$) probably belonged to this group, although only one individual was sampled. The remaining 9 species had $\delta^{13}\text{C}$ values between these 2 groups, with *Delphinus* sp., *Globicephala macrorhynchus*, *Phocoena phocoena*, *Stenella frontalis* and *Orcinus orca* presenting lower $\delta^{13}\text{C}$ values than *Globicephala melas* and *Tursiops truncatus* (Table 1, Fig. 3).

Regarding the $\delta^{15}\text{N}$ values, significant differences were observed between the most $\delta^{15}\text{N}$ -enriched spe-

Stable isotopic composition of marine mammals

Statistically significant differences exist among the bivariate isotopic signals of the considered species of marine mammals (MANOVA test, Wilk's lambda = 0.413, $F = 14.131$, $p < 0.001$). ANOVA indicated that both nitrogen and carbon were involved in these differences ($\delta^{13}\text{C}$: $F = 16.982$, $p < 0.001$; $\delta^{15}\text{N}$: $F = 11.256$, $p < 0.001$; Table 2). The results of the post hoc test for pairwise comparisons between species for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are illustrated in Table 3.

Table 2. Summary of the results of the ANOVA to test for differences in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the marine mammal species investigated in the study area

Factor	SS	df	MS	F	p	r ²
$\delta^{13}\text{C}$ (‰)						
Model	140.791	9	15.643	16.982	<0.001	0.399
Intercept	7009.343	1	7009.343	7608.979	<0.001	
Species	140.791	9	15.643	16.982	<0.001	
Error	211.875	230	0.921			
Total	33183.103	240				
$\delta^{15}\text{N}$ (‰)						
Model	94.977	9	10.553	11.256	<0.001	0.306
Intercept	10178.900	1	10178.900	10857.200	<0.001	
Species	94.977	9	10.553	11.256	<0.001	
Error	215.631	230	0.938			
Total	43505.029	240				

Table 3. Results of the post hoc test for multiple comparisons between the marine mammal species collected along the Mauritanian coast (see Table 1 for codes). Carbon isotope ($\delta^{13}\text{C}$) p-values are reported below the diagonal and those for nitrogen isotopes ($\delta^{15}\text{N}$) are reported above the diagonal. Significant p-values (<0.05) are highlighted in **bold**

	DDEL	GMAC	MMON	PPHO	STEU	TTRU	SFRO	GMEL	ORCA	PMAC
DDEL		0.000	0.000	0.000	0.052	0.000	0.334	0.049	0.002	0.000
GMAC	0.008		0.271	0.259	0.104	0.778	0.154	0.940	0.458	0.009
MMON	0.000	0.008		0.006	0.004	0.064	0.020	0.577	0.988	0.042
PPHO	0.000	0.523	0.000		0.351	0.118	0.399	0.513	0.128	0.001
STEU	0.000	0.000	0.076	0.000		0.055	0.829	0.305	0.060	0.000
TTRU	0.000	0.199	0.026	0.001	0.000		0.143	0.825	0.314	0.003
SFRO	0.585	0.284	0.002	0.435	0.000	0.033		0.291	0.077	0.001
GMEL	0.060	0.588	0.332	0.363	0.054	0.960	0.218		0.633	0.048
ORCA	0.357	0.567	0.017	0.805	0.000	0.155	0.732	0.378		0.091
PMAC	0.024	0.001	0.000	0.001	0.000	0.000	0.029	0.003	0.018	

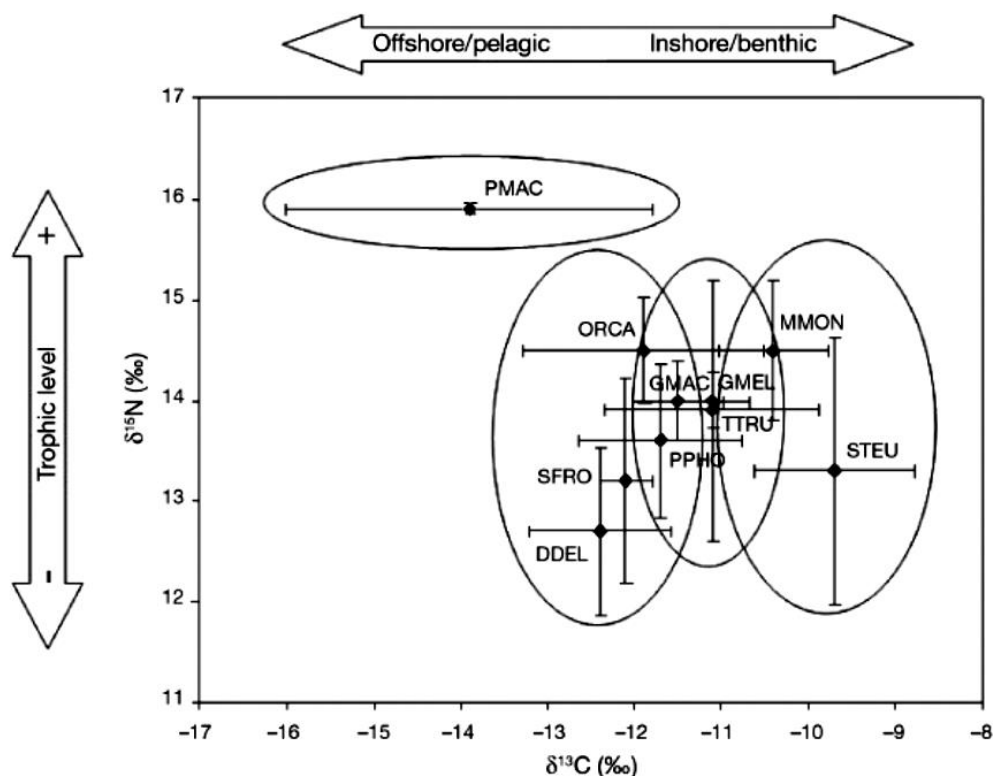


Fig. 3. Mean (\pm SD, ‰) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of the marine mammal species collected in Mauritania. The identification codes and number of individuals for each species are presented in Table 1; ellipses group the species that did not display significant differences in $\delta^{13}\text{C}$ values ($p > 0.05$)

cies (*Physeter macrocephalus*) and those species less enriched (e.g. *Delphinus* sp. and *Sousa teuszii*; Tables 1 & 3, Fig. 3). The only specimens of *Balaenoptera acutorostrata* and *Stenella coeruleoalba* analysed were also highly depleted for $\delta^{15}\text{N}$ —11.8 and 11.9‰, respectively. Differences in $\delta^{15}\text{N}$ values among species revealed differences in trophic level: species with higher $\delta^{15}\text{N}$ values were at a higher trophic level than species with lower $\delta^{15}\text{N}$ values. However, as previously stated, coastal species might present lower $\delta^{15}\text{N}$ values in comparison with oceanic species at the same trophic level. Accordingly, *P. macrocephalus* was the species with the highest trophic level, while *B. acutorostrata* and *S. coeruleoalba* were the species with the lowest trophic levels. It is important to notice that the mean trophic level of *Orcinus orca* ($\delta^{15}\text{N} = 14.5\text{‰}$) was equal to that of *Monachus monachus* ($\delta^{15}\text{N} = 14.5\text{‰}$) and lower than that of *P. macrocephalus* ($\delta^{15}\text{N} = 15.9\text{‰}$). However, direct comparisons between species may be slightly biased owing to the fact that *M. monachus* is a much more coastal species than *O. orca*.

DISCUSSION

Although the use of stable isotopes as diet tracers is a powerful technique, interpretation of the results is not always straightforward because the method is more reliable when large differences exist between the isotopic signals of the considered sources. Many researchers do not assess the variability in $\delta^{15}\text{N}$ among primary producers because it is assumed that they have similar $\delta^{15}\text{N}$ values. Although this is true in some ecosystems and regions, exceptions are common (Drago et al. 2009). The present study reveals a high variability in $\delta^{15}\text{N}$ among macrophyte species from the same region and also between macrophytes and phytoplankton, with differences often larger than the average increase expected per trophic level (Minagawa & Wada 1984, Caut et al. 2009). As a consequence, variability in the $\delta^{15}\text{N}$ of marine mammal bone may reflect not only differences in trophic level but also shifts among foraging grounds with primary producers that differ in $\delta^{15}\text{N}$ values. In the Mauritanian ecosystem, coastal marine mammals are expected to

have lower $\delta^{15}\text{N}$ values than their oceanic counterparts because macrophytes display lower $\delta^{15}\text{N}$ values than particulate organic matter. In species feeding on a variety of prey species with different nitrogen baselines, diet composition is difficult to establish simply through stable isotope analysis of nitrogen. Fortunately, the $\delta^{13}\text{C}$ of primary producers from Mauritania varied more consistently among groups, with phytoplankton less enriched in ^{13}C than macroalgae, and macroalgae less enriched in ^{13}C than seagrass. This allowed us to interpret the enrichment in ^{13}C of the bone of marine mammals as evidence of more-coastal foraging habitats.

Therefore, *Sousa teuszii* and *Monachus monachus* appear to be the most coastal species, a result consistent with published information about the distribution of these species off Mauritania. The main area of occurrence of *S. teuszii* is the Banc d'Arguin, a large system of shallow waters and channels extending along the northern coast of the country (Maigret et al. 1976, Maigret 1980a, Van Waerebeek et al. 2004). *M. monachus* is found off the Cape Blanc Peninsula, although it has also been observed to travel several miles offshore to obtain its prey (Gazo & Aguilar 2005). Despite the similarity in the $\delta^{13}\text{C}$ values of *M. monachus* and *S. teuszii*, the former was far more enriched for $\delta^{15}\text{N}$. This result is also consistent with stomach-content analysis, as *S. teuszii* consumes primarily detritivorous and zooplanktophagous fish that are abundant nearshore (Cadenat & Paraíso 1957, Van Waerebeek et al. 2004), whereas *M. monachus* principally eats lobsters, octopus and benthic, carnivorous fish (Marchesaux 1989). However, increasing fish landings have resulted in stocks, especially those of demersal fish and invertebrates (crustaceans and cephalopods), being overexploited or severely depleted (Christensen et al. 2004, Jouffre & Inejih 2005), most likely affecting the feeding behaviour of *M. monachus* and *S. teuszii*.

Also, the $\delta^{13}\text{C}$ values suggest that *Balaenoptera acutorostrata* and *Physeter macrocephalus* are the most oceanic species in the region; *B. acutorostrata* has been observed both in coastal and offshore waters off the Western Sahara (Van Waerebeek et al. 1999). Distribution data for *P. macrocephalus* off Mauritania are limited (Maigret 1980b), although the species is oceanic over most of its range worldwide (Rendell et al. 2004). The diet of these species off NW Africa is unknown, but elsewhere *P. macrocephalus* preys mostly on large, oceanic squid with a high trophic level (Clarke et al. 1976, Pauly et al. 1998b, Ostrom et al. 1993, González et al. 1994), which is consistent with its high $\delta^{15}\text{N}$ in the study area. By contrast, *B. acutorostrata* primarily consumes schooling fish (Das et al. 2003, Hassani et al. 1997, Pauly et al. 1998b, MacLeod et al. 2007). This kind of prey could explain its semblance with *S. coerulea*

with respect to $\delta^{15}\text{N}$ values, although only one specimen of the latter was analysed.

The remaining species lie between the former 2 groups, spaced along a continuous gradient. *Tursiops truncatus* is found in both coastal and offshore habitats along the Mauritanian coastline (Robineau & Vely 1998). This probably explains the large intraspecific variability in the $\delta^{13}\text{C}$ values and suggests the occurrence of persistent individual differences in foraging habits or even the existence of coastal versus offshore populations and/or ecotypes in the area, as has been found elsewhere (Segura et al. 2006).

Nevertheless, the mean $\delta^{13}\text{C}$ value of *Tursiops truncatus* was close to that of *Globicephala melas*, a typical deepwater species in the North Atlantic (MacLeod et al. 2007) that is also known to occur over the continental shelf off Banc d'Arguin and off the Grande Plage (Maigret et al. 1976, Robineau & Vely 1998). *G. melas* displayed a slightly higher $\delta^{13}\text{C}$ value than *Globicephala macrorhynchus*, a species usually inhabiting deeper water over the upper slope in the North Atlantic (Davis et al. 2002), although nothing is known about its distribution off Mauritania. Finally, these 3 species presented lower $\delta^{13}\text{C}$ values than those of *Sousa teuszii* and *Monachus monachus* and higher values than those of typical oceanic species, such as *Delphinus* sp. and *Physeter macrocephalus* (Robineau & Vely 1998, Rendell et al. 2004). However, *T. truncatus*, *G. melas* and *G. macrorhynchus* also showed similar $\delta^{15}\text{N}$ values, suggesting that they possibly forage for similar prey. Published dietary data, only available for *T. truncatus* off Mauritania, identified the detritivorous grey mullet *Mugil cephalus* as their main prey item (Robineau & Vely 1998). This observation does not seem to correlate with the typical prey of the 2 species of *Globicephala* (Overholtz & Waring 1991). It is possible, however, that only a fraction of the population of *T. truncatus* regularly consumes grey mullet, while most of the population has a diet similar to that of *Globicephala* spp. This scenario has been reported, for instance for northern Spain, where *G. melas* and *T. truncatus* feed mainly on neritic cephalopods (González et al. 1994). Likewise, in the Gulf of California, the coastal ecotype of *T. truncatus* displayed values of the $\delta^{13}\text{C}$ similar to those of coastal prey items such as the mullet *M. cephalus*, whereas the offshore ecotype of *T. truncatus* displayed values of the $\delta^{13}\text{C}$ more similar to those of pelagic fish and medium-sized squid (Segura et al. 2006).

The species below the previous 3 in decreasing order of $\delta^{13}\text{C}$ are *Phocoena phocoena*, *Orcinus orca*, and *Grampus griseus*. *P. phocoena* inhabits cold-temperate and subpolar regions in the North Atlantic, generally showing affinity for shelf waters (Skov et al. 1995). Mauritania is the southern limit of the distribution range of

the species probably because of the coastal upwelling, which produces relatively cold waters (Smeenk et al. 1992). Sightings suggest that *P. phocoena* lives close to the coast in the non-upwelling season and moves offshore in the onset of the upwelling season (Marchesaux & Campredon 1988, Smeenk et al. 1992, Robineau & Vely 1998). The diet of *P. phocoena* off NW Africa is unknown, but mean $\delta^{15}\text{N}$ values are similar to those of *Tursiops truncatus*, which is consistent with a fish-based diet (Fontaine et al. 1994). *Orcinus orca* has been reported in both coastal and oceanic areas off Mauritania, except in the very shallow waters of the Parc National du Banc d'Arguin (Duguy 1976, Maigret 1981). However, its $\delta^{13}\text{C}$ values reveal a preference for the deeper parts of the continental shelf and the shelf break, as they are close to the values for *Delphinus* sp. The Mauritanian population of *O. orca* appears to be the 'offshore' ecotype, which is an ecotype thought to eat fish (Ford et al. 2000, Saulitis et al. 2000). This is in agreement with visual observations that suggested that *O. orca* avoided other marine mammals, including the monk seal *M. monachus* (A. Aguilar unpubl. data). The mean $\delta^{15}\text{N}$ of this species suggests a diet based on carnivorous fish, as reported for southern Spain (De Stephanis et al. 2008). *Grampus griseus* is poorly reported in NW Africa (Robineau & Vely 1998), but isotopic values revealed an oceanic habitat preference that occurs seaward of the continental slope and is concentrated along the upper continental slope (Baumgartner 1997, Davis et al. 2002).

Delphinus sp. is seldom observed onshore in Mauritania (Robineau & Vely 1998) and displays a typical oceanic behaviour. *Stenella frontalis* also typically inhabits the shelf break (Nieri et al. 1999) because the quasi-permanent upwelling may act as a thermal barrier and prevent it from approaching the inshore coastal zone (Robineau & Vely 1998). Likewise, *Stenella coeruleoalba* is oceanic over most of its range (Aguilar 2000, Davis et al. 2002). Accordingly, these 3 species are more depleted for $\delta^{13}\text{C}$ than *Monachus monachus*, *Sousa teuszii* and *Tursiops truncatus*. *Delphinus* sp. and *S. coeruleoalba* presented some of the lowest $\delta^{15}\text{N}$ values of all marine mammals, which is similar to what has been observed for other marine trophic webs (Das et al. 2003, Walker and Macko 1999). The discrepancies in $\delta^{15}\text{N}$ values of these species compared with that of other pelagic marine mammals possibly reflects the higher proportion of low-trophic-level prey, such as *Sardinella aurita*, in the diet of these species off the coast of Mauritania. Additionally, dolphins are opportunistic feeders and thus take advantage of the local abundance of prey (Hassani et al. 1997).

The results obtained in the present study suggest that the outer continental shelf and the upper slope are the areas with greater marine mammal species diver-

sity. However, coastal areas are home to the most vulnerable and endangered species in the Mauritanian marine ecosystems. In the outer continental shelf and upper slope, there could be a certain redundancy between species because their isotopic signals are very similar, indicating that they occupy a very similar ecological niche. By contrast, the reduced number of species and the greater difference in isotopic signals indicate less redundancy in the coastal areas. From this point of view, conservation of coastal species (*Monachus monachus* and *Sousa teuszii*) in Mauritania should be a priority because, if these species were to disappear, they could not be replaced in the trophic web by other marine mammals. In the case of *M. monachus*, it is likely that this species is already extinct from the ecological point of view, given the small number of individuals of this population (Forcada et al. 1999). Artisanal fisheries could have an impact on *M. monachus* and *S. teuszii*, although industrial fishing operating in the deepest parts of the continental shelf are the only sources of incidental bycatch recorded to date in the region and *Phocoena phocoena* and *Stenella frontalis* are the only species known to be affected (Nieri et al. 1999). Nevertheless, overfishing of the local demersal species by the intensive bottom trawling fishing in Mauritania (Jouffre & Inejih 2005) may have limited the prey availability for *M. monachus*, which might explain, in part, the reproductive problems of this species (Pastor 2010). Therefore, the highest priority of conservation efforts should be to reduce the impact of artisanal and industrial bottom trawling fishing on the less abundant and most vulnerable species. Nonetheless, it will also be important to regularly estimate and control the populations and catches of fishes in the whole study area because this region shelters a great diversity of marine mammals. There are inevitable consequences of fishing down the food web: increased ecosystem instability, unsustainable fisheries and an inability for the ecosystem to support healthy, abundant populations of apex predators (Trites et al. 2006). Excessive catches of one species may lead to the collapse of an important predator or prey in the system, which may cause changes in the growth and survival patterns of other species in the food web (Walters & Kitchell 2001). Some groups of apex predators, such as marine mammals, may be affected by fisheries even when the distributions of prey and predators do not overlap, driving species at the end of the food chain to the brink of extinction (Trites et al. 2006). Conservation efforts should be made locally to protect these species because this area suffers a high fishing pressure (ter Hofstede & Dickey-Collas 2006, Christensen et al. 2004) and animals are constantly exposed to threats such as intensive fishing (Kenety 2001, Christensen et al. 2004), prey stock depletion (ter Hofstede & Dickey-



Collas 2006, Christensen et al. 2004), pollution and human disturbance (Colman et al. 2005). Conservation efforts should be unequivocally related to the local fishing policies, such as effective monitoring, control and surveillance of fisheries in the region (Atta-Mills et al. 2004); strengthening political interest to implement sustainable fisheries in NW Africa, because African leaders appear to be disregarding overfishing as a priority issue that needs to be addressed (NEPAD 2001); and lobbying the European Union and Asian countries, such as Japan and China, for policy reforms to minimize the impacts of European and Asian fishing fleets on NW African coastal marine resources.

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Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope values in common dolphins (*Delphinus* spp.) worldwide, with particular emphasis on the eastern North Atlantic populations

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RATIONALE: Distinguishing population units of small cetaceans continuously distributed in a widespread area is challenging but critical for their conservation and management. The use of chemical markers allows the investigation of foraging ecology and inter-specific variability, in order to detect population structure and niche segregation in the common dolphin (*Delphinus* spp.).

METHODS: The stable isotope ratios of carbon ($\delta^{13}\text{C}$ values) and nitrogen ($\delta^{15}\text{N}$ values) were measured in the bone tissue of common dolphins accidentally by-caught or stranded along the north-eastern and eastern Subtropical Atlantic Ocean, by means of continuous flow elemental analyser/isotope ratio mass spectrometry. Trophic positions were determined and compared, taking into account the local ecosystem trophic baseline for each study area. Data obtained for the study areas were qualitatively compared with those for common dolphin species/populations distributed worldwide.

RESULTS: The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were higher in the eastern Subtropical Atlantic as a consequence of the coexistence in the area of the common dolphin short- and long-beaked morphotypes. Individuals from the north-eastern Atlantic displayed lower $\delta^{15}\text{N}$ values, reflecting dissimilarities in diet and variation in local isotopic baselines. Comparisons with other areas around the world suggest that the species is extremely adaptive and feeds at different trophic levels to adapt to local variations.

CONCLUSIONS: Stable isotopes are a useful tool to investigate population structure and trophic niche segregation. The trophic behaviour of worldwide populations of common dolphins was fruitfully analysed and revealed substantial differences, probably reflective of both adaptive strategies of the genus and dissimilarities in the structure of the ecosystems. Copyright © 2015 John Wiley & Sons, Ltd.

Distinguishing population units of small cetaceans continuously distributed in a widespread area is challenging but it is critical for their conservation and management.^[1] The almost continuous distribution, alongside the inherent difficulties involved in the study of cetaceans, i.e. animals that spend long periods submerged and feed underwater and that are usually located at great distances from research sites,^[2] makes it hard to study their population structure, habitat use, trophic positions or feeding behavior.

Common dolphins (*Delphinus* spp.) are present in all oceans of the world between latitudes 40° and 60° N to about 50° S except in the western Atlantic, where they appear to be absent from much of its tropical/subtropical waters.^[3] Such an extensive geographical distribution has facilitated the differentiation of a number of morphotypes whose taxonomic status remains unclear still today.^[4] Banks and Brownell^[5] identified in the Pacific Ocean two morphotypes that could be easily differentiated by the relative length of their rostrum, and suggested that the anatomical differences were probably

due to dissimilarities in food habits. Thus, the long-beaked morphotype would be mostly of coastal or neritic distribution,^[6,7] while the short-beaked would be of both inshore and offshore distribution.^[5,7,8] Conversely, when the same differentiation was described for the eastern Atlantic Ocean, the long-beaked morphotype revealed a tendency for an offshore/pelagic distribution compared with the short-beaked morphotype.^[9,10] Decades later, the taxonomical status of these morphotypes is still a matter of debate and it is unclear whether the observed diversification is determined genetically or ecologically, or by both causes.^[4,9–15] Natoli and colleagues^[14] proposed that the long-beaked morphotype originated independently in different regions and suggested that adaptation to local environments was driving local speciation. Thus, skull morphology would be the consequence of adaptation to prey capture and habitat use. Nevertheless, no research has ever compared habitat use, feeding habits, trophic positions or distribution patterns of *Delphinus* spp. species/populations worldwide.

Stable isotopes constitute a useful tool for investigating diet, habitat use and distribution patterns of animals and in cetaceans they have been widely used to infer population structure.^[1,2,16–24] Stable isotopes of carbon (measured as $\delta^{13}\text{C}$ values) and nitrogen (measured as $\delta^{15}\text{N}$ values) have been used mostly as dietary tracers^[25] based on the fact that

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the isotopic composition of these elements present in the tissues of an animal depends on that of its food sources^[26,27] and therefore offers an integrated measure of the prey assimilated over a comparatively protracted period of time.^[28,29] In addition, the relative abundance of the heavier isotopes of both elements increases progressively through the food web due to the faster excretion rate of the lighter isotopes.^[30–32] Hence, the $\delta^{15}\text{N}$ value has been found to increase at each trophic level by 2–5 ‰ depending on species, tissue and other factors,^[32,33] and can therefore be used as a reliable indicator of trophic level. Increase in the $\delta^{13}\text{C}$ value is more moderate (estimated at ca 1 ‰ in each trophic level) but, because other factors than diet may also interfere with the $\delta^{13}\text{C}$ value (e.g., location and ecosystem properties related to plant type and carbon cycling),^[33] it is generally not used as an indicator of trophic level.^[32] Thus, the $\delta^{13}\text{C}$ value at the base of the food web is primarily driven by physical parameters (temperature and dissolved CO_2) and the type composition of primary producers, for which reason near-shore and benthic systems are typically more ^{13}C -enriched than offshore, pelagic systems.^[34,35] These $\delta^{13}\text{C}$ geographical differences transferred to animal tissues provide a valuable tool to infer marine mammal information, such as their origins and migration patterns.^[29]

In this study, we measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the bone of stranded common dolphins from different areas of the north-eastern Atlantic to investigate foraging ecology and potential intra-specific differences that could be associated with cranial morphology with the ultimate objective of defining population units of relevance to management and conservation. Trophic positions were calculated for the species in the different areas, and their isotopic values compared with those from other regions worldwide to investigate trophic behaviour and niche segregation within the genus. We hypothesise that there is a great trophic variability in the common dolphin and predict niche segregation between populations worldwide, probably reflecting differential use of habitat rather than taxonomy.

EXPERIMENTAL

Study site and sampling

Common dolphin bone remains (skulls) were collected between 1975 and 2007 from the Atlantic coast of the Iberian Peninsula, and between 1993 and 2008 from the north-west coast of Africa (Fig. 1). For comparative purposes, the study area was divided into three areas based on the characteristics of the habitat (upwelling areas, productivity, fresh water outputs, etc.) and taking into account the natural barriers between the sampling areas. The first area, Zone I ($n = 31$), extended over the northern Portuguese and Galician shelves up to Cape Finisterre ($42^\circ 54'\text{N}$, $9^\circ 16'\text{W}$), respectively, north and south of the Douro River. The second area, Zone II ($n = 38$), included the Portuguese and Andalusian shelves, from Cabo da Roca ($38^\circ 47'\text{N}$, $9^\circ 30'\text{W}$) to the southernmost tip of the Iberian Peninsula ($36^\circ 0'\text{N}$, $5^\circ 35'\text{W}$). Finally, the third area, Zone III ($n = 93$), extended along the continental shelf that ranges from the southern fringe of the Western Sahara ($20^\circ 46'\text{N}$, $17^\circ 3'\text{W}$) to the northern coast of Senegal, including Mauritania ($16^\circ 3'\text{N}$, $16^\circ 30'\text{W}$).

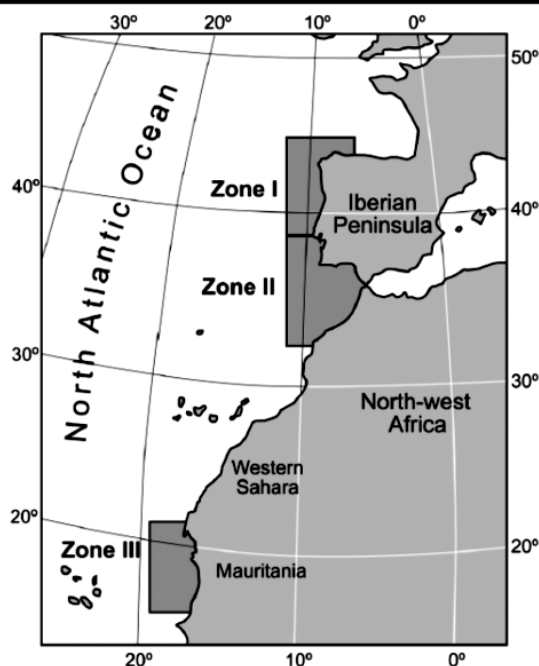


Figure 1. Map of the study area, with the three study zones indicated by shaded areas (Zones I to III).

The specimens used in this study belonged to museum collections held by Aquário Vasco da Gama (Portugal), the Museu Bocage (Portugal), the Parc National du Banc d'Arguin (Mauritania), the Institut Mauritanien de Recherches Océanographiques et des Pêches (Mauritania), the Centre National d'Élevage et de Recherches Vétérinaires (Mauritania), and the University of Barcelona (Spain). Most samples came from stranded animals, but some had been by-caught during fishing or old military-research activities. Specimens were identified as common dolphins on the basis of skull morphology (e.g., the presence of deep palatal grooves).^[4] Only adult animals were used in this study in order to avoid variability in stable isotope abundances due to ontogenic development. The physical maturity of the specimens was established as described by Pinela and co-authors.^[10]

Bone was the tissue of choice because of its low turnover rate, which reflects the animal's diet throughout several years.^[36] The bone part used for the study was a small fragment of the turbinate bones from the nasal cavity because of its ease of sampling and because the sampling did not affect the integrity of the skull with regard to posterior morphological studies. Until the moment of the analysis, the samples were stored dry and at ambient temperature ($15\text{--}25^\circ\text{C}$).

Stable isotope analyses

Stable isotope values (expressed as delta notation, δ), in which the relative variations in stable isotope ratios are expressed in per mil (‰) deviations from predefined international standards, were calculated as:^[37]

$$\delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} \right] - 1$$



where X is the value of the heavy isotope of the sample (^{13}C or ^{15}N), R_{sample} is the ratio of the heavy to the light isotope of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$), and R_{standard} is the ratio of the heavy to the light isotope in the reference standards, which were Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for carbon and atmospheric nitrogen (air) for nitrogen.

For the pre-treatment of the samples, approximately 1 g of bone tissue was rinsed with distilled water, dried for 3 days at 60°C and powdered with a mortar and pestle. Since lipids are depleted in ^{13}C compared with proteins, and may therefore mislead the analyses by decreasing the $\delta^{13}\text{C}$ value,^[38] they were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution.^[39] When conducting stable isotope analysis in bone, several authors have carried out preventive demineralisation of the tissue by treating it with either a 0.5 or 1 M hydrochloric acid (HCl) solution.^[40,41] However, a test using a subset of the samples revealed that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not differ between demineralised and untreated samples ($\delta^{13}\text{C}$: $t = 0.951$, $df = 19$, $p = 0.353$; $\delta^{15}\text{N}$: $t = 0.527$, $df = 19$, $p = 0.604$; $n = 20$), so this procedure was not followed. The C/N ratio was calculated in order to evaluate if the pre-treatment of bone tissue was effective^[42] and to ensure that the isotope values obtained were in the expected range for pure bone collagen protein.^[43,44]

After pre-treatment, approximately 1 mg of the treated bone was weighed in tin capsules (3.3×5 mm) and combusted at 900 °C. Isotope analyses were carried out by means of elemental analyser/isotope ratio mass spectrometry (EA-IRMS) using a ThermoFinnigan Flash 1112 (CE Elantech, Lakewood, NJ, USA) elemental analyser, coupled to a Delta C isotope ratio mass spectrometer via a ConFlo III interface (both from ThermoFinnigan, Bremen, Germany). Atropine was used as a standard for the elemental composition of carbon and nitrogen. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the IAEA (International Atomic Energy Agency, Vienna, Austria), namely polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$) and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios, namely $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$) and KNO_3 (IAEA NO3, $\delta^{15}\text{N} = +4.7\text{‰}$), were used for calibration at a precision of 0.3‰. The analyses were conducted in the Centres Científics i Tecnològics of the University of Barcelona (CCiT-UB).

Trophic level determinations

The $\delta^{15}\text{N}$ value provides an index for trophic position relative to the known trophic level of a primary producer, or of an organism whose trophic position in the local marine ecosystem is known ($\delta^{15}\text{N}_{\text{base}}$ value; i.e., the ecosystem baselines).^[31] In our study, the $\delta^{15}\text{N}_{\text{base}}$ value was estimated from published isotopic values of local widespread fish preys for each of the studied zones (different prey species were used as baseline, see Table 1) whose trophic level (TL) was previously known through the study of gut contents, as reported in FishBase^[45]. The trophic enrichment factor applied, specific for bone tissue ($\Delta_n = 2.03\text{‰}$), has recently been reported in a cetacean species (fin whale, *Balaenoptera*

Table 1. Published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, number of individuals (n), and trophic level (TL) for the species used as ecosystem baseline to determine the trophic position of common dolphins in each study area

Area	Species	n	BASELINE			Reference
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	TL ^a	
Zone I	<i>Sardina pilchardus</i> [†]	447	-17.0	10.7	3.1	Bode <i>et al.</i> ^[62]
Zone II	<i>Sardinella pilchardus</i>	5	-18.1	10.6	3.1	L. Cardona (unpublished)
Zone III	<i>Sardinella aurita</i> [†]	10	-15.0	10.4	3.4	Pinela <i>et al.</i> ^[9]

Common names of the baseline species: European pilchard (†), Round sardinella (†)
^aFishBase.^[45]

physalus) by Borrell and colleagues,^[46] and hence was assumed for trophic estimations for the common dolphins from the study areas (Zones I to III). For the consumer species, the trophic level (TL) was calculated as:

$$\text{TL} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}})/\Delta_n$$

where λ is the trophic level of the organism used to estimate the $\delta^{15}\text{N}_{\text{base}}$ value, Δ_n is the bone $\delta^{15}\text{N}$ fractionation value, and $\delta^{15}\text{N}_{\text{consumer}}$ is the direct measurement of the $\delta^{15}\text{N}$ value for the target species,^[47] i.e., the common dolphin.

Data analyses

Previous to data analysis, the raw $\delta^{13}\text{C}$ data was adjusted to take into account the Suess effect according to the year of sampling. The Suess effect describes the ^{13}C atmospheric depletion through time, due to burning of fossil fuels since the onset of the Industrial Revolution.^[48,49] Körtzinger *et al.*^[49] calculated that the $\delta^{13}\text{C}$ values in dissolved inorganic carbon (DIC) in the North Atlantic Ocean had decreased by a factor of $0.026 \pm 0.002\text{‰}$ per year. So, in order to minimise the Suess effect when comparing groups of samples from different periods, the raw $\delta^{13}\text{C}$ data was corrected adding 0.026‰ per year, since the first year of sampling and over a 33-year time period. Since all areas investigated in the present study are geographically situated in the North Atlantic, we used the same $\delta^{13}\text{C}$ increasing factor for the three areas.

The assumption of normality in the distributions of the sample groups was checked with the Kolmogorov-Smirnov test of goodness of fit, and the homogeneity of variances between them was checked with Levene's test. The variation in the results and the presence of outliers were tested graphically using boxplots, depicting the first, second (the median) and third quartiles, as well as the whiskers.

Differences in the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of common dolphins between sampling years were investigated through an analysis of variance (ANOVA), for each study area separately.

Differences in mean bivariate isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of common dolphins between areas were also assessed through a multivariate analysis of variance (MANOVA) because they followed a normal distribution. Univariate analysis of variance (ANOVA) was conducted separately for each isotope value. If a statistical significant interaction was found among areas for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, additional pairwise comparisons were made with a generalised linear model because it admits data with non-homogeneous variances. *Post hoc* analyses were made with least significant differences tests (LSD) based on estimated marginal means. As a method of estimation, the maximum likelihood (ML) was used in all cases. Normality distribution and identity as a link function was always used (for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values), since the data followed a normal distribution. Goodness of fit (Akaike's information criterion) was used to compare different models. The confidence interval level was set at 95% for all comparisons.

The isotopic ratio values determined in the bone of the common dolphins from the areas here studied were compared with those from other populations of common dolphins from other regions. Tissues other than bone were considered, but the tissue isotopic values were converted into those of bone by means of fractionation factors in order to avoid variability among tissues in the comparison. Trophic positions could only be determined in a qualitative manner for those worldwide populations.

Unless stated, data are presented as mean \pm standard deviation (SD) throughout the manuscript. All the statistical analyses were conducted using the SPSS version 15 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The C/N ratio for all samples varied between 2.96 and 3.39 (Zone I: 3.01–3.27; Zone II: 3.12–3.39; Zone III: 2.96–3.34). These values (lower than 4) show: (1) that the lipid extraction process in the bone samples was effective, and (2) that the samples were well preserved and the bone collagen integrity was guaranteed.^[43,44]

Two individuals were excluded from the dataset because they were significant outliers (Fig. 2). In all the common dolphin populations studied, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values followed a normal distribution ($p > 0.771$ for all areas) and were heteroscedastic ($\delta^{13}\text{C}$: $p < 0.001$, $\delta^{15}\text{N}$: $p < 0.001$).

ANOVA indicated that there were no significant differences between sampling years for both isotope ratios in Zone I ($n = 28$, $\delta^{13}\text{C}$: $p = 0.199$, $\delta^{15}\text{N}$: $p = 0.066$), Zone II ($n = 36$, $\delta^{13}\text{C}$: $p = 0.331$, $\delta^{15}\text{N}$: $p = 0.104$), and Zone III ($n = 87$, $\delta^{13}\text{C}$: $p = 0.157$, $\delta^{15}\text{N}$: $p = 0.140$). Therefore, we can assume that the trophic habits of this species have not changed across the years and potential differences in stable isotope values are probably due to dissimilarities between areas rather than the sampling period.

Stable isotope variability in Zones I–III

Individual bone $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the common dolphins gathered by study area are shown in Fig. 3, where lines are plotted around each group to aid in visualisation. The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the bone of common

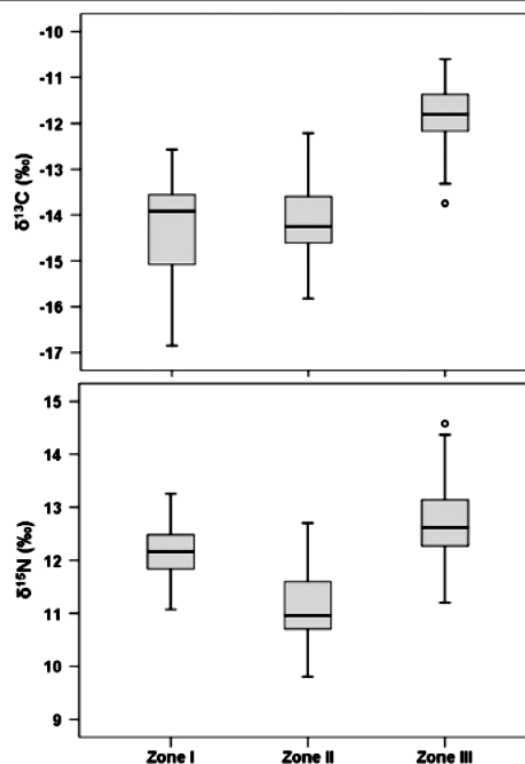


Figure 2. Boxplots illustrating the distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained from the isotopic analysis of common dolphin bone samples from Zones I to III.

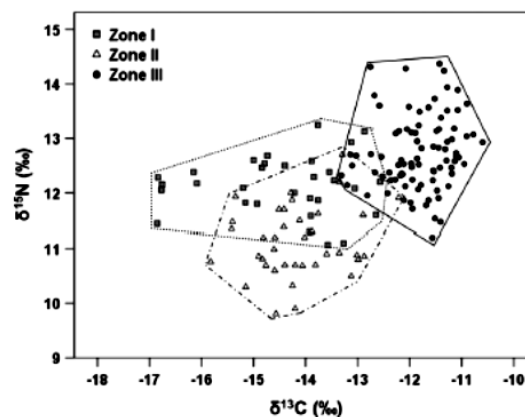


Figure 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the common dolphin individuals sampled in Zone I, Zone II, and Zone III (see figure legend for details). Lines were plotted around each group to aid in visualisation.

dolphins collected from each area, and other isotopic related information, are presented in Table 2. Figure 2 displays a boxplot depicting an intergroup comparison between the areas analysed for each isotope, allowing the visualisation of the tendency across common dolphin populations of the



Table 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) for common dolphin, *Delphinus* spp, samples collected along the study area and for populations worldwide

Area	Code	Species	n	Sex	Tissue	Lipids	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{15}\text{N} \pm \text{SD}$	TL	Reference
Mediterranean Sea										
Southwestern Mediterranean	SWM	<i>Delphinus delphis</i>	2	M	M	No	-17.8 ± 0.04	10.5 ± 0.4	ND	Borrell and Aguilar ^[61]
NE Atlantic Ocean										
Zone I	ZI	<i>Delphinus delphis</i>	31	M+F	B	Yes	-14.44 ± 1.3	12.14 ± 0.5	3.8	Present study
Zone II	ZII	<i>Delphinus delphis</i>	38	M+F	B	Yes	-14.16 ± 0.8	11.12 ± 0.6	3.4	Present study
Zone III	ZIII	<i>Delphinus</i> spp.	86	M+F	B	Yes	-11.81 ± 0.6	12.72 ± 0.7	4.5	Present study
Galicia	GAL	<i>Delphinus delphis</i>	5	M+F	M	No	-17.0 ± 0.4	13.1 ± 0.8	4.2	Bode <i>et al.</i> ^[62]
Bay of Biscay	BB	<i>Delphinus delphis</i>	10	M+F	M	No	-18.4 ± 0.5	10.3 ± 0.3	ND	Das <i>et al.</i> ^[63]
French Channel coast	FCC	<i>Delphinus delphis</i>	8	ND	M	No	-16.5 ± 0.5	12.1 ± 0.4	ND	Das <i>et al.</i> ^[52]
Irish coast	IRC	<i>Delphinus delphis</i>	14	ND	M	No	-17.1 ± 0.4	12.2 ± 1.0	ND	Das <i>et al.</i> ^[52]
Azores Islands	AZO	<i>Delphinus delphis</i>	55	M+F	S	Yes	-18.1 ± 0.5	10.4 ± 0.6	ND	Qu��rouil <i>et al.</i> ^[22]
Madeira Islands	MAD	<i>Delphinus delphis</i>	28	M+F	S	Yes	-17.8 ± 0.3	10.1 ± 0.3	ND	Qu��rouil <i>et al.</i> ^[22]
NE Pacific Ocean										
Gulf of California	GC	<i>Delphinus capensis</i>	16	M+F	T	Yes	-13.2 ± 1.5	18.1 ± 1.9	3.8–4.1	Ni��o-Torres <i>et al.</i> ^[6]
SE Pacific Ocean										
Chile	CHI	<i>Delphinus delphis</i>	15	M	T	Yes	-14.5 ± 0.3	12.4 ± 0.8	ND	Walker and Macko ^[64]
SW Pacific Ocean										
Southeastern Australia	SEA	<i>Delphinus delphis</i>	3	ND	M	Yes	-19.3 ± 0.8	13.3 ± 2.1	ND	Davenport and Bax ^[65]

Sex: M = male, F = female; ND = not determined; Tissue type: M = muscle, B = bone, S = skin, T = teeth.
 Additional data is presented, such as: the animals provenance (Area), the common dolphin species, the number of individuals (n), the sex of the individuals, the tissue used for the isotopic analyses and if the lipids were extracted or not from those tissues, the common dolphin trophic level (TL), and the reference whenever applicable.

north-eastern and eastern Subtropical Atlantic. The maximum and minimum isotopic values varied greatly across populations: Zone I displayed the lowest $\delta^{13}\text{C}$ value (-16.85‰) and Zone III the highest (-10.60‰); while Zone II presented the lowest $\delta^{15}\text{N}$ value (9.80‰) and Zone III the highest (14.37‰).

Multivariate analysis of variance indicated significant differences between areas for both isotopes (Wilk's $\lambda = 0.226$, $F = 82.192$, $p < 0.001$). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values contributed to the differences between common dolphin populations ($\delta^{13}\text{C}$: $p < 0.001$, $\delta^{15}\text{N}$: $p < 0.001$, Table 3). The results of the *post hoc* test for multiple comparisons between the three areas, for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, show that all pairwise comparisons were highly significant ($p < 0.0001$); except for dolphins from Zones I and II, which did not show differences in their $\delta^{13}\text{C}$ isotope signatures ($p = 0.401$). The common dolphins from Zone III had higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bone than those from animals belonging to other areas, and also the $\delta^{15}\text{N}$ values in the bone of common dolphins from Zone II were significantly lower than those from Zone I (Table 2).

Trophic position of common dolphins in Zones I–III

Table 2 presents the trophic levels (TLs) calculated for each population of common dolphin taking into account the baseline isotopic values of the local marine ecosystems. The trophic positions of dolphins sampled in this study were highly variable, showing a difference larger than one trophic level between Zone II (3.38 ± 0.23) and Zone III (4.52 ± 0.27). Dolphins from the northern area investigated (Zone I) displayed an intermediate trophic position (3.81 ± 0.26 , Table 2) between those from the other areas.

Stable isotope variability of common dolphins worldwide

The stable isotope values of common dolphins from other areas were extracted from the literature (Table 2). Most studies ($n = 7$) focused on the short-beaked common dolphin (*Delphinus delphis*) while only one focused on the long-beaked species (*Delphinus capensis*) (Table 2). When all tissues are considered, the values ranged from -19.3‰ to -12.9‰ for

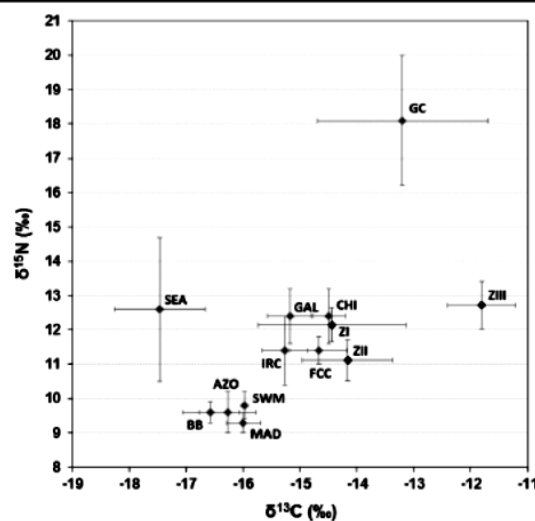


Figure 4. Comparison of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bone of common dolphins from the study area (Zones I to III, in black) and populations worldwide (in grey). Codes: SWM (south-western Mediterranean), GAL (Galicia), BB (Bay of Biscay), FCC (French Channel coast), IRC (Irish coast), AZO (Azores Islands), MAD (Madeira Islands), GC (Gulf of California), CHI (Chile), SEA (south-eastern Australia).

$\delta^{13}\text{C}$ and from 10.1‰ to 18.1‰ for $\delta^{15}\text{N}$ (Table 2). However, the $\delta^{13}\text{C}$ values are clearly lower and the $\delta^{15}\text{N}$ values higher in soft tissues (muscle and skin) than in hard tissues (teeth and bone),^[46] because tissue-specific values depend on the physiology and composition of the tissue.^[36] The data from muscle and skin were converted into bone by means of fractionation factors (see Borrell and colleagues,^[46] $\delta^{13}\text{C}_{\text{bone}} = \delta^{13}\text{C}_{\text{muscle}} + 1.83\text{‰}$, and $\delta^{15}\text{N}_{\text{bone}} = \delta^{15}\text{N}_{\text{muscle}} - 0.7\text{‰}$; $\delta^{13}\text{C}_{\text{bone}} = \delta^{13}\text{C}_{\text{skin}} + 1.83\text{‰}$ and $\delta^{15}\text{N}_{\text{bone}} = \delta^{15}\text{N}_{\text{skin}} - 0.8\text{‰}$),^[46] and the converted isotopic values for common dolphins from all the areas are displayed in Fig. 4. Dolphins from SWM, GAL, BB, FCC, and IRC tended to show lower $\delta^{13}\text{C}$ values than those investigated in the current study, probably because in these studies lipids were not extracted from the tissue. The results shown in Fig. 4 suggest that some populations have a more coastal/inshore distribution (GC and ZIII), while others occupy a more pelagic/offshore environment (SEA, AZO, MAD). When considering the nitrogen isotope values, the GC population segregates from the other populations, while the populations with lowest values appear to have a more pelagic distribution (AZO, BB, and MAD).

DISCUSSION

Marine systems are usually devoid of physical barriers, allowing top predators to be transient and to range beyond conventionally defined ecosystem boundaries.^[50] The large variability found within populations suggests substantial individual-specific dissimilarities in the exploitation of food resources and probably in habitat use.

Table 3. Summary of the results of the ANOVA to test for differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the bones of common dolphins from the three zones analysed (Zones I to III)

	SS	d_f	MS	F	p	r^2
$\delta^{13}\text{C}$						
Model	236.	2	118.22	170.74	<0.001	0.692
Intersect	23267.27	1	23267.27	33603.57	<0.001	
Zone	236.45	2	118.22	170.74	<0.001	
Error	105.25	152	0.69			
Total	26188.47	155				
$\delta^{15}\text{N}$						
Model	68.12	2	34.06	83.44	<0.001	0.523
Intersect	18446.19	1	18446.19	46154.35	<0.001	
Zone	68.12	2	34.06	83.44	<0.001	
Error	62.05	152	0.41			
Total	23253.84	155				



In our study, the variability in $\delta^{15}\text{N}$ values was particularly high in common dolphins from the north-west coast of Africa, a finding probably related to the presence of both the long-beaked and the short-beaked morphotypes in this area as opposed to the northern areas, where only the short-beaked morphotype is present. Variation in the rostral morphology of common dolphins, and consequently in their feeding apparatus, is associated with dissimilarities in feeding ecology and diet^[8,13] rather than to speciation,^[10,14] and this would have obvious consequences on the stable isotope values.

Environmental differences in temperature, light intensity, nutrient concentrations, and species composition can cause baseline changes in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of primary producers^[2] which cascade up food chains to reach primary and secondary consumers.^[25,28,51] Therefore, populations from different geographic locations, even when they have similar diets, can display contrasting isotopic signatures that can be used to assess habitat or resource use.^[52]

Moreover, the $\delta^{15}\text{N}$ values of higher consumers relative to a previously established baseline can provide time-integrated depictions of trophic structure and are a more comprehensive technique than traditional stomach content analyses, which only offer a snapshot of an animal's diet.^[47,50] Although in species feeding on a wide variety of prey diet composition is difficult to establish only through stable isotope analysis,^[46] inference of the trophic level at which the species feed is possible by comparing ^{15}N step-wise enrichment values.^[47,53]

Previously, trophic levels (TLs) of common dolphins have only been determined through stomach content analysis in a couple of local studies (Table 2). Here we assess the TLs in several populations of common dolphins by comparing the isotopic values determined in bone of dolphins with those of a basal local prey of previously known TL (Table 1). The TLs thus obtained are consistent with those generally determined for the genus from data on stomach contents and behavioral data.^[6,54,55] Although the results situated common dolphins between the third and the fifth TLs in most ecosystems (Table 2), which would imply preying on secondary consumers such as sardines,^[54,55] their position was not much higher than that of sardines, especially for the population inhabiting the southern Atlantic coast of the Iberian Peninsula. This suggests the existence of a relatively large degree of omnivory in these top predators globally.

The $\delta^{13}\text{C}$ values were significantly higher in dolphins from north-west Africa than in those inhabiting the Atlantic waters around the Iberian Peninsula (Fig. 4). This would in principle suggest a more in-shore distribution of the former population as opposed to the latter because the $\delta^{13}\text{C}$ values of coastal primary producers (macroalgae and sea grasses) are more elevated than those of the offshore primary producers (phytoplankton) and the difference is transferred through the food web.^[30,56] However, in north-west Africa, *Delphinus* spp. are seldom observed inshore but rather display a typical oceanic behaviour.^[9,57] In addition to this, other organisms inhabiting inshore waters of Mauritania display even higher $\delta^{13}\text{C}$ values than those of common dolphin bone,^[9] for which reason such elevated values are considered to be a characteristic of the general ecosystem rather than a peculiarity of common dolphins. Although a difference of this nature is not depicted in the zooplankton $\delta^{13}\text{C}$ isoscapes

proposed by McMahon *et al.* for the overall Atlantic Ocean,^[35] a possible explanation for such high $\delta^{13}\text{C}$ values may be that the elevated temperatures and the high productivity prevailing off the north-west African coast, which is one of the largest upwelling ecosystems in the world, would lead to high $\delta^{13}\text{C}$ values as occurs with the California current.^[58]

The $\delta^{15}\text{N}$ values were generally high, consistent with the progressive enrichment of the heavier stable isotope through food webs,^[33] and the fact that dolphins are top predators.^[59] However, and similarly to the $\delta^{13}\text{C}$ values, the $\delta^{15}\text{N}$ values were significantly higher in dolphins from Mauritania than in those from the Atlantic waters around the Iberian Peninsula and once more the difference is attributed to differences in $\delta^{15}\text{N}$ baseline although this is again not apparent in the zooplankton isoscapes proposed by McMahon and colleagues^[35] for the overall Atlantic Ocean. McMahon and colleagues^[35] showed that the nitrogen isotope values of zooplankton in the Mauritanian coast are approximately the same as or slightly lower than those around the Iberian Peninsula; despite that, the trophic level of sardines (used as baseline) is slightly higher in the African coast. We suggest that these differences could be associated with the feeding behaviour of the dolphins and their main prey items which would then have repercussions up the food chain.

In the Iberian Peninsula, the $\delta^{13}\text{C}$ values showed no differences between Zone I and Zone II; while the $\delta^{15}\text{N}$ values were lower in Zone II than in Zone I (Fig. 2), probably reflecting differences in diet and/or of variation in baseline,^[18,47,60] and therefore suggesting segregation.

When the above comparisons are extended to other areas not directly covered in the present study, but whose stable isotope values were transformed for comparison purposes (Fig. 4), the values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were extremely variable, suggesting feeding at different trophic levels and a large plasticity to adapt to local variations. The long-beaked common dolphin from the Gulf of California (GC) showed the highest $\delta^{15}\text{N}$ values followed by the north-west African population (ZIII) due probably to the presence of the long-beaked morphotype, which is present in both areas. Consistent with this, previous studies have shown that individuals with longer beaks tend to feed at higher trophic levels than animals with shorter beaks.^[9,10] The short-beaked common dolphins from Areas ZI+ZII+GAL showed the third highest $\delta^{15}\text{N}$ values of the populations analysed, consistent with the published TLs for the coast of Galicia.

CONCLUSIONS

The results substantiate the use of stable isotope ratios as a useful tool to investigate population structure and segregation between demographic subunits. Trophic positions were successfully determined for the common dolphin populations of the north-eastern and eastern Subtropical Atlantic, accounting for the local marine ecosystem baseline. The trophic behaviour of several populations of common dolphins worldwide was analysed, revealing substantial differences that are probably reflective of both adaptive strategies of the genus and dissimilarities in the structure of the ecosystems in which they live.



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Common dolphin morphotypes: Niche segregation or taxonomy?

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Keywords

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Abstract

In the extensive geographical distribution of the common dolphin, several morphotypes of uncertain taxonomic status, identified by the relative length of their rostra, have been established. We investigated variation in skull morphometrics and isotopic signatures of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in individuals from the subtropical waters of the North Atlantic Ocean, in order to assess population structure and taxonomic status. The distribution of the relative rostral lengths (RL) of individuals followed a cline with no subgrouping. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ showed high variability, which suggests that individuals use habitat heterogeneously. $\delta^{15}\text{N}$ correlated with RL, indicating that longer beaked individuals either feed at a higher trophic level and/or inhabit waters located further offshore than shorter beaked animals. Although $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were correlated, RL and $\delta^{13}\text{C}$ failed to show any correlation, possibly because the incremental effect of trophic level on $\delta^{13}\text{C}$ has been offset by the potential allopatric distribution of the morphotypes. We conclude that both the long-beaked and short-beaked forms of common dolphin do occur off Mauritania but, in contrast to other areas, the existence of more than one species in the region is questioned because both stable isotopes and skull morphometric appear to reflect differential use of habitat rather than taxonomy. Even though proposed previously by some authors, this is the first time that skull differentiation in common dolphins has been demonstrated to be likely due to niche segregation and not to speciation. This reveals that caution is needed when considering that long-beaked and short-beaked common dolphins from outside the eastern North Pacific fall into the taxonomic model described for this region.

Introduction

Common dolphins (*Delphinus* spp.) are present in all oceans of the world between latitudes 40 and 60°N to about 50°S, except the western Atlantic, where they are absent in tropical waters (Jefferson, Webber & Pitman, 2008; Jefferson *et al.*, 2009). Such an extensive geographical distribution has facilitated the differentiation of a number of morphotypes that until today, remain of unclear taxonomic status (White, 1999). In many areas, but not in all, two morphotypes have been distinguished: long-beaked, mostly of coastal or neritic distribution, and short-beaked, that occupies both inshore and offshore waters (Banks & Brownell, 1969; Evans, 1982; Perrin, 2009). Banks & Brownell (1969) differentiated the two forms through the ratio of zygomatic width (ZW) to rostrum length (RL), and suggested that the anatomical difference was likely due to dissimilarities in food habits. Later, the anatomical differentiation of the morphotypes was confirmed through additional multivariate analyses of skull measurements (Evans, 1982). In the eastern North Pacific, Heyning & Perrin (1994) and Rosel, Dizon & Heyning (1994), through morphological and genetic

analyses, respectively, distinguished the two morphotypes and split the genus into two species: the short-beaked common dolphin *Delphinus delphis* (Linnaeus, 1758) and the long-beaked common dolphin *Delphinus capensis* (Gray, 1828). Currently, the genus *Delphinus* comprises of two species and four subspecies: the short-beaked common dolphin *Delphinus delphis delphis*, distributed in continental shelf and pelagic waters of the Atlantic and Pacific Oceans; the Black Sea short-beaked common dolphin, *Delphinus delphis ponticus*; Gray's common dolphin (long-beaked form), *Delphinus capensis capensis*, distributed in nearshore tropical and temperate waters of the Pacific and South Atlantic Oceans; and the Indian long-beaked common dolphin, *Delphinus capensis tropicalis*, which occurs in the Indian Ocean (IWC, 2009).

The two most common morphotypes (short-beaked and long-beaked) also occur off the Atlantic coast of Africa. The range of the short-beaked form is believed to extend as far south as Senegal (Heyning & Perrin, 1994; Van Waerebeek, 1997; Rice, 1998), while the long-beaked is present from the waters off Mauritania to those off South Africa (Amaha, 1994; Van Waerebeek, 1997; Rice, 1998; Samaai, Best &

Gibbons, 2005). Thus, the waters off Mauritania are apparently a region of overlap in the geographical distribution of the two forms, and indeed both morphotypes occur sympatrically in this area, as is the case off the Californian coast (Heyning & Perrin, 1994).

However, a genetic study conducted by Natoli *et al.* (2006), based on analysis of the mtDNA D-loop region of a small number of samples, concluded that the Mauritanian population was highly differentiated from all other populations, including the long-beaked population from South Africa; and challenged the currently accepted hypothesis of a single long-beaked lineage worldwide. More locally, it raised questions about the identity of the *Delphinus* population off north-west Africa. Nevertheless, Natoli *et al.* (2006) based their study only on the genetics of six specimens from Mauritania, thus leaving some uncertainty as to the conclusiveness of their findings and about the actual genetic structure of the population. Additionally, the study proposed that the long-beaked form originated independently in different regions, suggesting that adaptation to local environments is driving local speciation. Therefore, skull morphology reflects adaptation to prey capture and the habitat used by the animals.

One of the most useful tools for investigating diet, as well as habitat use, is the analysis of stable isotopes. Stable isotope analyses are based on the assumption that the isotopic composition of an animal depends on that of its food sources (DeNiro & Epstein, 1978, 1981), and offers an integrated measure of all prey assimilated over a comparatively protracted period of time (Michener & Schell, 1994; Hobson, 1999). Additionally, it assumes that the relative abundance of the heavier isotopes of some elements increases in each trophic level, thus rising progressively through the food web (Peterson & Fry, 1987; Cabana & Rasmussen, 1996; Caut, Angulo & Courchamp, 2009), increasing their levels from prey to predator owing to the preferential excretion of the lighter isotopes (Caut *et al.*, 2009). The nitrogen isotope $\delta^{15}\text{N}$ is enriched at each trophic level by 3–5‰ (Minagawa & Wada, 1984) and has been

successfully used as an indicator of diet and trophic level. $\delta^{13}\text{C}$ is usually enriched by each trophic level by *c.* 1‰. However, few studies have used it as an indicator of trophic level, because such small levels of enrichment could be misinterpreted and confounded by other explanatory factors. In addition, $\delta^{13}\text{C}$ changes along ecological gradients as different plants discriminate it to a different extent when compared with $\delta^{12}\text{C}$, and it has been most commonly used as an indicator of habitat use, that is, to differentiate between pelagic versus benthic and offshore versus near-shore origins (Pinela *et al.*, 2010).

In this paper, we report the results of a study on the population of common dolphins from Mauritania, using two independent tools: the relative abundance of nitrogen and carbon stable isotopes, and the morphometrics of the skull.

Material and methods

Sampling

Samples were collected between 1990 and 2008 along the *c.* 700 km of Mauritanian coastline that extends from Cape Blanc Peninsula to the Senegal River, north-western Africa (Fig. 1). The study site was divided into three areas: area 1, from the north to the south tip of Cape Blanc Peninsula, including *c.* 50 km of the Western Sahara border; area 2, comprises the Parc National du Banc d'Arguin (PNBA); and area 3, from Cape Timirist (the southern border of the PNBA) to the Senegal River (the border between Mauritania and Senegal). The beaches were surveyed opportunistically or in dedicated expeditions and, when the remains of a dead dolphin were found, the skull was preserved. Sex, body length, geographical position and any indication of the cause of death were recorded whenever possible. However, most specimens were found in an advanced state of decomposition or as skeletal remains, so detailed biological information is lacking for a large part of the sample. In total, 72 specimens were examined and a sample of pterygoid bone was collected from all individuals for stable isotope

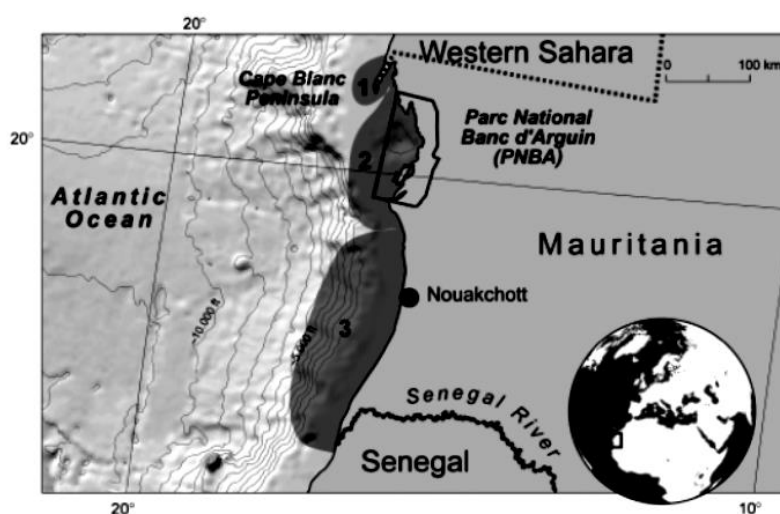


Figure 1 Map of the study site, including bathymetry (in feet), outline of the continental shelf and the three main sampling areas of the Mauritanian coast as indicated by dark grey areas with numbers (from 1 to 3).

analyses. The pterygoid bone was used because it is a structure present in all skulls, it is easy to break, and did not damage the skull for subsequent morphometric analysis.

Stable isotopes analyses

Stable isotope levels (expressed as delta notation, δ), in which the relative variations of stable isotope ratios of carbon and nitrogen are expressed in parts per thousand (‰) deviations from predefined international standards, were calculated as

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where X is the value of the heavy isotope of the sample (^{13}C or ^{15}N), R_{sample} is the ratio of the heavy to the light isotope of the sample ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) and R_{standard} is the ratio of the heavy to the light isotope in the reference standards, which were the Vienna Pee Dee Belemnite calcium carbonate for carbon and the atmospheric nitrogen (air) for nitrogen.

For the pre-treatment of the samples, c. 1 g of bone was rinsed with distilled water, dried for 3 days at 60 °C and powdered with a mortar and pestle. Because lipids are depleted for ^{13}C compared with other molecules (DeNiro & Epstein, 1977), they were removed from the samples by rinsing the powdered tissue several times with a chloroform/methanol (2:1) solution (Bligh & Dyer, 1959). Bone was the selected tissue because, owing to its low turnover rate, it is possible to average out several years of dietary history (Hobson & Clark, 1992). When conducting stable isotope analysis in bone, several authors have carried out preventive demineralization of the tissue by treating it with 0.5 and 1.0 M hydrochloric acid solution (Bocherens *et al.*, 1997; Newsome *et al.*, 2006). However, a test using a subset of the samples ($n = 20$) revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not change between demineralized and untreated samples, so this procedure was not followed. Approximately 1 mg of lipid-free bone was weighed in tin capsules (3.3×5 mm), combusted at 900 °C and analysed in a continuous-flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan, Thermo Finnigan, Bremen, Germany). Atropine was used as a standard to check the elemental composition for carbon and nitrogen. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ ratios, as given by the International Atomic Energy Agency (IAEA), namely polyethylene (IAEA CH7, $\delta^{13}\text{C} = -31.8\text{‰}$), graphite (USGS24, $\delta^{13}\text{C} = -16.1\text{‰}$) and sucrose (IAEA CH6, $\delta^{13}\text{C} = -10.4\text{‰}$), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $^{15}\text{N}/^{14}\text{N}$ ratios, namely $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1, $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2, $\delta^{15}\text{N} = +20.3\text{‰}$) and KNO_3 (IAEA NO3, $\delta^{15}\text{N} = +4.7\text{‰}$), were used for calibration at a precision of 0.3‰.

Morphometric measurements

Measurements were obtained from 72 common dolphin (*Delphinus* sp.) skulls and mandibles from the Mauritanian

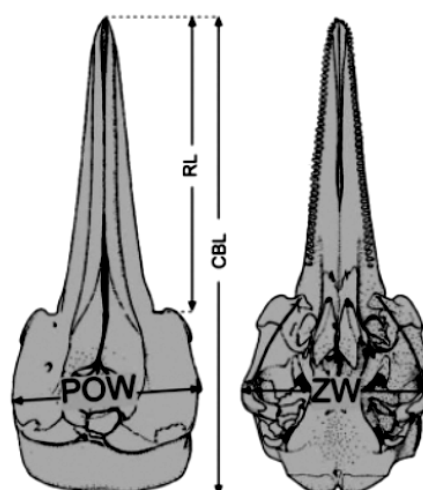


Figure 2 Common dolphin skull morphometric measurements: condylobasal length (CBL), rostral length (RL), skull post-orbital width (POW) and zygomatic width (ZW).

coast housed at the Headquarters of the PNBA, the Institut Mauritanien de Recherches Océanographiques et des Pêches and the Centre National d'Élevage et de Recherches Vétérinaires (CNERV). Specimens, either deposited in scientific collections or found during beach surveys, were identified as common dolphins on the basis of skull morphology (e.g. the presence of deep palatal grooves; Evans, 1994; Heyning & Perrin, 1994).

Because skull proportions change allometrically with growth, we selected from Heyning & Perrin (1994) the morphometric measurements that had been identified previously as being of taxonomical relevance or that indicate maturity. These included the RL/ZW ratio (Fig. 2) and the left upper tooth count [UTC (left)], which have been reported as the most diagnostic measurements for discriminating between short-beaked and long-beaked forms of common dolphins (Banks & Brownell, 1969; Van Bree & Gallagher, 1978; Amaha, 1994; Evans, 1994; Heyning & Perrin, 1994; Bell, Kemper & Conran, 2002; Jefferson & Van Waerebeek, 2002; Samaai *et al.*, 2005; Westgate, 2007; Tavares *et al.*, 2010). All measurements were made using metal vernier callipers (0.1 mm accuracy). In order to test measurement accuracy, repetitions of all measurements used were made on several specimens (28% of the total sample) until a level of precision of ± 0.5 mm was obtained (Bell *et al.*, 2002). All measurements were conducted by AMP. Additionally, the overall skull quality, overall degree of cranial fusion and the degree of fusion of the pre-maxilla and maxilla were also annotated (Westgate, 2007). Incomplete or badly damaged skulls, which prevented reliable measurement, were excluded from analyses ($n = 6$).

The final sample only included skulls that were considered to have completed their growth. Physical maturity of the specimens was assessed by examining the degree of fusion between the pre-maxillary and maxillary bones at the distal tip of the rostrum. However, Perrin & Heyning



(1993) reported that this method may be subject to some error in the assessment of physical maturity, so additionally the condylobasal length (CBL, Fig. 2), degree of fusion of the alveoli and the degree of overall cranial fusion were used as supplementary indicators of physical maturity, owing to the lack of data on the age and total body length of the specimens (Westgate, 2007). Correlations between the skull length (condylobasal length minus the rostral length, i.e. CBL–RL), skull post-orbital width (POW) and RL were investigated to remove any potential influence of skull allometric growth on the relationship between skull morphometrics and isotopic signatures (Fig. 2). Nine individuals were removed from the initial dataset, because their skulls did not have closed sutures, closed alveoli and distal fusion between the pre-maxillae and maxillae at the tip of the rostrum, and thus were considered physically immature. Sexual identification was also not available for most of the specimens, because the main objective was to detect differences at the taxonomical level we analysed the dataset as a whole. Moreover, despite that on an average, male common dolphins had significantly larger skulls in the North Atlantic and Pacific Oceans (Heyning & Perrin, 1994; Murphy *et al.*, 2006; Westgate, 2007), the RL/ZW ratios were very similar between sexes in those areas (Heyning & Perrin, 1994; Westgate, 2007) and in southern Australia (Bell *et al.*, 2002).

Data analyses

The normality of the data was tested by the Kolmogorov–Smirnov test and heteroscedasticity by Levene's test. Relationships between variables were analysed through regression analysis. A principal component analysis (PCA) was carried out on all taxonomically [RL/ZW and UTC (left)] and ecologically ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) relevant variables in the study, in order to check whether these variables are associated with different common dolphin morphotypes. The statistical software package SPSS 15.0 was used for all analyses.

Results

From the 72 common dolphin skulls collected along the Mauritanian coastline (Fig. 1), we were able to analyse 57 individuals, owing to damaged skulls or immature individuals. The minimum CBL that was deemed as physically mature was 394 mm, because above this size skulls displayed degree of fusion of alveoli, and the degree of overall cranial fusion, consistent with physical mature individuals. Measurements from skulls of individuals followed a normal distribution ($P = 0.56$) and variances were homogeneous for all analysed variables, both isotopic and morphometric.

The average isotopic values of the individuals sampled in this study were 11.40 ± 0.62 (‰, mean \pm SD) for $\delta^{15}\text{N}$ and -12.23 ± 0.65 (‰, mean \pm SD) for $\delta^{13}\text{C}$. High intraspecific variability was observed for both stable isotopes: $\delta^{15}\text{N}$ ranged between 11.4 and 14.31‰, which corresponds to a difference of approximately one trophic level, while $\delta^{13}\text{C}$ ranged between -14.07 and -10.93 ‰, which indicated

differences in habitat use. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were significantly and negatively correlated ($R = 0.51$, $P < 0.001$; Fig. 3).

Investigation of possible effects of allometric growth did not produce significant results for any of the combinations tested: skull length (CBL–RL) versus RL ($R = 0.22$, $P = 0.10$, $n = 57$), (CBL–RL) versus POW ($R = 0.26$, $P = 0.06$, $n = 57$) and RL versus POW ($R = 0.05$, $P = 0.73$, $n = 57$). This suggests that the age of the sampled skulls did not affect the results. RL/ZW ratios followed a normal distribution (Fig. 4), but there was no apparent separation between two putative groups, related to relative RL. Figure 5 shows a comparison between RL/ZW ratios and left UTC in this study and those published previously for short-beaked and long-beaked forms elsewhere. RL/ZW ratios (mean = 1.46, range = 1.27–1.76, Fig. 5a) and UTC (mean = 46.62, range = 40–55, Fig. 5b) overlap with those of *D. delphis* and *D. capensis* populations from other parts of the world.

RL was significantly and positively correlated with $\delta^{15}\text{N}$ levels: RL versus $\delta^{15}\text{N}$ ($R = 0.44$, $P = 0.001$, $n = 57$). This indicates that individuals with longer rostra tend to feed at higher trophic levels than those with shorter rostra. Also, a positive significant correlation ($R = 0.41$, $P < 0.001$, $n = 57$)

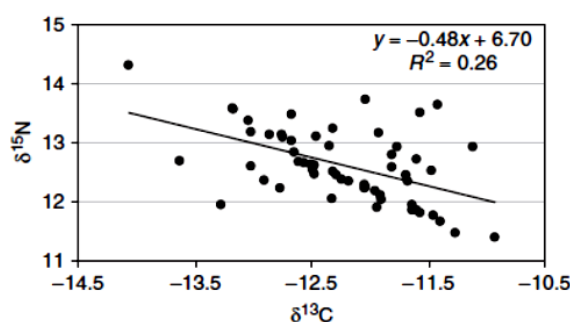


Figure 3 Correlation between nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) levels for common dolphins inhabiting Mauritanian waters ($R^2 = 0.26$, $P < 0.001$, $n = 57$).

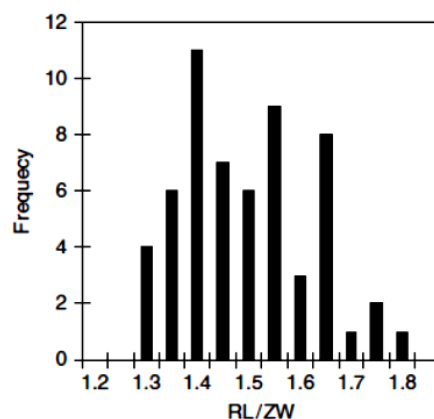


Figure 4 Distribution of the rostrum length/zygomatic width (RL/ZW) ratios for the sampled common dolphins ($n = 57$).

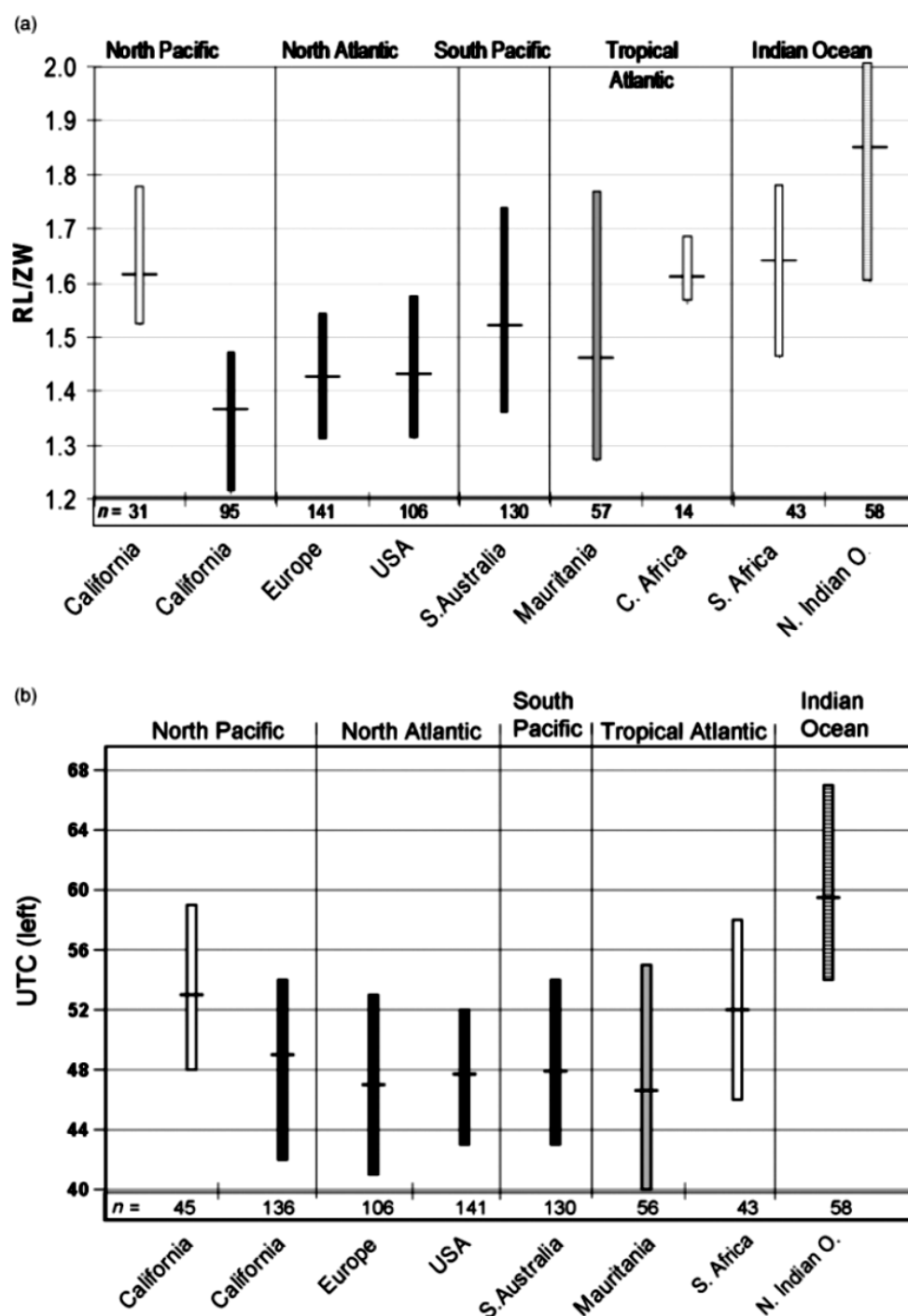


Figure 5 Comparison of (a) rostrum length/zygomatic width (RL/ZW) ratios and (b) upper tooth counts (UTCs) from *Delphinus* spp. populations worldwide: North Pacific Ocean common dolphin (California, Heyning & Perrin, 1994), North Atlantic common dolphin (Europe and USA, Westgate, 2007), South Pacific (Australia, Bell *et al.*, 2002), Mauritania (present study), Eastern Central North Atlantic (Central Africa, Van Waerebeek, 1997) and Indian Ocean (South Africa and North Indian Ocean, Jefferson & Van Waerebeek 2002). The bars represent different common dolphin taxa: *Delphinus delphis delphis* (black bars), *Delphinus capensis capensis* (white bars) and *Delphinus capensis tropicalis* (striped bars).

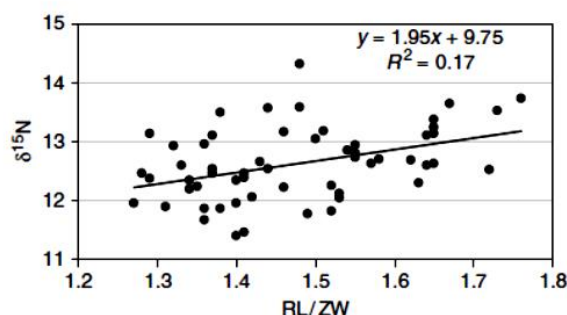


Figure 6 Correlation between nitrogen ($\delta^{15}\text{N}$) levels and rostrum length/zygomatic width (RL/ZW) ratios in common dolphins inhabiting Mauritanian waters ($R^2=0.174$, $P<0.001$, $n=57$).

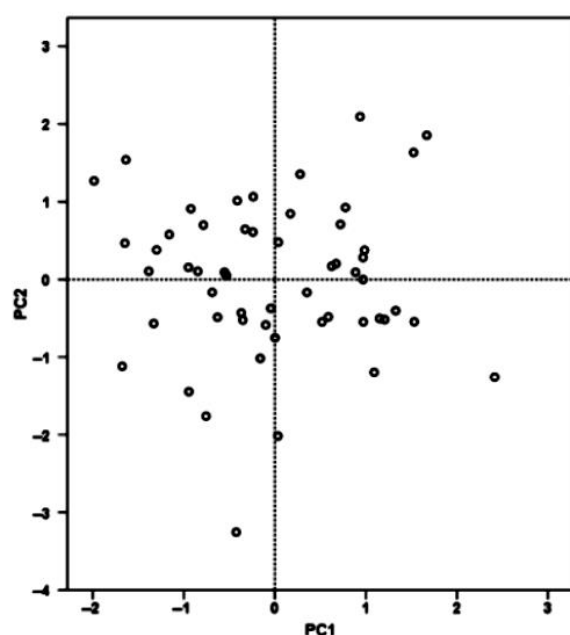


Figure 7 Principal components (PC1–PC2) extracted from the relative contributions of the taxonomic and isotopic variables.

was found between $\delta^{15}\text{N}$ and RL/ZW ratio (Fig. 6), while no correlation was found between the $\delta^{13}\text{C}$ and RL/ZW ratio ($P = 0.34$, $n = 57$).

In the PCA, we obtained two principal components, PC1 and PC2, accounting for 42.84 and 29.44% of the variance, respectively. With this small number of variables, we managed to explain 72.28% of the variability in the sample. The component matrix indicated that PC1 was positively associated with $\delta^{15}\text{N}$ and RL/ZW (ordered from higher to lower coefficients), and negatively associated with $\delta^{13}\text{C}$ and UTC (left). PC2 was positively associated with $\delta^{13}\text{C}$, RL/ZW, and $\delta^{15}\text{N}$ and negatively associated with UTC (left). The graphic representation of PC1 against PC2 did not display the formation of two clear and distinct groups, corresponding to short-beaked and long-beaked morphotypes (Fig. 7).

Discussion

In the present study, we report stable isotope data from common dolphin bone samples collected from 1990 to 2008. Statistical analyses were performed to detect differences in levels of stable isotopes of carbon and nitrogen on sampling decades (1990–1999 and 2000–2008), but no differences were found (data not presented). We thus assumed that the current distribution and habitat use of individuals, as well as feeding preferences, in the study area have not changed in recent decades. However, the results from the stable isotope analyses do not enable us to identify causal factors for the differentiation of two morphotypes on an evolutionary scale, as has been reported from morphometric and genetic markers. Thus, these three factors provide different insights into the differentiation of species, populations and ecological stocks; while bone isotopes integrate part of the total lifespan of common dolphins, morphometric and genetic markers can be used to detect differences at the population and species level.

Stable isotopes of carbon and nitrogen were significantly and negatively correlated, thus reflecting variation associated with habitat use. Furthermore, the large variability found in our common dolphin sample for both isotopes suggests substantial differences between individuals in the exploitation of food resources and, most likely, their habitat. $\delta^{15}\text{N}$ correlated with RL, indicating that longer beaked individuals either feed at a higher trophic level than shorter beaked individuals, and/or they exploit mostly an offshore-pelagic habitat, according to $\delta^{15}\text{N}$ baseline in Mauritanian ecosystems. As reported, in primary producers levels of $\delta^{15}\text{N}$ are higher (c. 5‰ $\delta^{15}\text{N}$), and those of $\delta^{13}\text{C}$ are lower in offshore habitats compared with coastal habitats (Pinela *et al.*, 2010), thus fractionation through food webs would show higher levels of $\delta^{15}\text{N}$ in dolphins feeding offshore (considering the offshore habitat as an open-ocean pelagic habitat).

Relative RL (RL/ZW) and $\delta^{13}\text{C}$ failed to show any correlation possibly because the large variability between individuals masked the effect of trophic level on $\delta^{13}\text{C}$. However, another hypothesis, which is consistent with the results obtained for $\delta^{15}\text{N}$, is that the incremental effect of trophic level on $\delta^{13}\text{C}$ is offset by the allopatric distribution of the morphotypes, the shorter beaked individuals inhabiting waters closer to coast than the longer beaked ones. This hypothesis is inconsistent with previous observations in Chile (Sanino, Van Waerebeek & Yanez, 2003) and Baja California (Niño-Torres *et al.*, 2006) indicating that the long-beaked form is more coastal than the short-beaked form (Perrin, 2009). Indeed, in the north-eastern Pacific Ocean, the region where the species *D. delphis* and *D. capensis* were first differentiated (Rosel *et al.*, 1994), the long-beaked form (also known as the Baja neritic form) shows a preference for shallower waters (inside the 180 m isobath) than the short-beaked form (Leatherwood *et al.*, 1982). On the other hand, Bell *et al.* (2002) found similar findings to ours in *D. delphis* from southern Australia. Apart from the high variability of skull morphometric parameters, they found a tendency for



dolphins with larger skulls and longer rostra to be closer to deep water, and those with smaller skulls and shorter rostra to be in shallow coastal waters.

Nevertheless, the variability in relative beak lengths for the Mauritanian common dolphins was very large compared with those of studied *Delphinus* populations from other parts of the world (Heyning & Perrin, 1994; Van Waerebeek, 1997; Murphy *et al.*, 2006; Westgate, 2007) especially in regions where only one of the species is present. Although our sample could not be differentiated by sex, the observed variability in RL/ZW ratios and UTCs is unlikely to be explained by sex, because sexual dimorphism in *Delphinus* spp. skulls is very limited (Heyning & Perrin, 1994; Van Waerebeek *et al.*, 1994), despite the differences in skull size displayed by the males from England and Wales (Murphy, 2006). The RL/ZW ratios for the Mauritanian animals are more similar to those of animals from the Atlantic Ocean than to those from the Pacific Ocean, but overall the range of variation is so large as to be only slightly smaller than the composite variation in RL/ZW ratios of the two North Pacific species, *D. delphis* and *D. capensis*, put together (Heyning & Perrin, 1994, Fig. 5a). The lower RL/ZW ratios were similar to those found typically in *D. delphis* from the eastern North Atlantic (Murphy *et al.*, 2006; Westgate, 2007), while the higher ratios were similar to those found in Central West Africa, more precisely in Senegal, Gabon, Angola and Congo-Brazzaville (Van Waerebeek, 1997), and South Africa, which have been attributed to *D. capensis* (Jefferson & Van Waerebeek, 2002). The clinical variation in relative rostrum size that we observed in the Mauritanian common dolphins has also been observed in the Indo-Pacific (from the Middle East to China) *D. c. tropicalis*, with its extremely long rostrum, as one moves east or west from India (Jefferson & Van Waerebeek, 2002); and in southern Australia, as one moves from inshore/coastal to offshore/pelagic waters (Bell *et al.*, 2002). Common dolphin skulls off southern Australia overlapped in size with both the long-beaked and short-beaked species in the eastern North Pacific (Bell *et al.*, 2002). The Australian specimens showed a greater range of variation in RL/ZW ratios when compared with *D. delphis* in North Pacific and North Atlantic, especially considering that they all belong to the same species (Fig. 5a). Tooth count per upper tooth row in Mauritanian *Delphinus* spp. also showed slightly greater variability compared with that of *D. delphis* from the North Atlantic, southern Australia or even California (Fig. 5b). This result also seems to indicate that there is no stratification in our sample, because Mauritanian common dolphins showed a smaller range of UTC values than that obtained for *D. capensis* from South Africa and California, even though they overlapped slightly (Fig. 5b).

Concerning the geographical distribution of common dolphin strandings along the Mauritanian coast, most specimens were found stranded north of the tip of Cape Blanc (area 1 in Fig. 1) and south of Cape Timirist (beginning of area 3 in Fig. 1), even though the complete coastline was surveyed. Therefore, common dolphins appear to be absent or to occur at very low population densities in the shallow

waters of the Banc d'Arguin (area 2 in Fig. 1). Also, there is a clear bias towards strandings along the Grand Plage (area 3 in Fig. 1). We did not detect any differences between geographical areas in relation to the length of the beak of stranded dolphins (data not presented). Unfortunately, sightings data from Mauritania are scarce and opportunistic, and simply suggest that common dolphins are rarely sighted off Mauritania close to the beach, so that there is no indication of any habitat partitioning between the short-beaked and long-beaked forms (Robineau & Vély, 1998). Currently, there is practically no information on the ecology of common dolphins from north-west Africa. Also, the published literature does not show obvious differences in the diet of either short-beaked or long-beaked forms or species in other oceans (Ohizumi *et al.*, 1998), so further research is required both off Mauritania and elsewhere to clarify potential allopatry and trophic niche partitioning between morphotypes.

PCA analysis indicated that a combination of RL/ZW ratios and δ -stable isotopes values explained 72.28% of the variability in the sample. However, the principal components were not able to display a clear and distinct separation of individuals into morphotypic groups (Fig. 7). RL/ZW ratios, UTCs, and the isotopic levels found in Mauritanian dolphins formed clines, but in neither case did the data cluster into two distinct groups as typically occurs in studies of skull morphometrics from the eastern North Pacific (Heyning & Perrin, 1994). Therefore, despite the occurrence in the area of individuals that can be ascribed to the two *Delphinus* morphotypes by their relative RL, neither morphometric nor isotopic signatures support the hypothesis that the area is inhabited by more than one single species of common dolphin with intrinsic large variations in both body proportions and foraging behaviour. This apparent absence of differentiation is consistent with preliminary genetic research conducted by Natoli *et al.* (2006), which suggested that the long-beaked form from Mauritania is taxonomically closer to the short-beaked form of the North Atlantic rather than to *D. capensis* from the Pacific Ocean.

Therefore, we conclude that both the long-beaked and short-beaked forms of common dolphin do occur off Mauritania, but in contrast to other areas, their occurrence appears to reflect the differential use of habitat rather than taxonomic differentiation. Several authors have suggested that geographical variation in rostral morphology of common dolphins, and consequently in their feeding apparatus, could indicate variations in feeding ecology, most likely owing to differences in diet, even though this has never been demonstrated (Evans, 1982; Amaha, 1994; Murphy *et al.*, 2006). Nevertheless, and despite the need for genetic analyses on larger samples, our data, together with the genetic study conducted by Natoli *et al.* (2006), suggest that common dolphin's skull differentiation might be related to niche segregation and not to speciation. Taxonomic splitting appears not to be justified in the region. Hence, the existence of two putative populations or species (*D. delphis* and *D. capensis*) should be questioned until further research, particularly on genetics and using other taxonomically relevant



morphometric measurements, is conducted. Moreover, our findings call for caution when assuming as a general pattern that long-beaked and short-beaked common dolphins from outside the eastern North Pacific fall into the taxonomic model described for that region by Heyning & Perrin (1994) and Rosel *et al.* (1994).

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Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotope values in common dolphins (*Delphinus* spp.) worldwide, with particular emphasis on the eastern North Atlantic populations.



Common dolphin morphotypes: niche segregation or taxonomy?



Geographic variation in size and shape components of the skull of common dolphins (genus *Delphinus*) from the eastern Atlantic Ocean using landmark-based geometric morphometric methods.



Taxonomic status of the common dolphin (genus *Delphinus*) in the eastern Sub-tropical Atlantic Ocean.