University of the Azores

Department of Oceanography and Fisheries

Master of Science Degree

"Biology, ecology and conservation of Mobulid rays in the Azores"


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by

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Abstract

Mobulids are large migratory elasmobranchs, some of which appear to aggregate at specific locations and times. Due to their conservative life history traits, mobulids have low intrinsic capacity to sustain and recover from current threats globally, including directed fisheries, incidental capture, habitat destruction, pollution and unregulated tourism. Yet, although they are nowadays iconic species that support important ecotourism industries, most questions regarding their biology and ecology remain unanswered, hampering the identification and implementation of adequate conservation and management measures. This study provides an updated checklist of the family Mobulidae for the Azores, mid-North Atlantic, based on an extensive photographic, video and visual sighting database, and on the re-evaluation of previous citations for the region. There are, at least, three species of mobulids occurring in the Azores, possibly four: *Manta birostris* (occasional), *Mobula tarapacana* (seasonally frequent), and one or both of the *Mobula mobular/M*. *japanica* species complex (rare). This is the first study to provide detailed information on the spatial ecology of *M. tarapacana* across its circumglobal distribution. It demonstrates that, in the region, this species shows a marked preference for shallow seamounts, that some of these habitats support large and continuous aggregations of adults (Princesa Alice bank and Ambrósio reef), and that these aggregations seem to be restricted to the warmer months. A photographic database for mobulids and a program for the photo-identification of *M. tarapacana* based on ventral color patterns were also developed and implemented, in what constitutes the first and promising worldwide attempt to use photo-ID as a tool for *Mobula* demographic studies. Considering the highly migratory nature of these species and the current vulnerability of the most important, known aggregations sites, the findings in this study most possibly have direct implications for the conservation of the north Atlantic populations of Mobulas, in particular of *M. tarapacana*. Future research is urgent to learn more about the ecology and biology of the Mobulidae in the region and in the Atlantic.

Resumo

Os Mobulídeos são elasmobrânquios altamente migradores, alguns dos quais aparentemente agregam em locais e épocas específicos. Devido às suas estratégias de vida conservadoras, têm uma baixa capacidade intrínseca de suportar e recuperar das actuais ameaças a nível global, incluindo pescarias direccionadas, captura acidental, destruição de habitat, poluição e turismo não regulamentado. Contudo, apesar de, hoje em dia, serem espécies icónicas que suportam importantes indústrias na área do ecoturismo, a maioria das questões relativas à sua biologia e ecologia permanecem por responder, impedindo a identificação e implementação de medidas de conservação e gestão adequadas. Este estudo providencia uma *checklist* actualizada da família Mobulidae para os Açores, Atlântico Norte, baseada numa base de dados extensiva de fotografia, vídeo e avistamentos visuais, e também na re-avaliação de citações existentes para a região. Existem, pelo menos, três espécies de mobulídeos que ocorrem nos Açores, possivelmente quatro: *Manta birostris* (ocasional), *Mobula tarapacana* (frequente sazonalmente), e uma ou ambas do complexo de espécies *Mobula mobular/M*. *japanica* (rara). Este é o primeiro estudo a fornecer informação detalhada acerca da ecologia espacial de *M. tarapacana* na sua distribuição circumglobal. Demonstrando que, na região, esta espécie mostra uma preferência marcada por montes submarinos de baixa profundidade, que alguns destes habitates suportam grandes e contínuas agregações de adultos (banco Princesa Alice e Ambrósio), e que estas agregações parecem estar restritas aos meses mais quentes. Uma base de dados fotográfica para mobulídeos e um programa para a foto-identificação de *M. tarapacana,* baseado nos seus padrões de coloração ventrais, foram também desenvolvidos e implementados, no que constitui a primeira, e promissora, tentativa mundial de uso da foto-identificação como ferramenta para estudos demográficos de uma espécie do género *Mobula*. Considerando a natureza altamente migratória destas espécies e a actual vulnerabilidade dos locais de agregação conhecidos mais importantes, os resultados deste estudo têm, muito provavelmente, implicações directas para a conservação das populações de mobulas no Atlântico Norte, em particular de *M. tarapacana*. Investigação futura é urgente para obter mais informação acerca da ecologia e biologia da família Mobulidae na região e no Atlântico.

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Chapter 1. Introduction

The family Mobulidae presently accounts for eleven recognized species grouped in two genera: *Manta* or manta rays (two species) and *Mobula* or devil rays (nine species). Mobulid rays are pelagic elasmobranchs occurring worldwide in tropical, subtropical and warm temperate waters (Eschmeyer *et al.*, 1983; Notarbartolo-di-Sciara, 1988; Marshall *et al.*, 2009; Heinrichs *et al.*, 2011; Couturier *et al.*, 2012; Croll *et al.*, 2012). All mobulid rays have diamond shaped bodies, wing-like pectoral fins and five pairs of gill slits. One of the distinctive characters of mobulid rays is their cephalic fins, which look like horns when rolled up, therefore the name "devil rays". The primary function of these cephalic lobes is to enhance the entrance of plankton and small fishes into the mouths of the rays when they are feeding. Although the subject of little directed research, all mobulids are believed to undertake relatively large-scale movements between productive feeding areas and some species are known to aggregate at specific locations and times (Notarbartolo-di-Sciara, 1988; Couturier *et al.*, 2011). Most species have been observed travelling and feeding in schools of a few to hundreds of individuals and aggregate seasonally in large numbers at different locations throughout their ranges. Known aggregations of mobulids are thought to be mainly related to local productivity and food availability (Notarbartolo-di-Sciara, 1988; Marshall *et al.*, 2009, 2011*c*; Couturier *et al.*, 2011).

Mobulids records can be traced back to at least the 17th century, still little is known about their biology and ecology (Couturier *et al.*, 2012). The reason for this is that these are epipelagic animals that are difficult to study in their wide oceanic habitat, and also because there are relatively few locations worldwide where mobulids are easy to observe and approach. Even at well-known aggregation sites, obtaining detailed biological information can be difficult because of the fast swimming abilities of most mobulid species (Couturier *et al.*, 2012), and also the fact that some aggregation sites are located in remote places where the presence of the animals is seasonal. Thus, a lot of questions remain unanswered regarding these species and their life history characteristics.

Even though longevity remains unknown for all species, mobulids appear to be long-lived animals. Individuals of *Manta alfredi* and *Manta birostris* were re-sighted through photo-ID up to 30 and 20 years, respectively, after their initial identification (Marshall & Bennett, 2010; Clark, 2010; Marshall *et al.*, 2011*a,b,c*). Mobulids are thought to mature late (8-10 years) (Heinrichs *et al.*, 2011). *M. alfredi* males are already known to mature around three to six years (Clark, 2010), but age at maturity is unknown for most species. The gestation period is also generally unknown but it is likely to last for about one year, sometimes with resting periods between pregnancies of up to two years (Notarbartolo-di-Sciara, 1988; Marshall & Bennett, 2010; Couturier *et al.*, 2012). They give birth to as few as a single pup (Gill, 1908; Bigelow & Schroeder, 1953; Notarbartolo-di-Sciara, 1988; Fowler *et al.*, 2002; Compagno & Last, 1999; Dulvy *et al.*, 2008). Therefore, they have low reproductive potential and capacity for population increase (IUCN SSC Shark Specialist Group, 2007). These characteristics are thought to pose serious limitations on the ability of populations to sustain and recover from depletion caused by human exploitation or other impacts (Graham & Roberts, 2007). In fact, these rays are affected by numerous human activities, including directed fisheries, incidental capture as by-catch, habitat destruction, marine debris, boat strikes, entanglement and unregulated tourism (Heinrichs *et al.*, 2011; Couturier *et al.*, 2012). Even though mantas and mobulas are charismatic species, their survival is threatened by growing fisheries pressure due to the increasing demand for their gill rakers by the Asian market. Global annual documented landings are ~3.400 mantas (*M. birostris* only) and ~94.000 mobulas (all species) but unreported and subsistence fisheries point to landings likely being much higher (Heinrichs *et al.*, 2011).

In the past, these rays were seen as diabolic creatures that were responsible for stealing boats and deliberately killing fishermen and divers (Gill, 1908; Sáenz-Arroyo *et al.*, 2006) However, they are now recognized as harmless and iconic species, mainly due to the tourism industry that emerged directed to some of these species. A total estimated annual tourism value of over US$100 million per year compares favorably to the estimated market value of US$11 million per year for the global gill raker trade (Heinrichs *et al.*, 2011).

The potential for long-term sustainable income through responsible dive eco-tourism can provide a strong incentive for coastal communities to protect manta and mobula rays (Heinrichs *et al.*, 2011).

The assessment of the conservation status of these species of elasmobranchs on the IUCN Red List for Threatened Species resulted in species listed from 'Data Deficient' to 'Endangered' (Pierce & Bennett, 2003; Bizarro et al., 2006; Clark et al., 2006*a,b*; Notarbartolo-di-Sciara et al., 2006; White et al., 2006*b*; Bizarro et al., 2009*a,b*; Valenti & Kyne, 2009; Marshall et al., 2011*a,b*).

To date, there are no formal stock assessments done on any of the mobulid species, and the current global status of their populations is unknown. Further research is urgently needed to acquire knowledge of the habitat use and movements of mobulid species in order to evaluate their exposure to and overlap with human activities such as fisheries and pollution, and to identify areas of particular ecological relevance for their populations, or Essential Fish Habitats (EFH). For example, identifying the location of aggregation sites and migration routes can provide important information for their protection through the establishment of marine-protected areas. Ultimately, studies are much needed to understand the reasons behind mobulid aggregations. The Azores is one of the few places in the world where large schools of *M. tarapacana* appear to aggregate seasonally. Located in the middle of the Atlantic Ocean (37°-40°N; 24°-32°W), the nine volcanic islands of the Azores form the most isolated archipelago in the North Atlantic (Figure 1). These islands rise from the so called Azores Plateau, a thick and irregular area of the oceanic crust roughly limited by the 2000 meters bathymetric curve (Needham & Francheteau, 1974). In addition to its remoteness, the complex geological and geodynamic setting of the Azores islands makes them unique because of the variety of marine habitats, diversity of marine life and surrounding deep waters. As a result, the islands are of great nature conservation and marine biological interest (Schmiing *et al.,* 2009). The archipelago is located at the northern edge of the North Atlantic Subtropical Gyre, between two ramifications of the Gulf Stream: the cold and nutrient rich – southern branch of the North Atlantic current, north of the Azores, and the warm Azores Current with its main axis located at around 34°N.

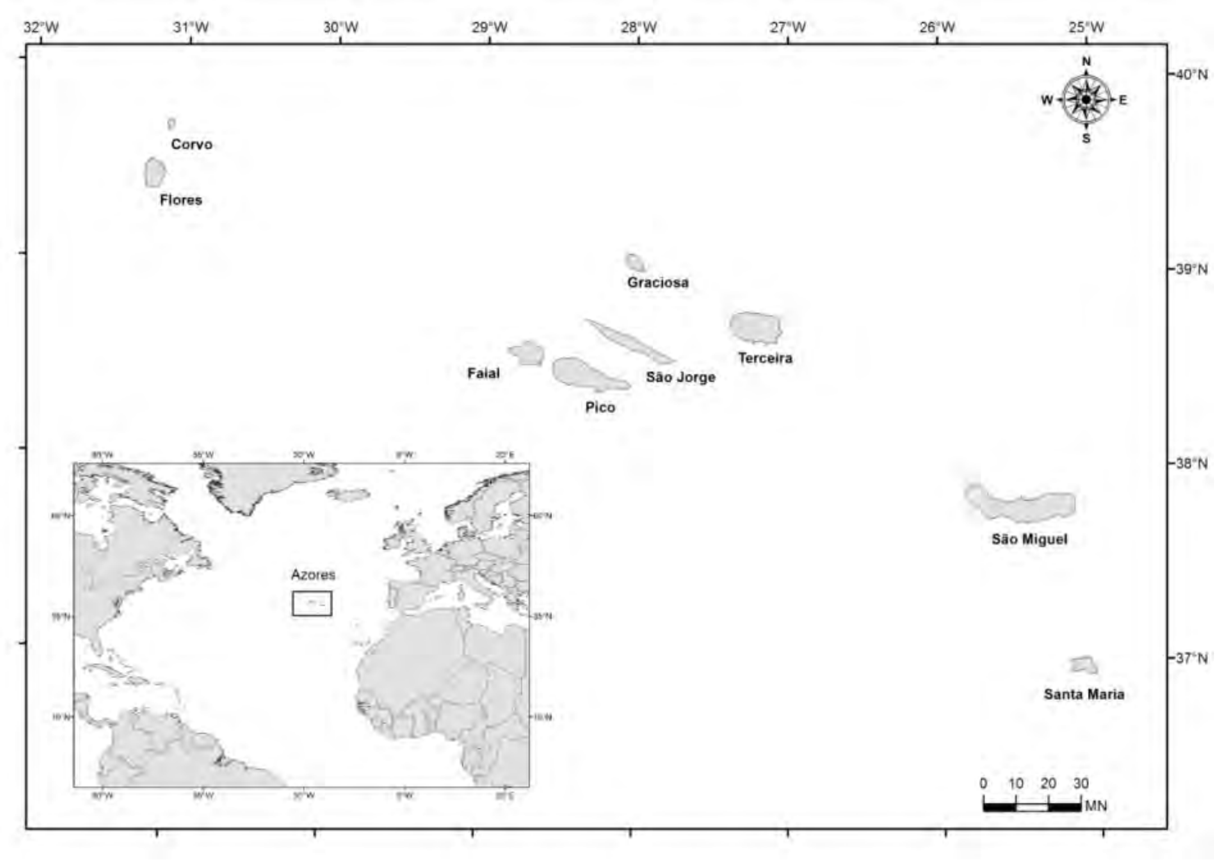


Figure 1. Location of the Archipelago of the Azores - R. Medeiros © ImagDOP

These unique conditions make the archipelago an important habitat for many large pelagic species that undertake Atlantic migrations and here find good conditions to breed, feed and grow. The region appears to constitute the uppermost limit of distribution for many of these large migrators. Currently, there are three species of mobulid rays cited for the Azores: *M. birostris*, *M. mobular* and *M. tarapacana*. However, difficulties in the identification of animals in the wild, the general absence of preserved specimens in collections or museums and even of detailed descriptions or depictions of the specimens in older references raise obvious doubts as to the identity of the species actually occurring in the region.

The genus Manta was recently re-described by Marshall *et al.* (2009) with at least two distinctive species (*M. birostris* and *M. alfredi*), and a third putative species (*Manta sp. cf. birostris*). In spite of such recent developments, an assessment of the taxonomic status of both genus for the wider Azores is still lacking. A revision of the taxonomy of mobulid species across their distribution range, including in the Azores region, is urgent given that it might have main implications for the conservation status of their Atlantic populations. In particular, comparatively less is known about their distributional ranges in oceanic regions at higher latitudes such as the Azores which are thought to constitute their distribution limits.

This work aims to:

1. Clarify which are the mobulid species occurring in the Azores archipelago and provide an annotated checklist for the region;
2. Understand the seasonal dynamics of their presence in the Azores;
3. Identify ecologically important habitats for each species in the region;
4. Learn about the location and importance of *M. tarapacana* aggregation sites;
5. Implement a public, regional photographic database for Mobulids, with a focus on *M. tarapacana*
6. Develop and test a photo-ID software and protocol to study individual occurrences of *M. tarapacana* globally;
7. Evaluate the threats these species face in the region and suggest possible conservation strategies.

Chapter 2. Occurrence of Mobulidae in the Azores

Introduction

The family Mobulidae includes the largest of the rays, some of them growing over seven meters disc width (Compagno & Last, 1999; Marshall *et al.*, 2009). The large size achieved by most of the species of this family accounts for their reduced representation in ichthyological collections. The descriptions of those species often having been based on a single museum specimen, frequently at an early stage of development or in a poor state of preservation (Notarbartolo-di-Sciara, 1987). In addition, mobulid species are difficult to identify due to their external resemblance, which has led to taxonomic ambiguities. The taxonomy of this family has a convoluted history, with numerous generic and species synonyms described in the literature. The misidentification of *Mobula* species is frequent even in the current literature, where synonyms referring to several recognized species are still used (Couturier *et al.*, 2012).

Due to the external similarities, manta and mobula rays are often confused in the field (Marshall *et al.*, 2011*b,c*). However, mobula rays are smaller than the mantas and can also be distinguished by morphological differences in their mouths and cephalic lobes. While manta rays have paddle-like cephalic fins, mobulas have shorter pointed ones. They can also be distinguished by the position of the mouth which is sub-terminal in mobulas, that is, it is located underneath the head, whilst mantas have terminal mouths, located at the front of the head (Figure 2).

Figure 2. Main morphological external differences between the giant manta (*Manta birostris*) and the Chilean devil ray (*Moula tarapacana*)

Currently there are three species of mobulid rays cited for the Azores: *Manta birostris*, *Mobula mobular* and *Mobula tarapacana*. The giant manta ray (*M. birostris*) occurs in tropical, sub-tropical and temperate waters of the Atlantic, Pacific and Indian Oceans and it's commonly sighted along productive coastlines with regular upwelling, oceanic islands and, particularly, offshore pinnacles and seamounts (Yano *et al.*, 1999; Marshall *et al.*, 2009, 2011*b*). Also, rare or seasonal sightings of *M. birostris* at sparse locations lead to the conclusion that this species must undergo significant seasonal migrations (Marshall *et al.*, 2009, 2011*b*).

The giant devil ray (*M. mobular*) occurs throughout the Mediterranean Sea and possibly in the nearby North Atlantic. It is also cited to the coast of Africa, from Morocco to Senegal; remote offshore islands as the Canary Islands, Madeira, the Azores and Cape Verde (Santos *et al.*, 1997; Wirtz *et al.*, 2008); Portugal (Nobre, 1935); and, as a transient, off southern Ireland (Notarbartolo-di-Sciara, 1987). Many of these records need to be verified as they are unconfirmed and as a consequence, may constitute misidentifications due to the close morphological resemblance between *M. mobular* and *M. japanica*, a species known from the tropical Atlantic (Notarbartolo-di-Sciara, 1987; Notarbartolo-di-Sciara *et al.*, 2006). Also, in the Azores *M. tarapacana* was considered as being *M. mobular* by different authors, those references were re-analyzed in this work. *The* Chilean devil ray *(M. tarapacana)* is probably circumglobal in temperate and tropical waters but is known from scattered locations in the Indian, Pacific and Atlantic Oceans. It is primarily oceanic, and one of the least known mobulids. The Azores seems to be one of the few locations in the world where *M. tarapacana* aggregates in such large schools. A distribution map of the species accounted in this study is given in Appendix I.

According to Santos *et al.* (1997) the first reference to Mobulid rays in the Azores dates from 1777, when Duhamel du Monceau described a fish named “raie cornue” from the Azores and “Mobular” from the Caribbean. Thereafter, only two mobulid species were cited for the Azores (*M. birostris* and *M. mobular)* until 2001, when a third species, *M. tarapacana* was recorded (Afonso, 2001). However, prior to Santos *et al.* (1997), nearly all the references to mobulids for the Azores, have no preserved specimens or detailed descriptions. Due to the taxonomic ambiguities of this family and their occurrence in the wider north Atlantic, it is possible that some of those sittings constitute misidentifications.

The genus *Manta* was recently re-described by Marshall *et al.* (2009) with at least two distinctive species (*M. birostris* and *M. alfredi*), and a third putative species (*Manta sp. cf. birostris*). An assessment of the taxonomic status of both genus for the wider Azores is lacking. A revision of the taxonomy of mobulid species across their distribution range, including in the Azores region, is very important given that it might have significant implications for the conservation status of their Atlantic populations. This work aims to clarify which are the mobulid species occurring in the Azores archipelago and provide an updated checklist of the family for the region. Previous citations for the area together with an extensive collection of photos and videos collected from 1990 to 2013 by scientific projects, underwater photographers and dive operators were critically re-analyzed.

Methods

Photographic database

Photos and videos from divers, photographers and dive operators from the islands of Faial, Pico and Santa Maria were gathered. The pictures mainly report to individuals sighted at the shallow banks of Princesa Alice, Formigas and Ambrósio, where *M. tarapacana* are known to aggregate in larger numbers and where the directed diving operations occur. The photos deposited at the image database of the Department of Oceanography and Fisheries of the University of the Azores (ImagDop), mostly also from these sites, were also analyzed. A photographic database of mobulid rays from the Azores was then created containing all the photos and videos collected, comprising in a total of 2252 photos and 188 videos, from 1990 to 2013. The identification of the species was based in the distinctive features described in the work of Notabartolo-di-Sciara (1987) for *M. mobular* and *M. tarapacana*, Marshall *et al.* (2009) for *M. birostris* and Stevens (2010) for *M. tarapacana*. Those distinctive characters are described in Table 1.

Previous citations for the Azores

Citations of Mobulid rays for the Azores Economic Exclusive Zone (EEZ) in scientific journals, underwater guides and public articles were reviewed. Personal information (communications, old photographs) was also incorporated in the list. The rules adopted for species identification, nomenclature and synonym were based on Santos *et al.* (1997). The resulting annotated checklist is organized in Classes, Orders and Families according to Compagno (1999). The scientific names are organized in alphabetical order within the families. Synonyms, misspellings and/or misidentifications found in the literature in reference to the occurrence of mobulid species in the Azores are mentioned in a separated field, together with the name of the author and the year of publication. When the record of a species was of primary order, that is, the reference included specimens or individuals that were directly studied and observed by the author, the symbol "°" follows the citation (e.g. Afonso, 2001°). Otherwise, the reference is of secondary order and is based on another author.

Table 1. Distinctive characters of the three mobulid species occurring in the Azores

Distinctive Characters	*M. mobular/M. japanica**	*M. tarapacana*	*M. birostris*
Caudal Spine	Serrate spine at base of tail	No caudal spine	Reduced, predominantly encased in a calcified mass posterior to the dorsal fin
Tail	Very long, wire-like	Short and thick, rod-like	Slender whip-like
Cephalic Fins	Short	Short	Long
Dorsal Surface	Dark blue, occasionally with a crescent-shaped light area on each 'shoulder'	Brown to olivaceous green dorsally; Distinctive pronounced ridge along dorsal midline	Black, with large, conspicuous, white shoulder patches approximately triangular in shape, which occur on either side of a dark midline.
Ventral Surface	Generally white	White anteriorly, grey posteriorly, with an irregular but definite demarcation line	White with dark grey to black spots and patches; Spots do not occur medially between the five gill slits or on the pectoral fins lateral to the body cavity; Charcoal-colored V-shaped margin along the posterior edges of the pectoral fins
Spiracle	-	Dorsal to plane of pectoral fins; Above and behind the margin of the pectoral fin where it joins the body; Under a distinct ridge and in an elongated longitudinal slit	-
Mouth and gill covers	-	Often with grey shading/flaring	Black mouth. That coloration often extends to the anterior edge of the first gill slits; Black semi-circular spots posterior to the fifth gill slits
Other	Top of the head has a thick dark black band which stretches from eye to eye;	Elongated 'neck'; Margin of rostrum lunate, slightly convex near the base of cephalic fins; The trailing edge of the pectoral fins are falcate or crescent moon-shaped	Pale to white chevron shaped patch, of variable size, extends anteriorly from the anterior insertion point of the dorsal fin; White coloration is often visible on the dorsal tips of the pectoral fins
Dorsal fin	White tip	Plain colored	Plain colored

*** *M. mobular* and *M .japanica* share the same morphological characters**

Results

Photographic database

A total of 2252 pre-screened photos and 188 videos of Mobulids, taken from 1990 to 2013, were analyzed. With the analysis of these underwater photos and videos it was clear that the entire database was constituted essentially by two species: *M. birostris* and *M. tarapacana*. However, in July, 2013, the first photographic record of a third species in the Azores entered the database. It was not possible to make a positive confirmation of that species, which is here considered as belonging to the *M. mobular/M. japanica* species complex, hereafter called *M. cf. mobular*.

The distinctive characters mentioned in Table 1 were used for the identification of the species through videos and photographs. Some of those characters present in photographs are demonstrated in Figure 3, 4 and 5 for each species.

Manta birostris

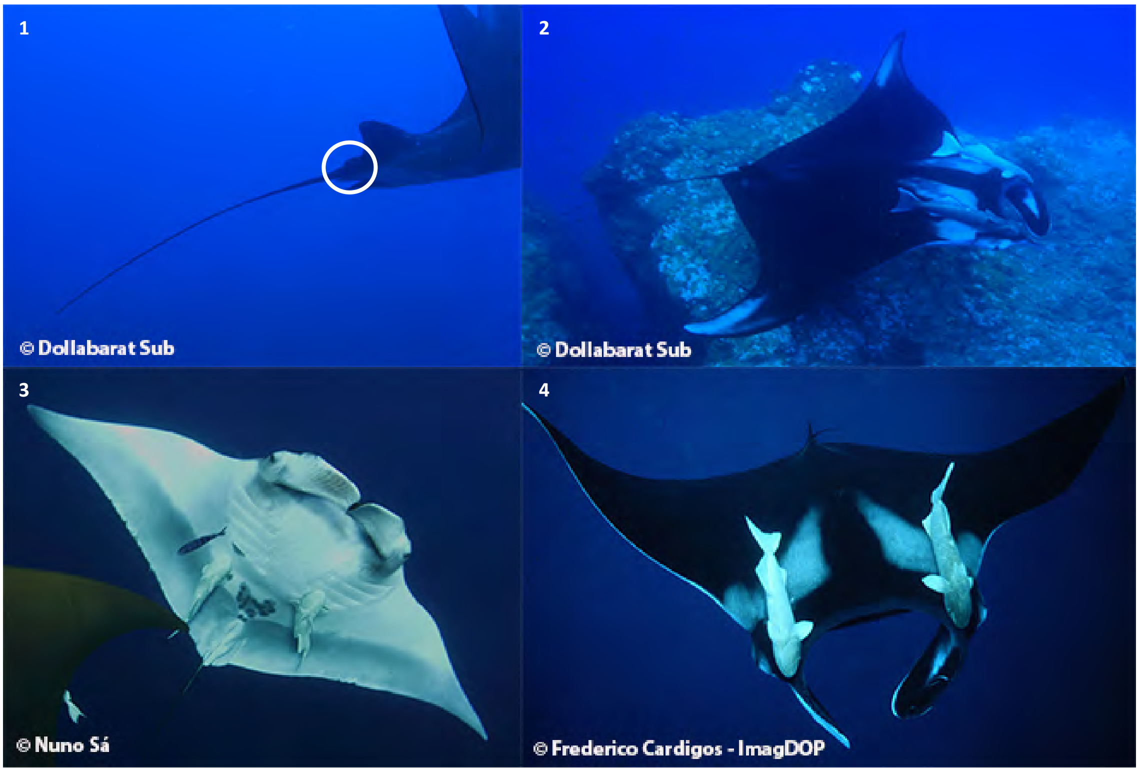


Figure 3. *M. birostris* in the Azores, evidencing distinctive features: (1) Slender whip-like tail, caudal spine predominantly encased in a calcified mass (2) Pale to white chevron shaped patch, blazes of white color on the dorsal tips of the pectoral fins (3) Coloration around mouth extends from the base of the cephalic fins to the first gill slits, ventral surface largely cream to white with dark grey to black spots and patches occurring between gill slit openings and the opening of the cloaca, black semi-circular spots posterior to the fifth gill slits, posterior edges of pectoral fins charcoal-colored forming a V-shaped margin (4) Dorsal surface black, with large white shoulder patches on either side of a dark midline, mouth black to charcoal grey in coloration

Mobula tarapacana

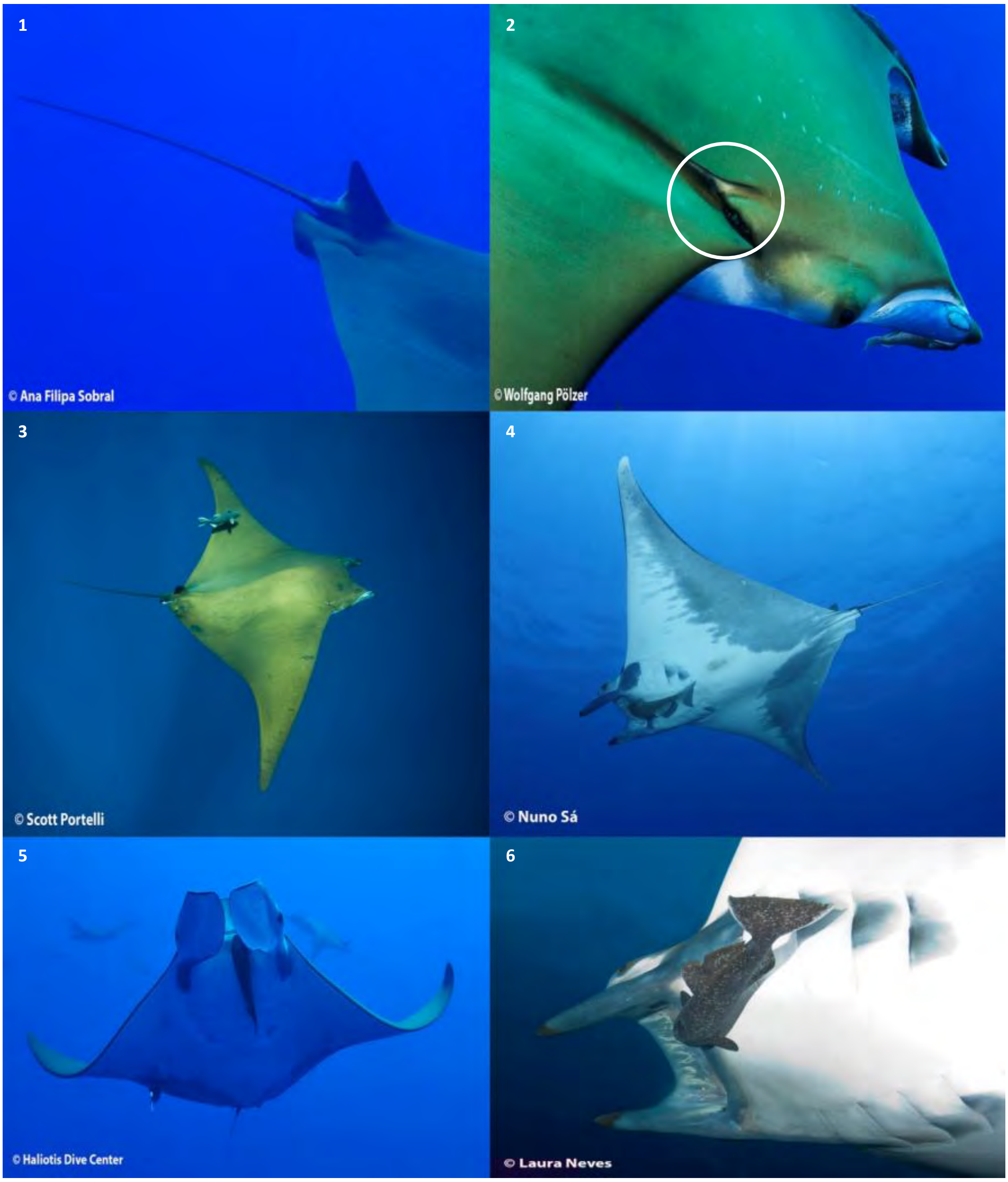


Figure 4. *M. tarapacana* in the Azores, evidencing distinctive features: (1) No caudal spine, Tail short and thick (2) Elongated 'neck', Spiracle in an elongated longitudinal slit above and behind the margin of pectoral fin (3) Brown to olivaceous green dorsally, distinctive pronounced ridge along dorsal midline (4) Ventral side white anteriorly, grey posteriorly, with an irregular but definite demarcation line (5) Short cephalic fins (6) Gill covers often with grey shading/flaring

Mobula cf. mobular

Figure 5. *M. cf. mobular* in the Azores, evidencing distinctive features (images are snapshots taken from video): (1) Very long tail, wire-like and white tip of the dorsal fin (2) Dark blue dorsal surface, with crescent-shaped light area on each "shoulder"; Top of the head with thick dark black band stretching from eye to eye (3) Short cephalic fins (4) White ventral surface

An annotated checklist of Azorean mobulids

Class **CHONDRICHTHYES**

Order **MYLIOBATIFORMES**

Mobulidae

Manta birostris (Walbaum, 1792)

The references of the species for the Azores have no morphological descriptions of the specimens.

Collins 1954° – The first confirmed citation of this species for the archipelago, which reports a specimen harpooned in Horta harbor in 1951. The specimen was identified based on photographs and details supplied by Collins to the British Museum (Natural History) in London (Appendix II).

Ré 1979° - Species mentioned in the checklist of observed and captured fishes of a Bio-oceanographic campaign made in the Azores.

Wirtz 1994 - Species included in fish guide. Brief description of the characteristics of the family Mobulidae. Pictures of *M. birostris* specimens.

Saldanha 1995° - Misidentification of *M. birostris*. Photo illustrating the species *M. mobular* is in fact a photo of a specimen of *M. birostris*.

Garcia 2008 - Based in older references the author considers *M. birostris* as a species that occurs in the archipelago. A photo is used to identify the species, however there is no information about whether the photo used was taken in the archipelago or somewhere else.

Barreiros & Gadig 2011 - Species included in the illustrated catalogue: Sharks and rays from the Azores.

Wirtz 2011 - Species included in fish guide. Brief description of the characteristics of the family Mobulidae and differences between the genus *Manta* and *Mobula*. Pictures of *M. birostris* specimens.

Gallagher *et al.* 2013 - In the description of the species *M. tarapacana* the author refers that it might be confused with *M. birostris* which also occurs in the region.

Synonyms, misspellings, misidentifications: Saldanha 1995

Mobula cf. mobular (Bonnaterre, 1788)

Most of the *M. mobular* references do not have a morphological description and the ones who do only provide a simplistic description of the specimens, preventing a definitive identification. For that reason, and also because of the morphologic similarities shared with *M. japanica*, it is here considered *M. cf. mobular*.

Duhamel du Monceau 1777° – the author describes a fish named “Raie cornue” in the Azores and “Mobular” in the Caribbean in his *Traité Général des Pêches*. Despite the fact that he includes the reference to the Azores in the designation of the species, the geographic localities specified are the coast of Normandy and the Caribbean. Even though the is a description of different measures of the animal, the description of the morphological characters is vague and the illustration is quite fantasized (Appendix III). The author does not mention the presence of a caudal spine which is characteristic of *M. mobular*. In the illustration the mouth of the animal is in the ventral side which is a distinctive character of the genus *Mobula*.

Bonnaterre 1788 - The description of this species was based on Duhamel du Monceau (1777), according to Bigelow & Shcroeder (1953) and Notabartolo-di-Sciara (1987).

Walbaum 1792 - In a section called *Species adhuc dubiae,* the author includes "Raja, *Vespertilio*", the description is based in Duhamel du Monceau (1777).

Lacepéde 1798 - Description of "Raie mobular" based in Duhamel du Monceau (1777).

van Beneden 1892 - The author describes a parasite collected from the skin of a *Ceratopterus sp.* in the Azores.

Silva 1893 - According to Santos *et al.* (1997) the author refers the presence of *Caeratopterus* sp. (*raia* or *jamanta*) in Museu Carlos Machado (Ponta delgada).

Sampaio 1904° - Considered "taxonomically very awkward and indeterminate" by Santos *et al.* (1997).

Fowler 1936 - Description of *M. mobular*, reference for the Azores based in Duhamel du Monceau (1777).

Ferreira 1939° - Describes a specimen of *Cephaloptera giorna* (Risso), a synonym of *M. mobular*. He mentions several features which are characteristic of the species: long and thin tail and the presence of a caudal spine. However when he describes a specimen harpooned in the Azores in 1929 he only mentions the full length (around three meters) in comparison with the caudal length (around two meters).

Bigelow & Schroeder 1953 - Description of *M. mobular*, the reference for the Azores is Duhamel du Monceau (1777).

Collins 1954 - Considers Ferreira's (1939) reference doubtful but makes no detailed description of the species.

Albuquerque 1954-1956 - Description of the genus *Mobula* and the species *M. mobular* which is said to be the only of this genus to occur in Portuguese waters. Reference for the Azores based in Fowler (1936).

Harmelin & Harmelin – Vivien 1979° - Species included on the checklist of the 1979 Bio-Oceanographic mission in the Azores.

Sanches 1986 - It was not possible to consult this publication.

Harmelin & Harmelin – Vivien 2001 - Re-analysis of the data from Harmelin & Harmelin – Vivien (1979).

Synonyms, misspellings, misidentifications: "Raie cornue" by Duhamel du Monceau 1777 ; "Raja, *Vespertilio*" by Walbaum 1792; "Raie mobular" by Lacepède 1798; *Ceratopterus* sp. by van Beneden 1892; Silva 1893; Sampaio 1904; *Cephaloptera giorna* by Ferreira 1939.

Mobula tarapacana (Philippi,1892)

Wirtz 1990° - Species included on the checklist of the scientific expedition 'Expedição Açores/89'. According to the author it was in fact *M. tarapacana* (Peter Wirtz, pers. comm. 2013).

Wirtz 1991° - Die Küstenfische der Azoren, magazine article. With picture of *M. tarapacana*. According to the author it was in fact *M. tarapacana* (Peter Wirtz, pers. comm. 2013).

Wirtz 1994° - Misidentification of *M. tarapacana*. Brief description of the characteristics of the Small devil ray (common name given by the author). In the description one of the characteristics given is: "The topside of its body is a green hue", which is a characteristic of *M. tarapacana*. Photo illustrating *M. mobular* represents a specimen of *M. tarapacana*. According to the author it was in fact *M. tarapacana* (Peter Wirtz, pers. comm. 2013).

Santos *et al.* 1994 - The author refers the occurrence of *M. mobular* in Madalena Islets (Pico Island) and in Baixa do Canal, between Pico and Faial Island. The species was in fact *M. tarapacana* (Ricardo Santos, pers. comm. 2013). Ghost paper of Santos *et al.* (1995).

Santos *et al.* 1995 - The author refers the occurrence of *M. mobular* in Madalena Islets (Pico Island) and in Baixa do Canal, between Pico and Faial Island. The species was in fact *M. tarapacana* (Ricardo Santos, pers. comm. 2013).

Afonso 2001° - This is the first reference of *M. tarapacana* for the Azores.

Garcia 2008 - The author considers *M. tarapacana* as a species that occurs in the archipelago. A photo is used to identify the species, however there is no information about whether the photo used was taken in the archipelago or somewhere else. Based in older references, the author also considers *M. mobular* as a species that occurs in the archipelago. Yet, the image illustrating this species clearly represents a specimen of *M. tarapacana* which leads to the conclusion that *M. mobular* is misidentified as *M. tarapacana*.

Barreiros & Gadig 2011 - Species included in the illustrated catalogue: Sharks and rays from the Azores. The author mentions that the references to *M. mobular* are possibly misidentifications of *M. tarapacana*.

Wirtz 2011 - More detailed description of *M. tarapacana* to which the author gives the common name of Small devil ray. Photos represent *M. tarapacana* specimens. According to the author it was in fact *M. tarapacana* (Peter Wirtz, pers. comm. 2013).

Gallagher *et al.* 2013 - The author makes a description of the key features to identify *M. tarapacana* individuals and gives a few facts on their biology.

Synonyms, misspellings, misidentifications: Wirtz 1990; Wirtz 1991; Wirtz 1994; Santos *et al.* 1994; Saldanha 1995; Santos *et al.* 1995; Garcia 2008.

Discussion

This study confirmed the current notion that two species (*M. birostris* and *M. tarapacana*) occur in Azorean waters. Importantly, it also provides novel data that unequivocally allows an update of this figure to three, maybe four species of Mobulids occurring in the Region.

In general, older references of Mobulids for the Azores are, in fact, impossible to ascertain given the lack of detail in the descriptions and the inexistence of preserved specimens in collections. In particular, it is important to have in consideration the external similarities and difficult distinction between *M. mobular* and *M. japanica*. The fact that some of the first original descriptions mention a caudal spine (Ferreira, 1939) does not imply that *M. mobular* was correctly identified. In fact, the records of Mobula specimens with a caudal spine reported for the Atlantic Ocean need to be verified, when possible, as their misidentification cannot be excluded (Notarbartolo-di-Sciara, 1987; Couturier *et al.*, 2012). Further studies are crucial to clearly distinguish *M. mobular* from *M. japanica* because of their close morphological resemblance and particularly because the division of both species is only based on the examination of a single specimen of *M. mobular* (Notarbartolo-di-Sciara, 1987). According to Notarbartolo-di-Sciara (1987) the two species may be distinguished by their maximum size (*M. mobular* being larger) and morphometrics (*M. mobular* reaching greater disk width relative to the size of the body). However, observing these features in the wild is practically impossible. Notarbartolo-di-Sciara (1987) noted that 'the overlap in their morphological features potentially indicative of single species status'. Genetic and morphological analysis of additional specimens from the Atlantic are needed in order to better understand the distribution ranges of these species.

Since *M. tarapacana* represents, by far, the most abundant species in the photographic database over a time lapse of 23 years (2125 out of 2252 photos), we can assume that it most certainly always occurred in the region but was repeatedly misidentified as *M. mobular*. By the analysis of the photos submitted to the database it is possible to conclude that at least three species occur in the Azores, with the chance of a fourth species, if *M. mobular* and *M. japanica* are considerate as separate species. Each of these species has a different occurrence level.

Three different occurrence levels were considered here: **Frequent** (More than one occurrence per week during season); **Occasional** (At least one occurrence every year), and **Rare** (Less than one occurrence every year). Considering that this classification is based on a minimum of c. 100 events spread across the archipelago and a period spanning 23 years, and that this period covered the advent of touristic diving in the region (and therefore of numerous photographic records in areas where mobulids are actively looked for), it seems reasonable to assume that *M. birostris* occurs occasionally in the Azores with several encounters reported every year. All reports have been from solitary individuals in offshore reefs but mainly in shallow banks. This is consistent with the habitat preference and distribution of this species described in the literature. According to Marshall *et al.* (2009, 2011*b*) *M. birostris* has a circumglobal distribution and is commonly sighted along productive coasts, oceanic island groups and particularly seamounts. This species seems to be more solitary, more oceanic and more migratory than its sister species *M. alfredi* (reef manta ray). Sightings of the Giant manta are often seasonal or sporadic but there are locations where their occurrence is more common. Observations of *M. birostris* frequenting remote seamounts and off remote islands such as the Azores islands suggests that this species undergoes significant seasonal migrations (Marshall *et al.*, 2009, 2011*b*).

M. tarapacana occurrence in the Azores is seasonal and frequent with aggregations of dozen individuals in specific locations seen every year and consistent encounters during the season. This ray probably has a worldwide distribution but is known from scattered locations in the Indian, Pacific and Atlantic Oceans. It is of primarily oceanic occurrence, and one of the least known mobulids (Clark *et al.*, 2006*a*). The Azores seems to be one of the few locations in the world where *M. tarapacana* aggregates in such large schools.

M. cf. mobular occurrence is considered rare due to the fact that there were only two records in the time lapse encompassed by the photographic database . We can't discard the chance that there could have been more encounters with this species and that they were not recorded or were misidentified, but there are only two encounters registered during the time lapse of the study that allow us to confirm it.

Wirtz (2011) suggested that species as *M. mobular* and *M. japanica* may occur in the area of the Azores but have not yet been recorded. *M. japanica* is probably circumglobally distributed in temperate and tropical waters (Notarbartolo-di-Sciara, 1987; White *et al.*, 2006; Couturier *et al.*, 2012), but detailed information on its distribution remains scarce (White *et al.*, 2006; Bustamante *et al.*, 2012). Besides the reports from the Pacific, Atlantic and Indian Oceans the species possibly occurs in the North Atlantic (Notarbartolo-di-Sciara, 1987; White *et al.*, 2006). Information on the biology of *M. japanica* is extremely limited. The species is found inshore, offshore and possibly in oceanic environments, but the movement patterns within its range are poorly understood (White *et al.*, 2006). Pupping grounds appear to be offshore, Ebert (2003) suggests around offshore islands or seamounts. *M. mobular* is an offshore, epipelagic ray that occasionally frequents shallow coastal waters (White *et al.*, 2006; Couturier, 2012). It occurs throughout the Mediterranean Sea and possibly in the nearby North Atlantic (Notarbartolo-di-Sciara, 1987; White *et al.*, 2006). Outside the Mediterranean it occurs along the coast of Africa from Morocco to Senegal, the Canary Islands, Madeira, the Azores, Portugal, and as a vagrant, off southern Ireland (Notarbartolo-di-Sciara, 1987). Expert examination is needed to distinguish *M. mobular* from *M. japanica* thus the confusion between these species limits current knowledge of their respective distribution ranges. For the same reason reports of devil rays from the Atlantic need to be verified because they might constitute misidentifications.

The present study provides an annotated checklist of the mobulid species present in the waters of the Azores. However, it is clear that more research is needed on this group in order to clarify the potential occurrence of other species in the region and also to clarify the distinction between *M. mobular* and *M. japanica*. The use of new technologies and methodologies for the study of these species has already proved of great importance and will be crucial to learn more about the biology and ecology of the most understudied species of the family Mobulidae.

Chapter 3. Distribution, seasonality and aggregation of mobulids in the Azores

Introduction

The spatial ecology and demography of mobulids is poorly understood, particularly of the genus *Mobula* and especially in areas as the Azores which constitute fringes of the limit distributions of these species.

Manta birostris has a widespread distribution across the Atlantic, Pacific and Indian Oceans. It is frequently sighted at oceanic islands and, particularly, at offshore pinnacles and seamounts (Yano *et al.*, 1999; Marshall *et al.*, 2009, 2011*b*). It is thought to undertake considerable migrations (Marshall *et al.*, 2009, 2011*b*). This species appears to visit coastal and offshore sites seasonally. Even though their presence is common in some locations, their sightings are often seasonal and sporadic (Marshall, 2001*b*). In the Azores the occurrences seem to be of solitary individuals given that the few previous records never report the presence of various individuals simultaneously.

Mobula tarapacana is thought to be circumglobal but it is known from scattered locations in the Indian, Pacific and Atlantic Oceans (Compagno & Last, 1999; Clark *et al.*, 2006*a*). Although this species is primarily oceanic, it has been occasionally reported to occur in coastal waters (Clark *et al.*, 2006*a*). Our knowledge on the basic ecology of *M. tarapacana* is extremely limited, especially in terms of the habitat patterns, migration routes, aggregation dynamics and drivers of their distribution. As other species of Mobulids, *M. tarapacana* aggregates at specific locations, a behavior that has been poorly studied. In the archipelago of the Azores, this species is known by diving operators and fishermen to aggregate seasonally at different locations where they can be observed in large schools of up to dozens of individuals. These aggregations appear to occur consistently in the warmer months, when the water temperatures are higher, and in specific offshore locations.

The highly predictable spatial and temporal aggregations, coupled with the curious behaviour of these animals, supported the rapid development of a directed eco-tourism activity.

Obtaining daily data on abundance and behaviour of wide-ranging pelagic species is challenging (Couturier *et al.*, 2012; Jaine *et al.*, 2012). It is economically and logistically impossible for researchers to regularly conduct underwater visual censuses (UVC) due to the fact that these aggregations are usually located in remote places. However, it is possible to enroll dive instructors and dive masters to collect and report important data on the occurrence and abundance of these species. Such approach in data collection, often known as citizen science, can be a valuable alternative to obtain relevant ecological information (Cohn, 2008; Dickinson *et al.*, 2010, 2012). In particular, through the submission of photos which allow to obtain key information (e.g. behaviour, threats). Multi-year observational records are relatively easy to collect and can provide vital insights into patterns of occurrence and behaviour of these species. Coupled with in situ environmental observations and appropriate statistical models, such datasets offer a great opportunity to explore and identify key drivers, such as sea surface temperature (SST), for the presence of these species at specific sites (Jaine *et al.*, 2012).

The objective of this work was to provide baseline information on the ecology of mobulas in the Azores using data collected by dive operators and from the IMAR-DOP underwater visual census monitoring program. More specifically, the aim was to describe the spatial and temporal patterns of Mobulid occurrence in the region, including their aggregation dynamics, and use these data to discuss the ecological significance of Azorean habitats for the north Atlantic populations of Mobulids.

Methods

Data collection

Geographic and temporal patterns

The spatial patterns and seasonality of mobulid occurrence across the archipelago and its marine shallow habitats were investigated by underwater visual census (UVC). The data was obtained as part of the IMAR – DOP/UAz reef fish monitoring program. A total of 662 belt transects performed over 14 years (1997-2011) provided Mobulid presence/absence data (Schmiing *et al.*, 2013). Census were conducted at coastal and offshore reefs ('baixas') around the islands of Corvo, Faial, Pico and Santa Maria, and in three offshore shallow seamounts: the Formigas bank (a marine protected area, MPA, since 1988), the Dom João de Castro bank (an MPA since 2010), and the Princesa Alice bank (Figure 6)(see Afonso, 2001 and Schmiing *et al.*, 2013 for a detailed description of the sites). This design was made and used here to evaluate the differences in occurrence between these three major habitat types. In Faial, the UVC were made across the year, although unequally sampled, whereas in all other islands they were made mostly in the summer and less intensively in the winter. On offshore seamounts UVCs were only made in the summer (with the exception of four UVCs in Formigas bank in March 2008). Therefore, these data are used to evaluate the seasonality of mobulid occurrence across habitats but with the obvious limitations of such an unbalanced design.

Aggregation Dynamics

The occurrence, relative abundance and seasonality of Mobulids in general and *M. tarapacana* in particular were studied in three distinct dive sites which are renown for supporting mobula aggregations (Figure 6): Princesa Alice Bank, Formigas Bank, Ambrósio Reef. Princesa Alice Bank is a shallow bank located 50 nm (92.6 km) to the southwest of Pico Island and 45 nm (83.3 km) to the southwest of Faial Island in the Azores. The bank rises from depths of more than 1500 m to about 35 m below the surface. The Formigas Islets and Dollabarat Reef are two sites distancing ca. 3 nm (5.5 km) from each other at the Formigas Bank, a shallow seamount in the eastern group of the Azores archipelago. The bank is located 23 nm (42.6 km) northeast of the island of Santa Maria covering a surface area of approximately 9 km^2.

Ambrósio Reef is a volcanic pinnacle located 3 nm (5.5 km) off the north coast of Santa Maria, rising to about 45 m below the surface and with a minimum depth of 200 m between shore and the peak.

Data on the individual abundance of Mobulids was obtained from the dive operators. The data was collected for two consecutive years (2012 and 2013) on the Islands of Faial, Pico and Santa Maria, which support the industry of Mobula diving in the archipelago. Sighting sheets (Appendix IV) were distributed to the dive operators to be filled by the dive masters and dive instructors after each trip. The data included: species identification, behavioural data (e.g. feeding, interaction with divers), number of individuals, size, sex, numbers of boats and divers. The program was implemented to gather as much information as possible covering all the activity across the Mobula diving season, which normally extends from July to September.

Data obtained through photography and video (see Methods in Chapter 2) was also used to acquire additional qualitative information on behaviours, faunal associations and sexual maturity of mobulids occurring at the sites.

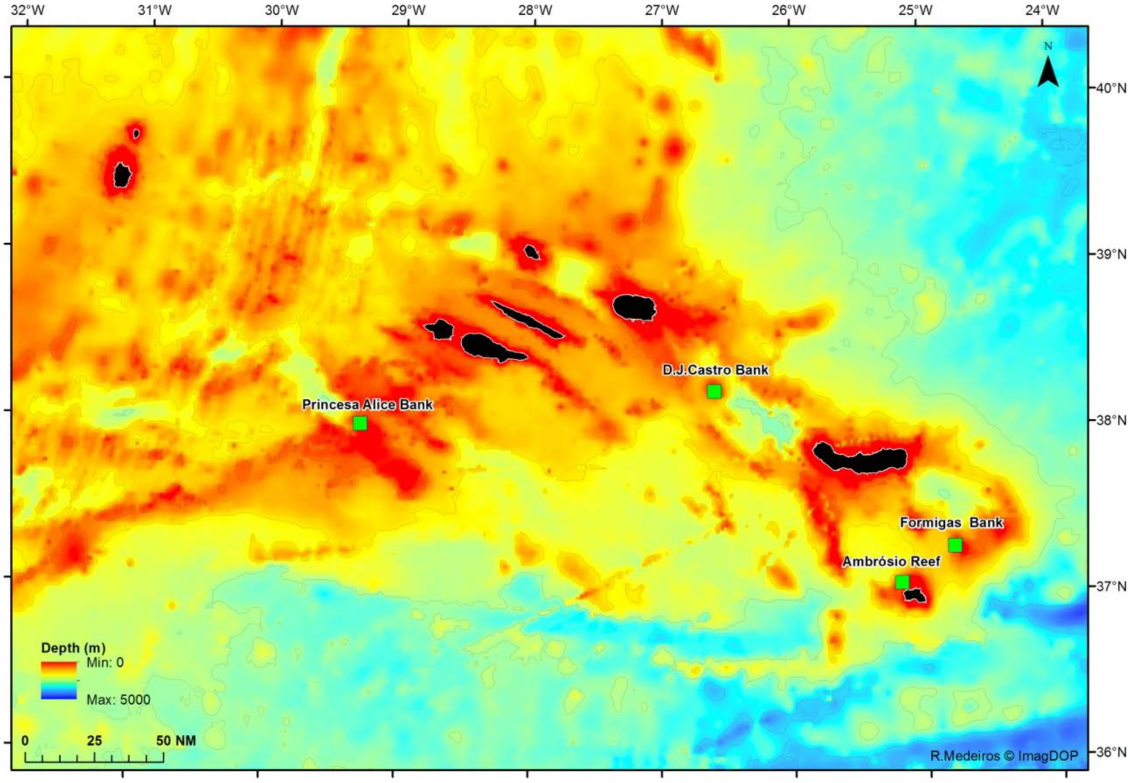


Figure 6. Map of the location of the shallow banks were mobula aggregations occur in the archipelago of the Azores

Data analysis

Geographic and temporal patterns

The sites sampled by UVC where divided into three different habitat types: Coastal Reef, Offshore Reef and Seamount. We assessed the relationship between the probability of species presence (response variable) and the habitat type (categorical predictors) at the sampled locations using binomial generalized linear models (GLM) with a logit link function, which is used for binary data and logistic regression (Quinn & Keough, 2001). For logistical regression, we assume that the binomial distribution is appropriate, which is likely for binary data (Quinn & Keough, 2001). The analysis was performed using presence/absence data from 662 UVC performed in all habitat types using the statistical programming environment R v3.0.2 (R Development Core Team, 2013).

The ability of the model to discriminate between presence and absence states was determined by using the Area Under the Curve (AUC) of the Receiver Operating Characteristics (ROC) plot test statistics. The ROC curve compares the proportion of true positive predictions (sensitivity) and the proportion of false positive predictions (i.e. 1–specificity) obtained from the model. The ROC curve is analyzed by the area under curve and the shape of the curve. If the ROC curve rises towards the upper left corner then the value of area under the curve will be greater and test performance is good (true positive rate is high). The AUC ranges from 0 to 1, where a score of 1 indicates perfect discrimination and a score of 0.5 implies predictive discrimination no better than a random guess. If the curve decline from upper left corner to lower right corner it means the test performance is bad (false positive rate is high) (Elith *et al.*, 2006). According to Hosmer & Lemeshow (2000) interpretation in Pittman & Brown (2011), an AUC value of 0.7–0.8 is considered an ‘acceptable’ prediction, 0.8–0.9 is ‘excellent’ and >0.9 is ‘outstanding’. Therefore, the AUC value obtained in this analysis was between 0.8-0.9 which tells us that the prediction is 'excellent'. The ROC curve and respective AUC value of the model are represented in Appendix V. By default, in a GLM with a categorical predictor, R sorts the levels in alphabetical order, so it was necessary to set each habitat as base to have all p-values for differences between all levels.

Because the p-value is calculated several times a post-hoc testing was necessary to test which levels were different from each other. According to Zuur *et al.* (2012) each time a test at the 5% significance level is conducted there is a 5% chance that a type 1 error is made. By doing this, in this case N=3x(3-1)/2=6 times, the chance of error rapidly multiplies. Using the Bonferonni correction the test was not at the 5% level but at the 5/N% level, which was 1.6667% in this data.

Aggregation Dynamics

The Mann–Whitney Rank Sum Test is a non-parametric test of the null hypothesis that two populations are the same against an alternative hypothesis, especially that a particular population tends to have larger values than the other. It is considered more efficient and robust on non-normal distributions than the t-test. Since in this case the data had a non-normal distribution this test was chosen to compare data of temperature (SST data) and abundance (Dive operators data) of two aggregation sites.

Notched Box Plots were used to understand the statistically significant differences between the two biggest aggregations in terms of SST temperature and mean number of individuals per dive in the overall of the two years of data (Appendix VI). The notch marks the 95% confidence interval for the medians. There is a 95% certainty that the actual median for the underlying population actually is within the interval marked by the notches. If the notches surrounding the medians from two box plots do not overlap, we can assume at the (0.05 significance level) that the medians are significantly different (McGill *et al.*, 1978).

Monthly average relative abundance was then visually compared with Sea Surface temperature (SST) for the sites. The software ArcGis, the package Marine Geospatial Ecology Tools (MGET) and the tool "Interpolate PO.DAAC MODIS L3 SST at Points" from the NASA Jet Propulsion Laboratory (JPL) Physical Oceanography Distributed Active Archive Center (PO.DAAC) were used to obtain the data. NASA JPL PO.DAAC publishes collections of SST images gathered by the Moderate Resolution Imaging Spectroradiometer (MODIS) carried by the Terra and Aqua satellites.

The following set of parameters was chosen in order to obtain a more complete and reliable information of the values of SST for the different aggregation sites:

MODIS satellite – Aqua: the Aqua datasets start in July 2002.

Temporal resolution - 8day - 8-day images: There are 46 images per year. The first image of the year starts on January 1. The duration of the last image of the year is five days during normal years and six days during leap years.

Geophysical parameter - NSST: nighttime SST derived from the thermal IR infrared (IR) bands.

Data to obtain - SST: the so-called "all-pixel" SST value, in degrees Celsius, estimated by averaging the AVHRR Global Area Coverage (GAC) observations made for the pixel during the time period, including those contaminated by clouds and other sources of error.

Spatial resolution - 9 km: the grid has a cell size of 1/12 geographic degree, or about 9.28 km at the equator, with 4320 columns and 2160 rows.

Finnally, remotedly sensed SST data at the aggregation sites was compared to the SST experienced by four *M. tarapacana* individuals externally tagged with satellite telemetry tags (miniPAT tags, Wildlife Computers, US) at Princesa Alice bank during 2012 (Throrrold *et al*. subm.). The tags were lodged under the pectoral fin musculature using a sling pole and stayed on the animals for an average period of 43 days, during which all migrated south to subtropical latitudes. This procedure was done to discuss the relevance of the aggregation sites in the context of the supposedly optimal thermal environmet for the species. SST tag temperature was grouped in 0.5°C intervals. Comparision was done using the mean of individuals of *M. tarapacana* registred per individual dive in each MODIS SST temperature interval versus the average percentage of days spent by the four tagged individuals in each temperature interval.

Results

UVC - Geographic and habitat patterns

A total of 543 UVC were made in coastal and offshore reefs of three islands of three different island groups: Corvo (Western Group), Faial (Central Group) and Santa Maria (Eastern Group) (Table 2; Figure 7). On those dives there were only seven presences of *M. tarapacana*. Regarding seamounts (D.J. Castro, Formigas and Princesa Alice) a total of 104 dives were recorded with a total of 31 presences. The frequency of occurrence [FO(%)] of *M. tarapacana* was much higher in seamounts, but the number of dives was lower than in island habitats.

Faial had the highest FO value (1.71%) and Santa Maria had a really close value (1.69%). Corvo had a FO value of 0% in coastal as well as offshore reef habitats. Both in Central and Eastern groups the FO was greater in offshore reef habitat, while Coastal reef had values of 0.43% and 0% respectively. In seamounts (Figure 8), Princesa Alice Bank had the highest FO (83.3%) followed by D.J. Castro (37.5%) and Formigas bank (16.7%).

Table 2. Data of frequency of occurrence (%) of *M. tarapacana* for Coastal Reef and Offshore Reefs in three different islands of three different island groups of the Azores archipelago and also for three Seamounts.

	Habitat	**No. Dives**	***M. tarapacana* presences**	**FO (%)**
Islands	Corvo - Coastal Reef	88	0	0.00
	Corvo - Offshore reef	45	0	0.00
	Faial - Coastal Reef	233	1	0.43
	Faial - Offshore reef	59	4	6.78
	Sta. Maria - Coastal Reef	46	0	0.00
	Sta.Maria - Offshore reef	72	2	2.78
Seamounts	D.J. Castro	8	3	37.5
	Formigas Bank	78	13	16.7
	Princesa Alice Bank	18	15	83.3
	Total Geral	**647**	**38**	

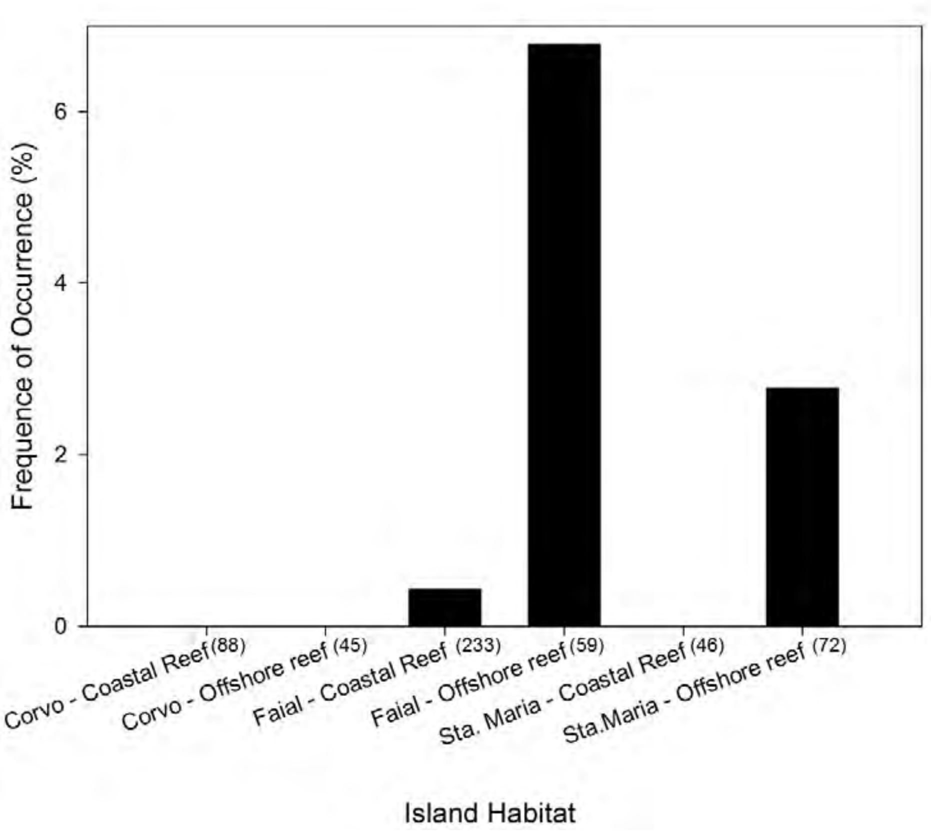


Figure 7. Frequency of occurrence of *M. tarapacana* at coastal and offshore reefs of three different islands across the archipelago of the Azores. Numbers in parenthesis represent the total number of transects made in coastal and offshore reefs of each island

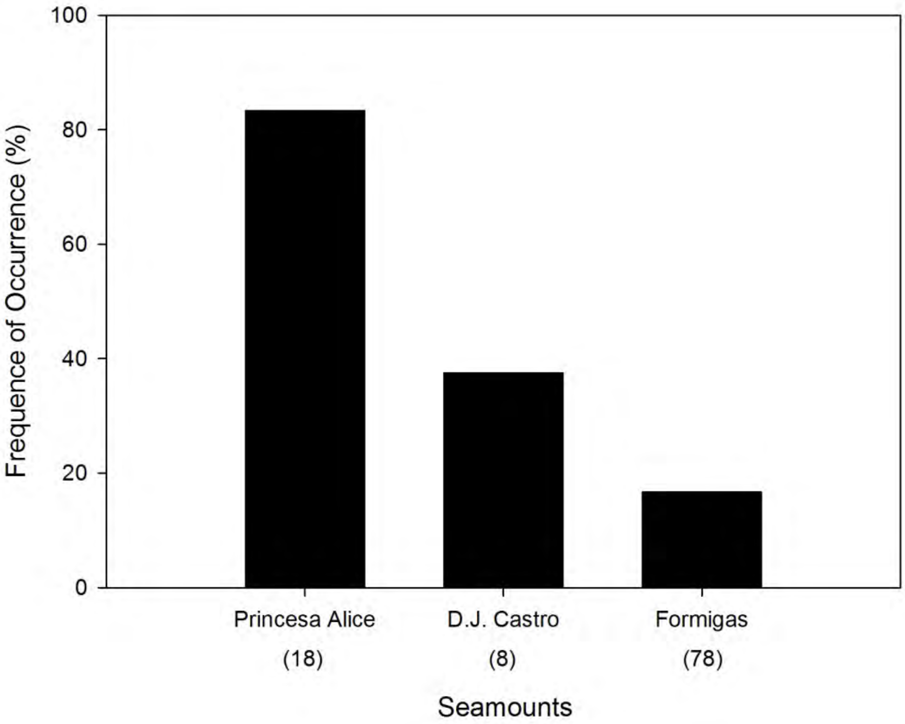


Figure 8. Frequency of occurrence of *M. tarapacana* at three different seamounts. Numbers in parenthesis represent the total number of transects made in each of the seamounts

M. tarapacana was present in 38 transects out of the 662 UVC, 31 in seamounts, six in offshore reefs and only one in coastal reefs. Accordingly, the highest frequency of occurrence (FO) (29.8%) corresponded to the seamount habitat (Figure 9).

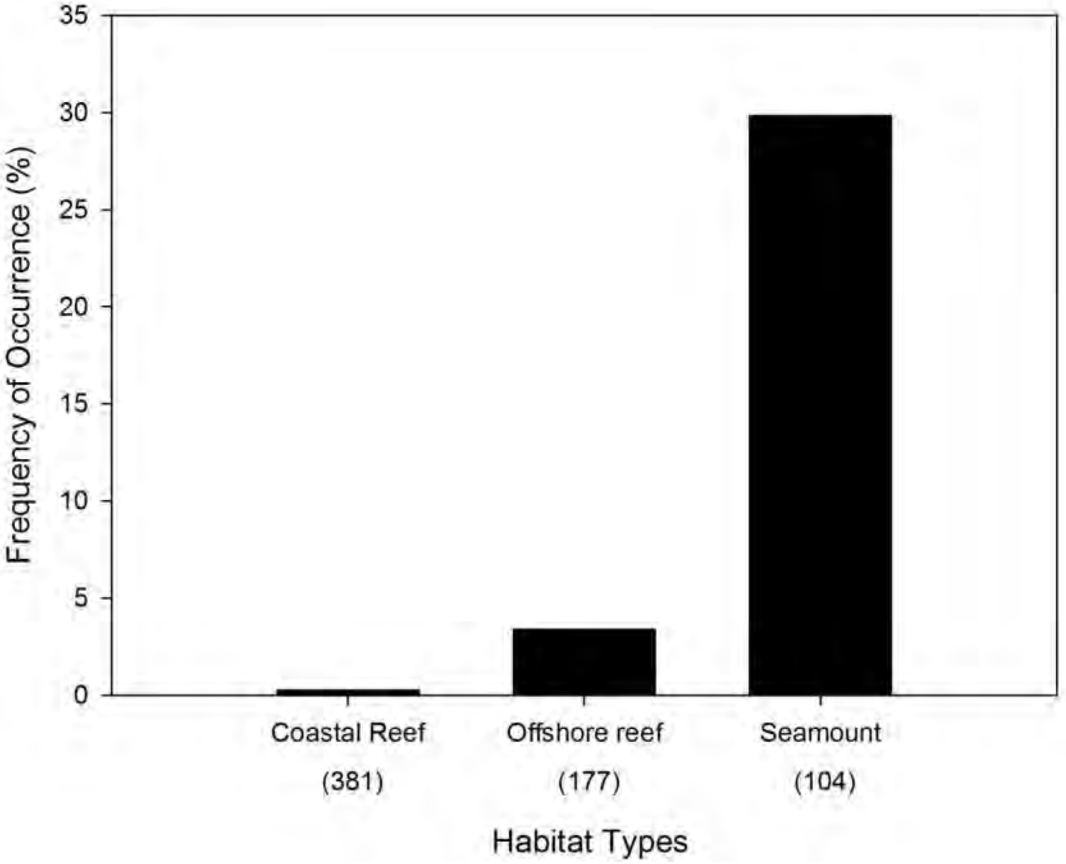


Figure 9. Occurrence of *M. tarapacana* in transects of the Underwater Visual Census program on three different habitat types across the Azores. Numbers in parenthesis represent the total number of dives in each habitat

The model result yielded the highest probability of *M. tarapacana* presence at seamount habitats (0.3) and the lowest at coastal reef habitats (approximately zero, Figure 10). There was a significant difference between the probability of presence of mobulas at seamounts and the other two habitats (Table 3), but not between coastal and offshore reef habitats.

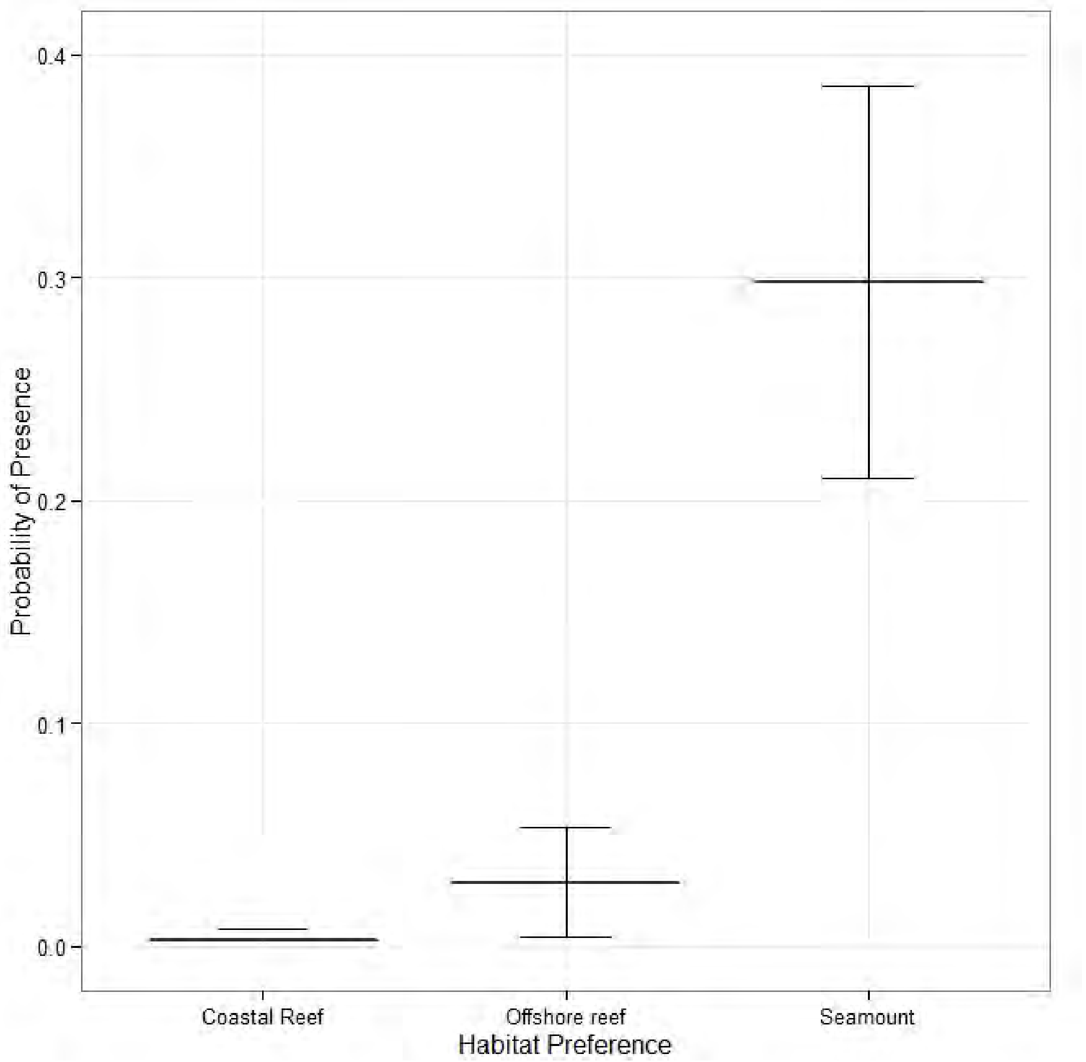


Figure 10. Mean probability of presence of *M. tarapacana* in different habitat types (with 95% confidence interval bars). Confidence bars of the seamount habitat do not overlap with those of the other habitat types, denoting a significant difference between the probability of presence of mobulas in seamounts and the other habitats

Table 3. Comparison of the probability of presence between three different habitat types. Significant differences (p<0.01, after Bonferonni correction) are represented in bold and marked with (*)

	Coastal Reef	Offshore Reef	Seamount
Coastal Reef	-	0.0281	**6.87e-07***
Offshore Reef	0.0281	-	**1.04e-07***
Seamount	**6.87e-07***	**1.04e-07***	-

UVC - Seasonality

The highest values of FO were obtained in the summer months (June to September, Figure 11,), which were also those with the highest number of dives (Table 4). In the remaining months (out of summer) there were no sightings of *M. tarapacana* individuals.

Table 4. Distribution of No. of dives and corresponding FO(%) of *M. tarapacana* during the year for the three different habitat types

	Coastal Reef			Offshore Reef			Seamount		
Month	**No. dives**	**No. individuals**	**FO(%)**	**No. dives**	**No. individuals**	**FO(%)**	**No. dives**	**No. individuals**	**FO(%)**
January	12	0	0,0	2	0	0,0	0	0	-
February	14	0	0,0	2	0	0,0	0	0	-
March	19	0	0,0	5	0	0,0	4	0	0
April	18	0	0,0	2	0	0,0	0	0	-
May	20	0	0,0	25	0	0,0	0	0	-
June	**20**	**0**	**0,0**	**13**	**0**	**0,0**	**21**	**2**	**9,5**
July	**96**	**0**	**0,0**	**37**	**2**	**5,4**	**8**	**3**	**37,5**
August	**92**	**0**	**0,0**	**46**	**1**	**2,2**	**44**	**16**	**36,4**
September	**53**	**1**	**1,9**	**34**	**3**	**8,8**	**27**	**10**	**37,0**
October	11	0	0,0	3	0	0,0	0	0	-
November	20	0	0,0	6	0	0,0	0	0	-
December	6	0	0,0	2	0	0,0	0	0	-
Total	**381**	**1**		**177**	**6**		**104**	**31**	

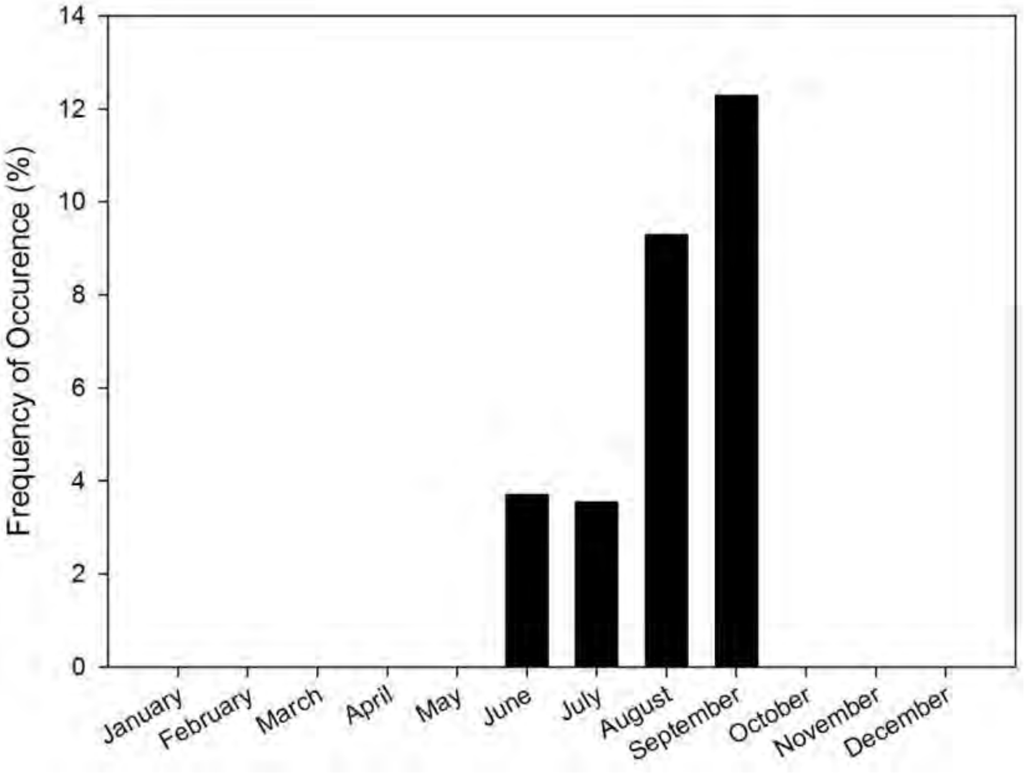


Figure 11. Monthly occurrence of *M. tarapacana* for all habitats combined

Dive operators data – Aggregation Dynamics

The data collected by the dive operators in 2012 and 2013 consisted of 510 dives which show that the aggregations occur mainly at seamounts. However, there were substantial differences in the number of individuals sighted between sites. Princesa Alice bank supported an average of 14.5 individuals per dive, three times higher than that of Ambrósio reef and much higher than the two sites at Formigas bank (Formigas islets and or Dollabarat reef), where less than one individual per dive was sighted (Figure 12).

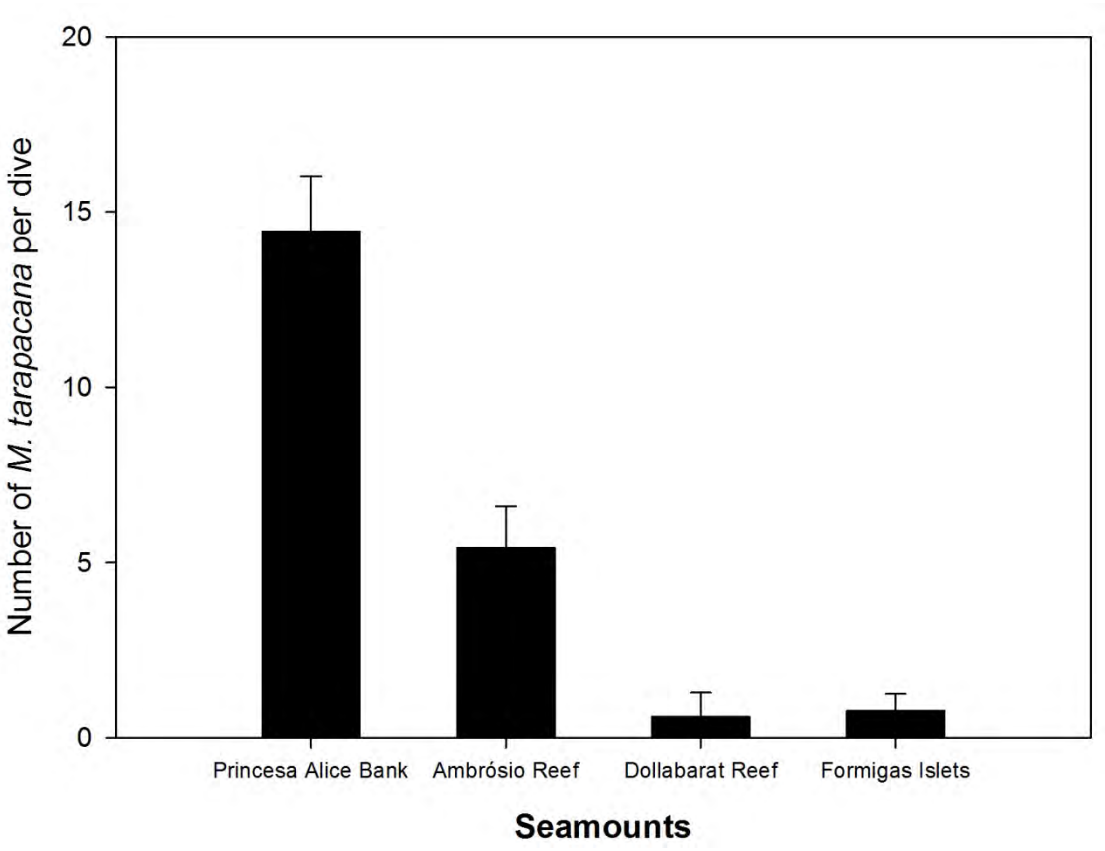


Figure 12. Average relative abundance of *M. tarapacana* (dive operators data) for the main aggregation sites (with 95% confidence interval bars)

When joining both years together (2012 and 2013), there was significantly more mobulas sighted in Princesa Alice Bank in comparison to Ambrósio reef (Mann-Whitney U Statistic= 10000; $p<0.05$). The mean temperature in Princesa Alice Bank during the summer months (22.5 ±0.13ºC) was also significantly higher than in Ambrósio Reef (21 ±0.13ºC; Mann-Whitney U Statistic= 871; $p<0.05$). However, in 2012, when SST was not different between both sites (Mann-Whitney U Statistic= 265; $p=0.151$), there were no differences in the mean number of individuals sighted per dive at both sites (Mann-Whitney U Statistic= 689; $p=0.359$). The situation changed in 2013, when the average SST in Princesa Alice was higher than at Ambrósio Reef (Mann-Whitney U Statistic= 162; $p<0.05$). Accordingly, the mean number of mobulas in Princesa Alice Bank was also significantly higher (Mann-Whitney U Statistic= 1025; $p<0.05$).

In June 2012, there was only data for one dive in Ambrósio Reef (no sightings) and two for Princesa Alice Bank (one individual). In July 2012 there was data of only five dives in Ambrósio and 28 in Princesa Alice. Despite this, the mean of individuals sighted was higher for Ambrósio than for Princesa Alice. August had data from 15 dives for Ambrósio and 41 for Princesa Alice and, the mean of individuals was higher in Princesa Alice. The sampling during September was quite unbalanced with just two dives in Ambrósio against 39 in Princesa Alice. However, as it happened in July, the mean of individuals was higher in Ambrósio than in Princesa Alice.

The temperature was higher in Princesa Alice across these months with a difference of c. 2°C in July, August and September. For June, the mean temperature was considerably lower than for the following months, although its validity is questionable as it is based on three dives alone.

In 2013 there was no data available for the month of June. The mean of individuals was higher in Princesa Alice from July to September. In July, the number of dives was the same for both aggregation sites (36 dives) and the temperature was slightly higher in Ambrósio. August had data of 47 dives in Ambrósio and 38 in Princesa. The month of September had data of 33 dives in Princesa Alice and 17 in Ambrósio. The temperature in August and September was almost the same for both sites.

For both years, the average number of individuals was higher in Princesa Alice, except for June when there is only data from 2012. Concerning the two years, the temperature was higher in Princesa Alice but the highest difference was of c. 1°C in August. The monthly data for both years is represented in Figure 13. The statistically significant differences between Ambrósio and Princesa in the overall of the two years are represented through Notched Box-plots in Appendix VI.

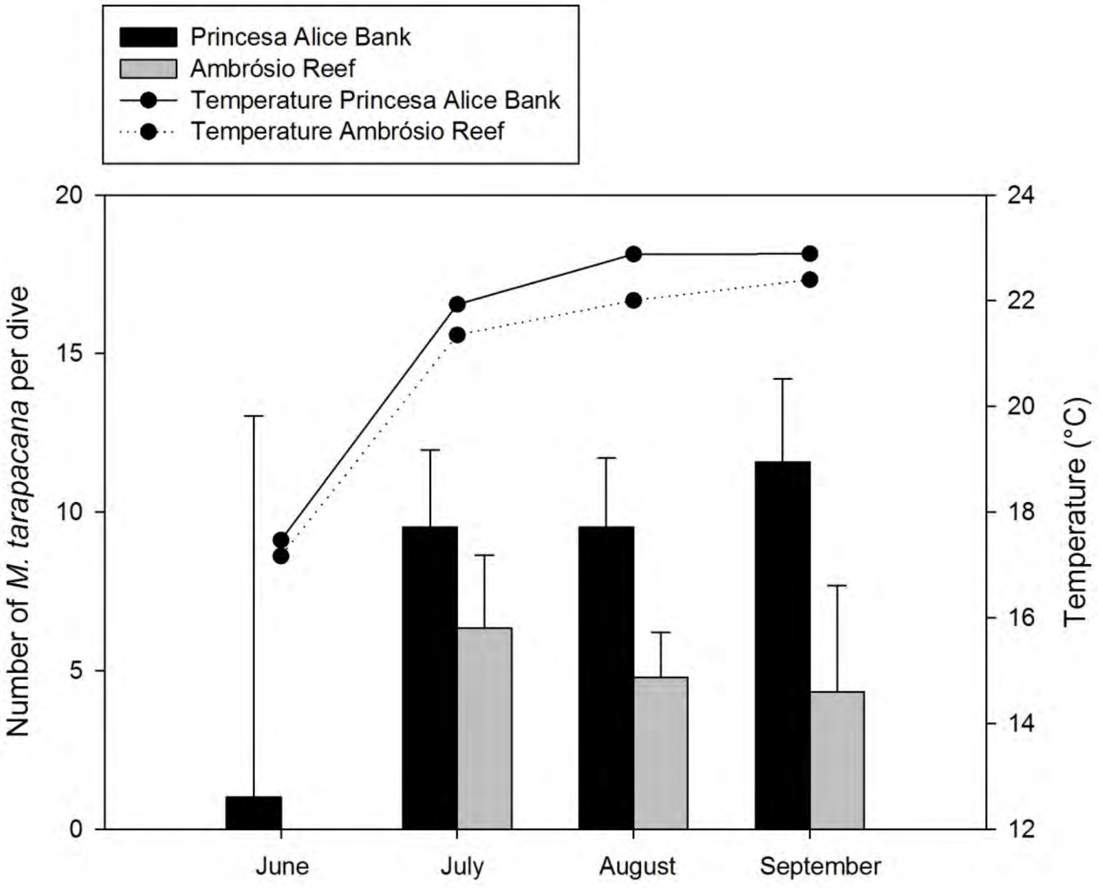


Figure 13. Comparison between the mean of individuals of *M. tarapacana* per dive and the mean temperature (°C) in June, July, August and September of 2012/2013

Demography and Behaviours

Various different behaviours of Mobulids could be distinguished from the photos and videos analyzed. These were: interaction with divers, feeding behaviour, inter-specific interactions between *M. tarapacana* and *M. birostris* and also a behaviour that consisted of males chasing a female that might constitute a putative courtship behaviour of *M. tarapacana*, which is not yet described.

Interaction with divers was considered when the animals got close to divers or to a particular diver, sometimes surrounding them or just swimming and staying in the area. Feeding behaviour was recognized when the animals were swimming with the cephalic lobes unrolled or rushing into bait balls [Figure 14(1,2)]. Inter-specific interactions between individuals were considered when there were two or more individuals of different species following or surrounding each other's [Figure 14(3,4)]. The putative courtship behaviour of *M. tarapacana* was recorded in video. The observed behaviour consisted of a "mating train" were several males following a single female mimicked her moves in a series of flips and turns and, after that, one of those males started chasing the female really close and they both increased greatly the swimming speed. The male was positioned over the dorsal surface of the female and both were really close together. Both individuals had bruises from abrasions which could be related with mating behaviour. The actual copulation was not observed perhaps because this behaviour was performed really close to the divers and a sudden stop in the chasing of the female happened after the animals got really close, at that time the swimming speed decreased but the male continued following the female around. Mating trains were observed in several other dives but the chasing was only recorded this one time [Figure 14(5,6)].

Figure 14. Photos representing different behaviours of Mobulid rays in the Azores region. (1,2) Feeding behaviour; (3,4) Inter-specific interactions; (5,6) Putative courtship behaviour (snapshots from video)

The photographic database documents faunal associations of both *M. birostris* and *M. tarapacana* with *Remora* spp., juvenile *Seriola* spp. and pilot fish (*Naucrates ductor*) [Figure 15(1,2)]. The most common faunal association is with *Remora* spp., with the majority of mobulid individuals carrying at least one individual.

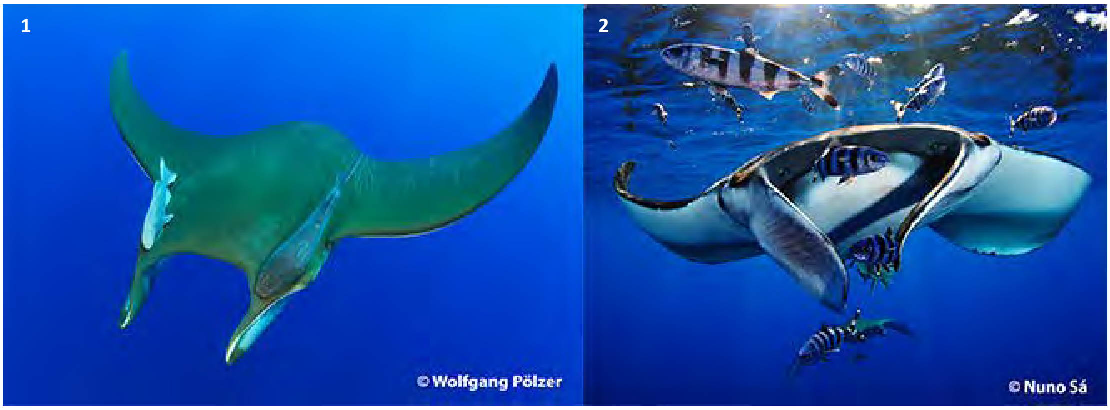


Figure 15. Underwater photos showing commonly seen faunal associations of Mobulid rays in the Azores: (1) *Remora* spp. on *Mobula tarapacana* and (2) pilot fish with *Manta birostris* (*Naucrates ductor*)

In all the photos analyzed were it was possible to identify the sex of the individuals and assess their sexual maturity there was only one record of a sexual immature male, an individual of *M. birostris*. All of the identified *M. tarapacana* males were mature individuals, as indicated by the size of the claspers, larger than the pelvic fins [Figure 16 (1)]. Regarding females, it was not possible to access their maturity except for the cases when they were seemingly pregnant. Indeed, there seem to be females in different gestation states recorded in the photographic database, as indicated by the different belly protrusion and proportion to the individual's size [Figure 16 (2)]. Most importantly, all *M. tarapacana* sightings during the course of the 14 year UVC program and reported by the dive operators, correspond to individuals that are, at least, 2 m disk width (DW), the majority being around 2.5 m DW or larger. This size is indicative of mature individuals, considering the few biological evidences known for this species (Notarbartolo-di-Sciara, 1988; Clark, *et al*., 2006*a*).

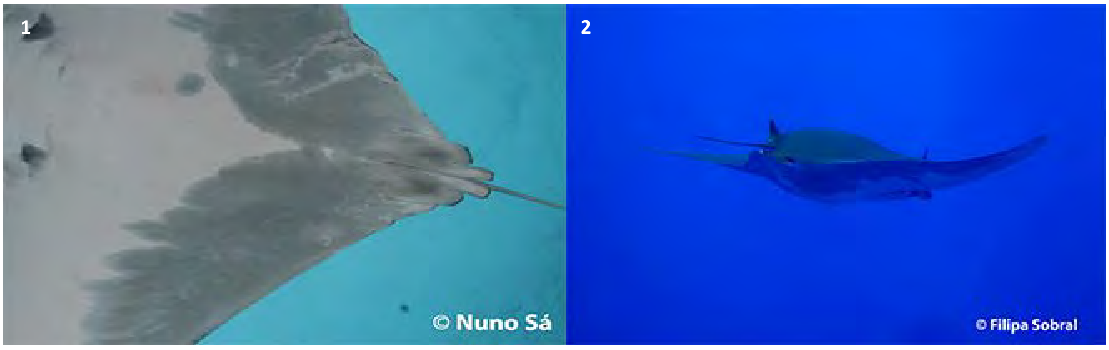


Figure 16. Photos representing a (1) Mature male; (2) Pregnant female in what seems to be a final stage of gestation

Temperatures experienced by M. tarapacana

The diving data and telemetry data are complementary and both peaks gave an optimal temperature range for this species, which goes from 22.5°C to 24.5°C (Figure 17).

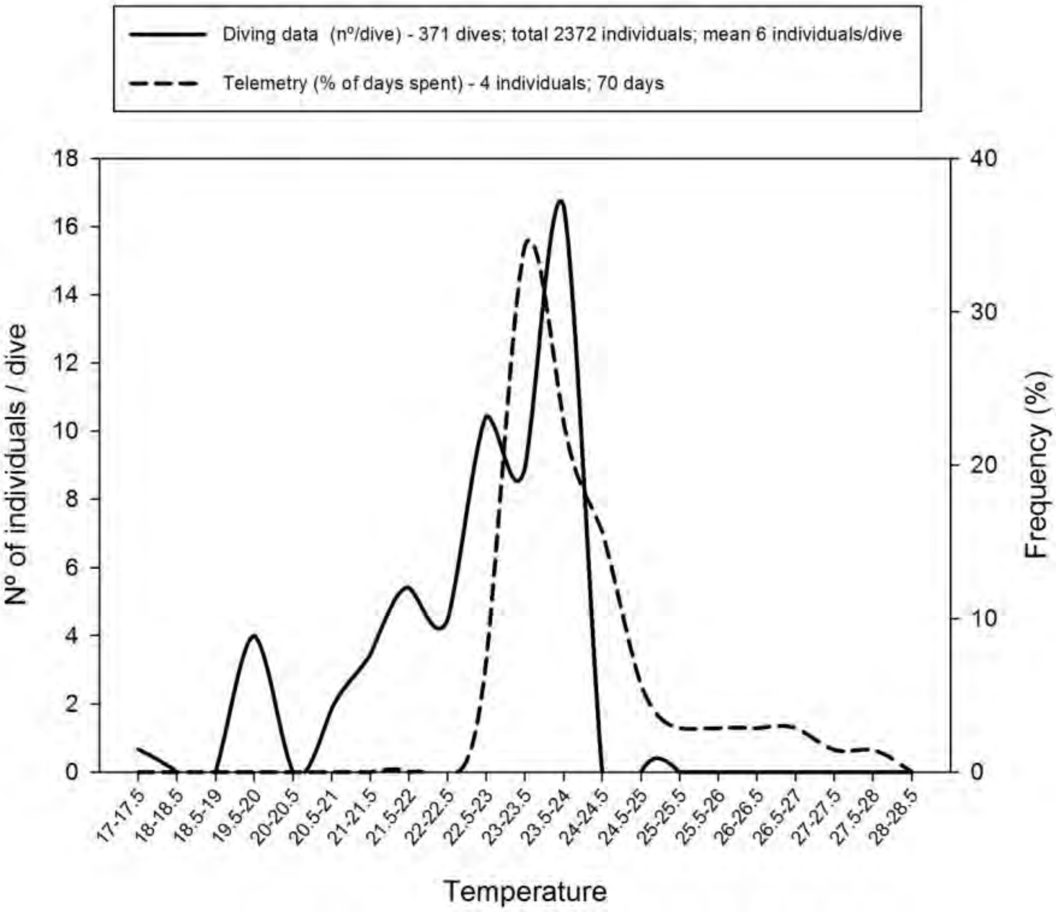


Figure 17. Optimal temperature range obtained by combining SST data from dives and SST data from satellite telemetry. Diving data consisted of a total of 371 dives in which the no. of individuals per dive was estimated for each temperature (°C) interval. The telemetry data consisted of information of 70 days, from four tagged individuals in which the percentage of days spent at each temperature (°C) interval was calculated

Discussion

This is the first study to provide detailed information on the distribution and habitat use of Mobulids in the archipelago of the Azores, and for *M. tarapacana* anywhere across its circumglobal distribution. It was demonstrated that this species seems to prefer the shallow banks of the archipelago as aggregation sites. This finding is of clear ecological and conservation significance and contributes to the growing body of evidence that Mobulids visit remote seamounts and oceanic archipelagos during the course of their large migrations (Marshall *et al.*, 2009, 2011*b*).

Data representativeness and limitations

Although personal and anecdotal information obtained from fishers' and dive operators clearly suggest that mobulids are only present in Azorean waters between June and October, it is not possible to exclude that possibility. The data used for seasonality assessment was obtained from a study oriented to coastal habitats, with unequally sampling at the different habitat types across time of year and years. Specifically, UVCs were conducted at coastal and offshore reefs in every month of the year whereas in offshore seamounts, where one would expect mobulas to be more easily found, they were essentially restricted to the summer months (June to September). Also, general effort was much higher during summer months reflecting the experimental design of the monitoring program. That mainly has to do with the fact that visiting these remote places requires good sea conditions.

The data used to understand the aggregation dynamics of this species was entirely collected by the dive operators, which has different limitations such has the time range of the data, the sites sampled and the amount and quality of data collected. In the first year of the study there was less data than in the second year, mainly because in the second year there was more dive operators collaborating, especially in Santa Maria island where there was only one operator in the first year and three in the second. Also, the companies that had already collaborated in the first year were more familiarized with the sighting sheets and with how to collect the data which leads to the assumption that in the future there will be more data available and more complete.

The dive operators have the season for mobula diving well defined, especially in the central group (July to September) were the aggregation sites are more remote and they do not want to risk a trip with a chance of encountering no mobulas. This is why we have very few data for the month of June and October, which would be crucial to understand the gradual increase in number of individuals in the beginning of the season as well as the decrease towards the end.

The UVC data showed that shallow banks are the preferable habitat type for aggregations of *M. tarapacana*. Princesa Alice bank, Ambrósio reef, Formigas islets and Dollabarat reef were considered the main aggregation sites in the archipelago but it is important to have in mind that these are the places which are most visited by the dive operators. It is possible that other aggregation sites exist and remain unknown or that are not visited as much as the ones mentioned before. Princesa Alice bank and Ambrósio reef were considered the largest aggregations of the archipelago with Princesa Alice having the highest overall mean of individuals in the total of both years. However, data of larger time windows is needed to understand if there are fluctuations between years.

With these limitations in consideration, it is possible to discuss the spatial and temporal patterns of mobulid occurrence in Azorean habitats, and in particular the aggregation of *M. tarapacana* at seamounts.

Mobulid habitat preferences and seasonality in the Azores

Shallow banks are seamounts where the summit reaches depths <100 m or even breaks the surface. Shallow banks can be important aggregation sites for highly migratory pelagic species, but their role in aggregating those species is largely unknown (Morato *et al.*, 2008, 2010). The importance of seamounts for visiting large pelagic organisms is poorly understood, mainly because drivers of spatial distribution and habitat selection in large highly migratory marine species are often hard to clarify (Jaine *et al.*, 2012). Seamounts generate characteristic conditions such as increased vertical flux of nutrient and retention of water masses that promote local productivity and support the higher trophic levels that feed upon it (White *et al.* 2007; Morato *et al.*2008).

They also may have distinctive “magnetic signatures” that might be used as cues to navigate and stop in the migrations of numerous pelagic species (Pitcher & Bulman, 2007; Morato *et al.*, 2009). It is most likely that the explanation for these aggregations lies in the combination of factors that makes seamounts ideal for these animals to aggregate (Morato *et al.*, 2010).

Essential Fish Habitat and aggregations

In any case, it is clear that adults of this species aggregate at these particular locations in much higher numbers than at other sites, during which they have increased chances of social interaction. The fact that the majority of M. *tarapacana* individuals seen in these aggregations in the Azores are adults, including numerous pregnant females, indicate that these sites may well play a role as mating and/or pupping grounds for this species. The observation of a putative mating behaviour supports the first hypothesis, whereas the consistently inexistent records of small juveniles contradict the second hypothesis. This mating behaviour described in this study is consistent with the already described courtship and mating behaviour of the *Manta* genus (Yano *et al*., 1999; Marshall & Bennett, 2010). Evident mating scars were not recorded but different stages of gestation are present in the database. Feeding behaviour was also observed at multiple occasions, indicating that the rays do use local feeding resources when at aggregations. These findings point to the role of these areas as EFH for the north Atlantic population of this species, and deserves further investigation.

It seems that there is a gradual increase in the number of individuals at the aggregations in the beginning of the season and a gradual decrease towards the end. This finding indicates that there are individuals arriving earlier and individuals leaving later. The increase and decrease of water temperature, day length or prey abundance may all be factors explaining such temporal dynamics in aggregation.

Even though NASA collects satellite observations continuously, the SST satellite dataset is not intended for near-real-time use. There is a delay before data is available, due to the calibration of the satellite measurements to in-situ measurements. For that reason it was impossible to obtain SST data for most of the month of September 2013, leaving the dataset with a gap in one of the warmer months which would have been important to understand if there is a correlation between the temperature and the abundance of individuals. Data of SST was compared with data of abundance and in the overall of both years, there was significantly more mobulas in Princesa Alice Bank in comparison to Ambrósio and interestingly, the temperature at Princesa Alice Bank was also higher than at Ambrósio. However, the obtained data is not sufficient to affirm with certainty that temperature influences the presence/abundance of *M. tarapacana at the sites,* as many other factors are important and can't be discarded.

Comparing SST data from diving to SST data from satellite telemetry it seems that *M. tarapacana* has a narrow SST optimal range. However, even though this optimal range is only a small band of averaged temperatures, they are not exclusive and the animals might frequent temperatures outside this range.

Therefore, it is important to have in mind that the possible effects of temperature and season, as well as other factors, are inevitably related. Previous investigations on how environmental variables affect the sightings of planktivorous elasmobranchs have focused primarily on temperature. In the Indian Ocean, SST was found to be the most suitable predictor of whale shark habitat while surface chlorophyll-a concentrations were less reliable. Chlorophyll-a appears to be a poor proxy for zooplankton availability because the trophic links between the two are not necessarily direct or temporally and spatially synchronized (Sequeira *et al*., 2012). The sightings of filter-feeding elasmobranchs are strongly related to environmental variables, as the distribution and abundance of zooplankton respond quickly to environmental changes. Also, large-scale factors influencing oceanographic processes have been linked to shifts in abundance and distribution of planktivorous elasmobranchs (Rohner *et al.*, 2013).

The present study is the first to provide information on different ecology aspects of mobulids in the Azores. Taken as a whole, it is crucial to continue to gather data on these species to understand the drivers of these aggregations and to determine whether observed fluctuations in sightings correspond to short-term environmental variability or whether they represent long-term trends. For that, an improved collaboration with the dive operators is necessary in order to have more and better data. Since the dive industry directed to mobulas is recent in the Azores, and is growing fast, the fact that this kind of collaboration is already starting is a great opportunity to start a long-term data gathering program that would allow to better understand this understudied species and a possible early identification of changes in populations and abundance.

These aggregations provide great opportunities for scientists to study these animals in the wild through genetic studies, tagging programs, dietary analyses, and behavioural studies and also to test new and non-invasive techniques (e.g. Photo-identification) as a complement of other studies. Collaboration between scientists of different parts of the world where aggregations occur is also vital for a combined effort to understand the migrations, movement patterns and role of aggregations sites for *M. tarapacana*.

Chapter 4. Photo-ID of *M. tarapacana*

Introduction

Photo-ID is a cost-effective and non-invasive technique widely used for the long-term studies of animal populations (demography) and individual behaviours. Photo-ID consists in the use of photographs of marks or patterns that are unique to each individual and that remain unchanged throughout its lifetime (Couturier *et al.*, 2012; Marshall & Pierce, 2012). These marks and patterns can be used as fingerprints to ID different animals, i.e., photo-ID is used as a mark-recapture technique.

Photo-ID research is frequently supported by eco-tourism operators and the dive community and therefore increases awareness and environmental education (Couturier *et al.*, 2011). Photo-ID has been used to study the biology and ecology of different elasmobranchs (Castro & Rosa, 2005; Domeier & Nasby-Lycas, 2007; Bansemer & Bennett, 2008; Dudgeon *et al.*, 2008) and recently, of *M. alfredi* and *M. birostris* (Couturier et al., 2011; Deakos et al., 2011; Marshall et al., 2011*a*). There is already a global database for the photo-identification of both species of mantas based on the spotted pattern they have on their underside. However, there is no similar project for any of the species of Mobula, mainly because *M. tarapacana* is the only species of mobula to show a distinct color pattern on the ventral area (Notarbartolo-di-Sciara, 1987), which might be used to photo-ID different individuals of this species. However, it is not yet clear that the markings of *M. tarapacana* are present from birth and constant through time, as it is the case for *M. alfredi*, which has been identified after more than 30 years.

Improved biological information as well as life-history traits are urgently needed for all mobulid species (Camhi *et al*., 2008). This photo-ID database is essential to learn more about the spatial ecology of these highly migratory animals and the threats they may face, in particular to learn where, when and why these animals aggregate at particular areas in the Atlantic, such as the devil ray diving hotspots in Azorean seamounts. Photos will help track individual animals when they visit these aggregations.

The larger and longer the photo-ID database, the greater the chances of tracking a returning animal. Such efforts can only be successful in delivering relevant data on a long-term basis and with significant efforts by the public.

The objective of this work was to create a photographic database and to develop and test a photo-ID methodology for *M. tarapacana*. It is part of a wider effort to implement a long-term program for the study of the ecology and conservation of Mobulid rays in the Azores and the northeast Atlantic.

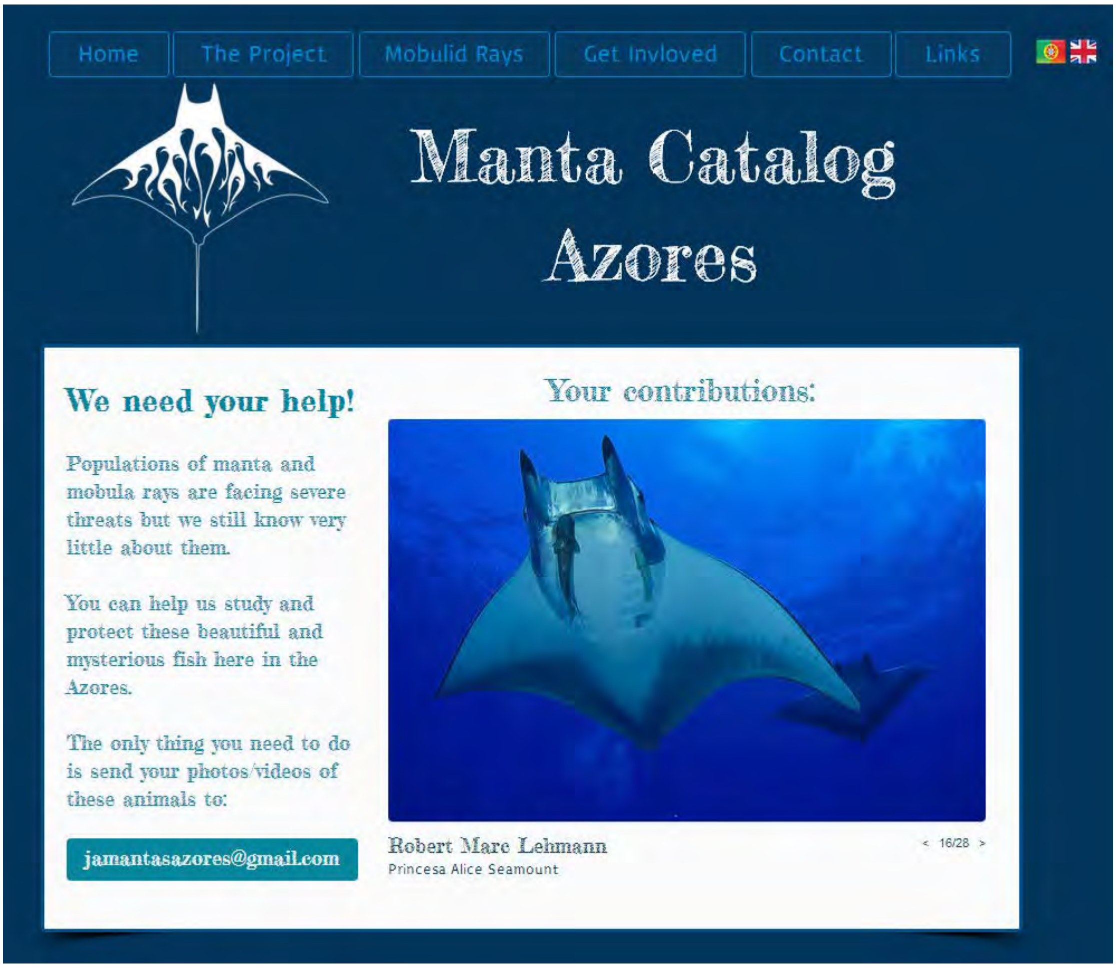
Methods

Manta Catalog Azores Project

Photographic database

The project "Manta Catalog Azores" was created in order to voluntarily collect photos and videos from divers, photographers and dive operators from Azores, mainly operating from the islands of Faial, Pico and Santa Maria. Divers were contacted by e-mail and personally at the dive centers through briefings. Posters briefly informing about the project and data needs were posted in the collaborating dive operators. The pictures were mainly taken at Princesa Alice bank, Formigas Islets and Dollabarat reef and Ambrósio reef which are the places where there is a diving operation directed to the Mobulas and where *M. tarapacana* aggregates in larger numbers (Chapter 3). The photos deposited at the image database of the Department of Oceanography and Fisheries of the University of the Azores (ImagDop), mostly also from these sites, were also analyzed. A photographic database of mobulid rays from the Azores was then created containing all the photos and videos collected, comprising in a total of 2252 photos and 188 videos from 1990 to 2013.

The larger the photo-ID database, the larger the chances of getting a returning individual. With that in mind and with the goal of getting a wide number of national and international divers to know the project and send photos, as well as having the general public to know more about these species, a website was created (Figure 18). The website has information about the project and its goals, about mobulid rays in general, on how to differentiate between species and on what kind of photo can be used for photo-ID and thus should be submitted to the database.



Manta Catalog Azores

We need your help!

Populations of manta and mobula rays are facing severe threats but we still know very little about them.

You can help us study and protect these beautiful and mysterious fish here in the Azores.

The only thing you need to do is send your photos/videos of these animals to:

jamantasazores@gmail.com

Your contributions:

Robert Marc Lehmann
Princesa Alice Seamount

Figure 18. Homepage of the "Manta Catalog Azores" project website (http://mantacatalogazores.wix.com/mobulaid)

Photo-ID software

Photo-ID was never done for *M. tarapacana*, which means that there is no specific software for the particular color pattern of this species. The pattern of these animals is basically a contour between a grey and white area in their ventral side. With that in mind, to understand if any already existing photo-ID software could be used to compare photos of this species a few different ones were tested. The software's used were directed to the analysis of cetacean flukes contours with the exception of one that is used to identify great white sharks from the contour between the grey and white area on the sides of their bodies. The software's that allowed the analysis to go further and actually enabled the comparison between different pictures were: Europhlukes and I^{3}S Contour.

Results

An ideal photo for photo-ID of M. tarapacana

After the analysis of the photographic database it was possible to conclude which would be the best standard ID area and also the requirements to take a good ID photo:

- The photo should be taken as perpendicular to the animal as possible. Angles may cause problems due to paralaxis if the photos are to be compared using a software;
- The standard area goes from the base of the cephalic lobes to the pelvic area, so it would be possible to ID the individual and also to know its sex as well as to assess its maturity. The standard ID area is represented in Figure 19;
- The animal should have its wings stretched so the complete pattern can be visible in the photo;
- The standard ID area does not encompass the tips of the wings because the pattern starts to blur in that area
- If the individual as any identifiable marks, scars or deformities those should also be photographed.

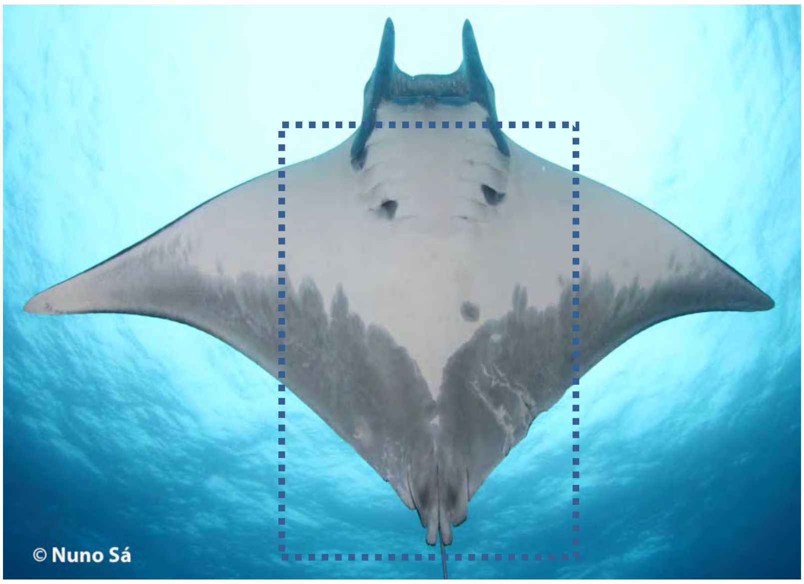


Figure 19. Example of ideal photo to be used for photo-identification of M. tarapacana individuals. The standard area considered for the photo-Id of this species is bounded by the blue dashed line

Matching through the use of softwares

I^3S Contour

The I^3S (Interactive Individual Identification System) Contour function uses the contour of flukes, dorsal fins or lining along the side of individuals for identification. This software has the possibility of automatically recognizing the pattern as well as to do it manually (Figure 20). I^3S has a tracing algorithm which tries to find the contour between the points selected by the user. The contours are analyzed as histograms which are used by the software to match one processed image with the other images in the identification database and then shows a ranked list of potential matches. The more the histograms overlap the better the match, which is given in the form of a score. The best possible score is zero or close to zero. The user is always required to make the final visual match between the unknown image and an image from the identification database.

A great advantage of this software is that it supports three database types: Whale tail (When two contours are simultaneously visible), Dorsal fin (For body parts which have a left and a right view such as a dorsal fin) and Generic (Suitable for any animal with a single contour per image which can only be viewed from a single side, such as the contour between the dark and the light half of a great white shark). Further, it allows the user to add other metadata fields of identification (e.g. marks, scars, sex, maturity, etc) which will, later, help refine the search within the database.

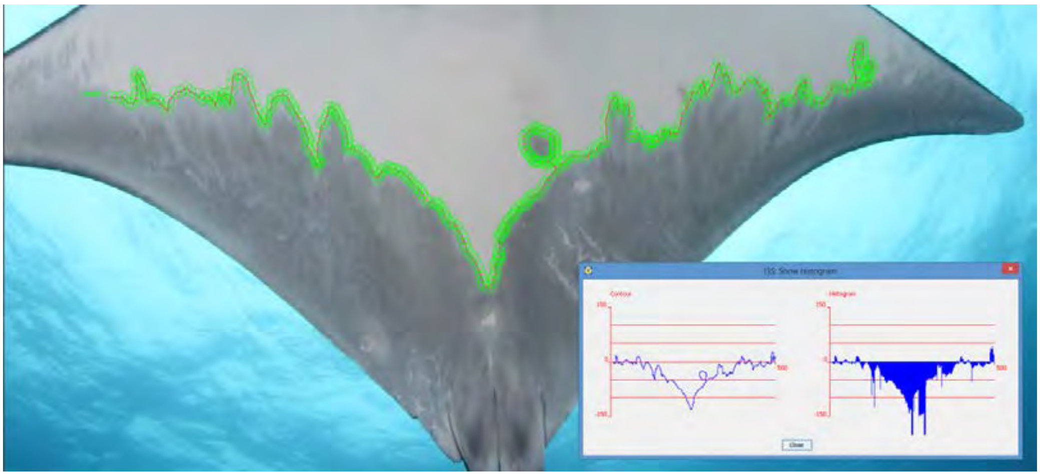
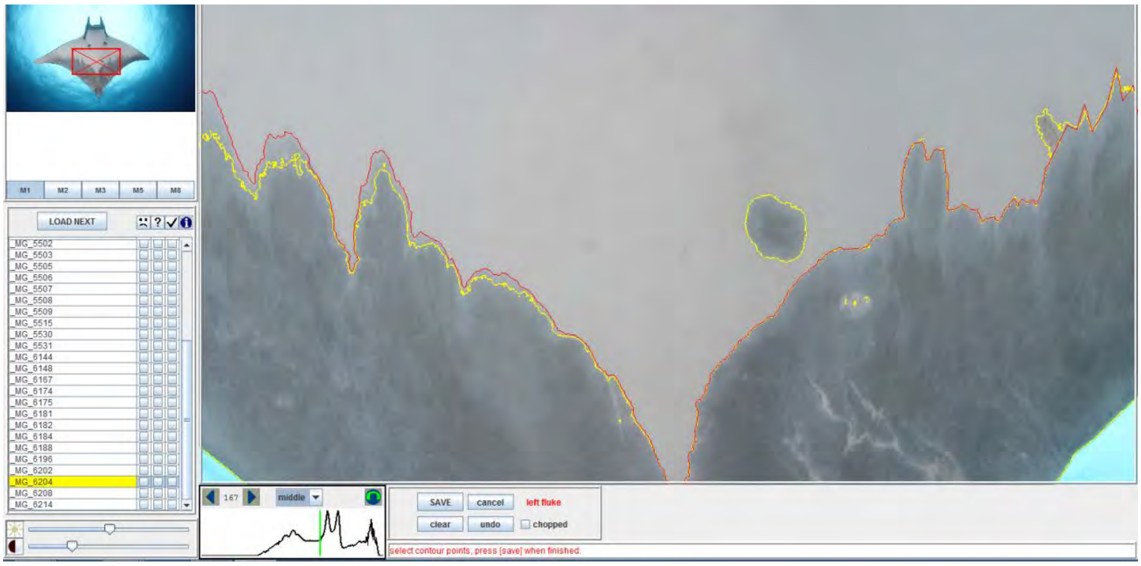


Figure 20. Processed image of an individual of *M. tarapacana* using I^3S software with corresponding contour and histogram

Europhlukes

This software was specifically designed for the study of sperm whales using the trailing edge of the fluke as an identifying feature. A great advantage of this software is that it allows to use the automatic and manual recognition of the contour combined (Figure 21). However, since it is directed to sperm whales flukes the contour has to be done for the left and right sides separately. Once the photograph is processed, it is compared against those already in the database and the program provides an ordinal list of the best possible matches. This software uses a matching algorithm which computes a match coefficient (R-value) for each comparison, ranging from 0 to 1. The photographs with the highest R-values wil rank at the top of the list., and the researcher has to visually check the proposed matches. A true match will always be ranked near the top of the R-value list. If there is no match, most R-values are small. The R-value advised for visual checking is ≥0.6 (Beekmans *et al*., 2005).



select contour points; press [save] when finished.

Figure 21. Processed image of an individual of *M. tarapacana* using Europhlukes software. The contour automatically recognized by the software is represented in yellow and the manually selected contour represented in red

Photographic database

All the photos received went through a first selection to ensure that the photos present in the database were only from specimens of the family Mobulidae. A total 2252 photos taken across 14 years were present in the database. From those 2252 photos, 2125 were from M. tarapacana specimens (94.4%) and 128 from M. birostris (5.7%). A second selection was then made to include only photos suitable for photo-ID resulting in a total of 601 photos (26.7%). Those 601 photos comprised 259 (43.1%) considered to be of medium quality (quality of a non-professional camera with no artificial light, but in which the pattern is identifiable. This quality might not be enough to be recognized by a software) and 342 (56.9%) considered of good quality (photos taken with artificial light, professional cameras or when the quality is enough for the pattern to be perfectly visible and to be recognizable by a software).

The 188 videos received comprising a total of 7 hours of video, were also analyzed.

Discussion

Choosing a standardized ID area and specifying what is the ideal photo for the identification of individual M. tarapacana individuals is crucial to improve the number and quality of the photos received for this purpose. Until the Manta Catalog Azores project was created, in 2012, there was no objective reason for divers to take such specific photos, so the great majority of the photos received from previous years were not suitable for photo-ID. Also, is important to have in mind that digital photography is quite recent and only more recently started to be affordable for the general public. The photos received from 1990 to 2006 where from digitalized slides and the quality of most of them was not suitable to use for individual identification. From 2007 onward there are already digital pictures and some of them were considered of medium quality for identification purposes. As technology evolves the quality of the photos gets better so the best quality photos received were from 2010 to the present. This technology is now much more accessible to the wider public. Therefore, it could be expected that the amount of photos received would be higher, especially after the project started. Having in consideration that between 2012 and 2013 a minimum of 447 dives with a mean of eight divers were made, in the dive sites from which we received photos, the predictable number of photos received would be much higher than 286.

The main reason for this discrepancy may be that the project did not get the necessary visibility and publicity to enjoy from a massive participation from the dive community. The fact that the website did not have the possibility for the divers to directly upload their photos and thus feel more involved in the process is also a disadvantage and deserves improvement. It is also important to consider the difficulties of the photo-ID for this species: the artificial light is ideal to get a high quality photo and to counteract the backlight when photographing from the underside of the individuals. On the other hand, the use of artificial light most certainly constitutes a factor of disturbance for the animals; the fact that these animals aggregate at offshore sites where the currents are often strong and the divers have to be holding to a cable is limiting in terms of freedom of movements to photograph. In the future It is necessary to improve the outreach of the project website and publicity.

Long term stability of individual markings has not yet been demonstrated for this species. Photos of fetus of this species taken in a market in Sri Lanka were analyzed with the purpose to understand if the pattern is present at birth. However, it wasn't conclusive because the photographed individuals were already dead for a few weeks before being photographed and the pattern was too faded (Daniel Fernando, pers. comm. 2013). This is a vital question to clarify in the future, since the long term stability of the markings is one of the most controversial and criticized points about photo-ID (Marshall & Pierce, 2012).

Regarding the tested software's, in *I^3S Contour* the generic type database was used since it allowed to do a single contour and also because it is was proven in a similar fashion with Great White Sharks (*Carcharodon carcharias*) (den Hartog & Reijns, 2011). For this species, the automatic recognizing tool did not work because the contrast between the grey and white of the pattern was not enough for the software to detect. Therefore, the contour had to be done manually point by point. Besides of being extremely time consuming that method ended up not enabling a positive and reliable match. Since the contour had to be done point by point, it induced a great source of error in the matching process because it was impossible to select the exact same point twice even when processing the same picture. That means that the match would also be affected by the quality of the image, position of the animal, etc. Having this, the lowest score obtained between photos of the same individual was 13490, when in other species the usual score for different individuals is around 1000 (Bruno Macena, pers. comm. 2013). This led to the conclusion that this software was not reliable for the identification of the kind of pattern present in this species.

On the other hand, *Europhlukes* was able to do a better automatic detection of the contour of the pattern, having to be adjusted manually only in some areas depending on the quality of the photo, which showed that the contrast of the pattern was enough to be detected by this program. Comparing good quality photos of the same individual, R-values around 0.76 were obtained and visual comparison confirmed it were true matches. Thus, this software seems to be reliable for the photo-identification of *M. tarapacana*.

Yet, the development of a specific software directed to the photo-ID of *M. tarapacana* would be ideal and fundamental for the expansion of this project throughout this species distribution range, since it would speed up the matching process of a larger database.

Photo-ID creates opportunities for the public to become directly involved in projects like this one through the submission of their photos. This benefits researchers by increasing the quantity and geographical extent of available data and also the citizen scientists who are offered an educational experience. This research technique facilitates collaboration between researchers in different locations which is fundamental for the study of highly migratory species like mobulid rays.

Chapter 5. Conservation of Mobulids in the Azores

Introduction

Mobulid rays roam the world's oceans and cross international borders. This implies that efforts for their conservation, even regionally, must have in consideration the global scale of their distribution ranges and movements. The fact that their Their conservative life history traits, including one of the lowest know fecundities of all elasmobranchs (Couturier et al., 2012), confers them a low intrinsic rate of population increase (Dulvy *et al*., 2008) and makes them highly vulnerable to any kind of impact. The current lack of biological and ecological information prevents the assessment of the status and population trends of most mobulid species (Table 5). Objectively, assessment and management of these species is hindered by a complete lack of information on their stock status.

The giant manta ray (*M. birostris*) was listed under Appendix I and II in the Convention on Migratory Species of Wild Animals (CMS) on November, 2011. In March 2013, both species of mantas were protected under Appendix II of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), meaning that the trade for manta products must be controlled in order to insure a sustainable exploitation of the species. However, there are no mandatory international protection for any manta or mobula species.

Table 5. Current IUCN Conservation Status and population trends of mobulid species

Common Name	Scientific Name	IUCN Status	Population Trend
Chilean devil ray	*Mobula tarapacana*	Data Deficient (DD)	Unknown
Atlantic devil ray	*Mobula hypostoma*	Data Deficient (DD)	Unknown
Shortfin devil ray	*Mobula kuhlii*	Data Deficient (DD)	Decreasing
Pygmy devil ray	*Mobula eregoodootenkee*	Near Threatened (NT)	Unknown
Spinetail mobula	*Mobula japanica*	Near Threatened (NT)	Unknown
Smoothtail mobula	*Mobula munkiana*	Near Threatened (NT)	Unknown
Bentfin devil ray	*Mobula thurstoni*	Near Threatened (NT)	Unknown
Lesser Guinean devil ray	*Mobula rochebrunei*	Vulnerable (VU)	Unknown
Giant manta ray	*Manta birostris*	Vulnerable (VU)	Decreasing
Reef manta ray	*Manta alfredi*	Vulnerable (VU)	Decreasing
Giant devil ray	*Mobula mobular*	Endangered (EN)	Decreasing

Threats

Mobulids face several threats related to human activities throughout their distribution ranges, such as: directed fisheries, incidental capture as by-catch, habitat destruction, marine debris, boat strikes, entanglement and unregulated tourism (Heinrichs *et al.*, 2011; Couturier *et al.*, 2012). The photos collected during this study were vital to determine that these species face most of these threats in the Azores region as well (Figure 22). There were photos of different animals entangled in, and hooked by, fishing gear, dead animals brought to shore after being caught as by-catch, injuries caused by boat propellers and also plenty of photos of divers chasing and touching the rays.

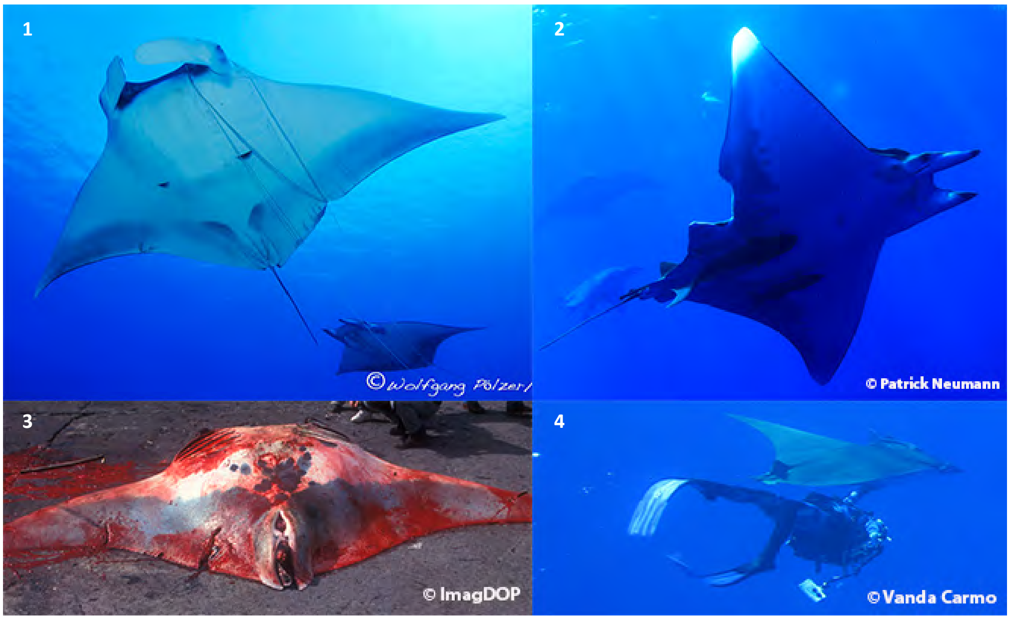


Figure 22. Photos representing threats to mobulid rays in the Azores region: (1) *M. birostris* individual entangled in fishing gear; (2) *M. tarapacana* injured by possible boat strike; (3) *M. birostris* specimen caught as by-catch; (4) Diver touching a *M. tarapacana*

Entanglement in fishing, mooring and anchor lines represents a problem since it can cause injuries and even amputations (e.g. of cephalic fins) which can be a source of mortality or affect the animals' fitness and ability to feed (Deakos et al., 2011; Heinrichs et al., 2011; Couturier et al., 2012). Also, if the animal gets trapped in these lines and is not able to move, it will die from suffocation given that elasmobranchs in general, including mobulids, need to be in near constant movement to promote the flow of water through the gills and therefore respiration.

Tourism can also pose threats, especially given that the aggregation sites became touristic attractions. High boat traffic, large numbers of people in the water and detrimental behavior of divers like chasing, touching or riding the animals may result in boat strikes and cause the animals excessive stress (Deakos et al., 2011; Heinrichs et al., 2011). Diving is a very important, rapidly expanding and high-yielding source of tourism income in the region. Mobula diving is one of the top attractions for dive tourism in the Azores nowadays. With such amount of economic impact, there is a considerable interest among dive operators in preserving these animals and their activity. Therefore, operators can be enrolled in the study and conservation of these species along with the recreational divers by providing information on behaviour and ecology, if properly organized by researchers. Dive operators can also raise divers' awareness and education on the importance of the conservation of these species and must inform divers of the possible problems of detrimental behaviours towards the animals.

Fisheries, both directed and incidental, are the biggest threat to manta and mobula rays (Heinrichs *et al*., 2011) worldwide. The high value of their gill rakers is driving a dramatic increase in the catches of these animals in some parts of the world (Heinrichs *et al*., 2011). Many of these species, especially the largest and more oceanic, (Manta spp., *M. mobular*, *M. japanica* and *M. tarapacana*), are also regularly caught in relatively large numbers as by-catch in pelagic longlines, purse seining and gillnet fisheries targeting tropical tuna and billfishes (Dulvy et al., 2008; Amandè et al., 2010) throughout their distribution ranges. The impact of by-catch on mobulid populations remains largely underestimated and unknown due to the fact that data on the by-catch of these animals is hardly ever recorded and when recorded is not classified by species. Since the current lack of basic information on these species poses limits to management and assessment of conservation status mobulids may face bigger threats than what is currently thought (Heinrichs *et al*., 2011). These species are occasionally caught in longline fisheries in the Atlantic Ocean (Cadenat, 1958; Rey & Munozchapulli, 1992). The Azores is no exception and mobulas and mantas are occasionally by-caught in the region, although in small numbers.

During an experiment to evaluate gear modification on rates of sea turtle by-catch in the swordfish longline fishery in the Azores, an individual of *M. mobular/japanica* was caught in 2003 (Figure 23) and an individual of *M. birostris* in 2004 (Marco Santos, pers. comm. 2013). These numbers correspond to a combined CPUE of 0.014 individuals/set (which typically cast around 1200-1500 hooks per day) of a single vessel. Additionally, this experiment took place in the months of September to December in both years, which is not the time of year were mobulids are more present in Azorean waters.

Figure 23. Photo of a mature male individual of *M. mobular/japanica* caught as by-catch in a longline vessel during an experiment to evaluate gear modification on rates of sea turtle by-catch in the swordfish longline fishery in the region of the Azores

On the other hand during the MADE project (Mitigating adverse ecological impacts of open ocean fisheries) a total of 134 sets were performed by three longline vessels in Azorean waters in 2008 and 2010 between July and October, which comprised the season of mobulid aggregation in the region, and no mobulid specimens were caught (Martins, 2013).

Having that, it is clear that a detailed assessment of the impact of the pelagic longline by-catch in the region is necessary.

Climate change can pose threats in the future primarily through ocean circulation and temperature changes (Chin et al., 2010). Chin et al. (2010), suggested that mobulids have low vulnerability to climate change, compared with other elasmobranchs, because they do not seem to have high habitat specificity or inflexibility to adaptation. However, as planktivors, it is probable that they are affected by the change in the abundance and distribution of their food sources as the oceans get warmer (Couturier et al., 2012).

Importantly, local and regional oceanographic conditions at seamounts, such as upwellings, currents and productivity, that may promote seasonal feeding aggregations, could be altered with climate change (Richardson, 2008) and possibly influence migration patterns (Chin et al., 2010) and seasonality. This scenario could have major impacts for the aggregation sites in the region.

Conservation measures in the Azores

This study shows that shallow seamounts are the preferable habitats for mobulids in the Azores region. The Princesa Alice bank and Ambrósio reef, and to a lesser extent the Formigas bank, represent the largest aggregation sites and, to the best of current knowledge, the most important specific habitats in the region for *M. tarapacana*. Together with the emerging results of telemetry studies (Pedro Afonso, pers. comm. 2013), it seems that Azorean seamounts play an important role as Essential Fish Habitat (EFH) in the annual migratory routes of these animals.

The Azores has been a leader in the development of offshore Marine Protected Areas (MPAs) (Santos *et al*., 2009), and as an outcome some of the Azorean seamounts have been declared as MPAs. One of these, designated by the Azores Regional Government in 1988, is the Formigas Islets and Dollabarat Reef MPA. In 2003, the area of this MPA was enlarged and the restrictions increased, and in 2005 it was nominated as an OSPAR MPA. All fishing activities are now banned within a large box encompassing all the seamount and its surrounding habitats, with exception of commercial pole-and-line fishing directed to tunas. Ambrósio reef was also constituted as a diving MPA in 2012 along with three other sites in Santa Maria island.

However, this MPA is very small, delimited by a circumference with a radius of 150 m where all fishing activities are prohibited with the exception of pole-and-line fishing to capture tuna and purse seine for the capture of life bait. Dive operators also need to inform the port authorities every time they intend to dive in this site so that only one boat at a time is present at the site. Each boat can only go once a day to Ambrósio and can only remain there for a maximum of 1.5 hours. Each boat can only carry a maximum of 10 divers including the dive masters/instructors.

The dive operators are obliged to report data from dives (e.g. date, site, no. of divers, species seen, abundance of individuals) annually, but there is no standardized form for that purpose. Even though these protections exist, a lack of oversight from the responsible entities has made possible that regulations are not always properly enforced. Furthermore, this study showed the importance of the seamount Princesa Alice, the largest seasonal aggregation of mobulids in the Azores, which is still not protected. It seems obvious that it would be important and urgent to evaluate the usefulness and implement a set of measures to protect this aggregation and avoid possible impacts. In this regard, information is needed on the economic importance of extractive vs. non-extractive activities taking place in this area so that a realistic set of management measures can be created. The high economic value of mobula diving, a non-extractive use, can act as an incentive to species conservation and to the implementation of an MPA, whether this is fixed or seasonal. However, the large home ranges and migratory behaviour of Mobulids imply that these animals cannot be protected only by means of small marine reserves and managed areas (Woodroffe & Ginsberg, 1998), highlighting the need for concerted international actions, for example within the context of Regional fisheries management organizations (RFMOs).

Proposed measures

The already existing regulations of MPAs should be reviewed and improved. There should be included specific measures regarding specific dive activities such as mobula diving, taking into account the characteristics of the target species. The establishment of codes of conduct for recreational diving activities (regulating operations and divers behaviour) as well as for research activities directed to these species should be created and implemented not only in the aggregation sites but for every encounter with these animals. All of these should be done in collaboration between the scientists, dive operators, fishermen and the Administration.

Data collection and monitoring are crucial for proper management measures to be taken. For that is important to:

- Prepare regional mobulid species identification guides
- Standardize data collection
- Implement training programs
- Implement data collection programs for dive operators

For the specific case of Princesa Alice seamount, an assessment of the socio-economic value of different activities is urgent so proper measures can be implemented. A few possibilities of measures to be taken regarding mobulid aggregations are:

- Fisheries restrictions at the aggregations during the aggregation periods (June to September);
- Management and regulation of tourism and research activities;
- Implementation of a permit system for diving and research activities (which imply the report of specific and standardized data)
- Application of a maximum boat and divers quota system

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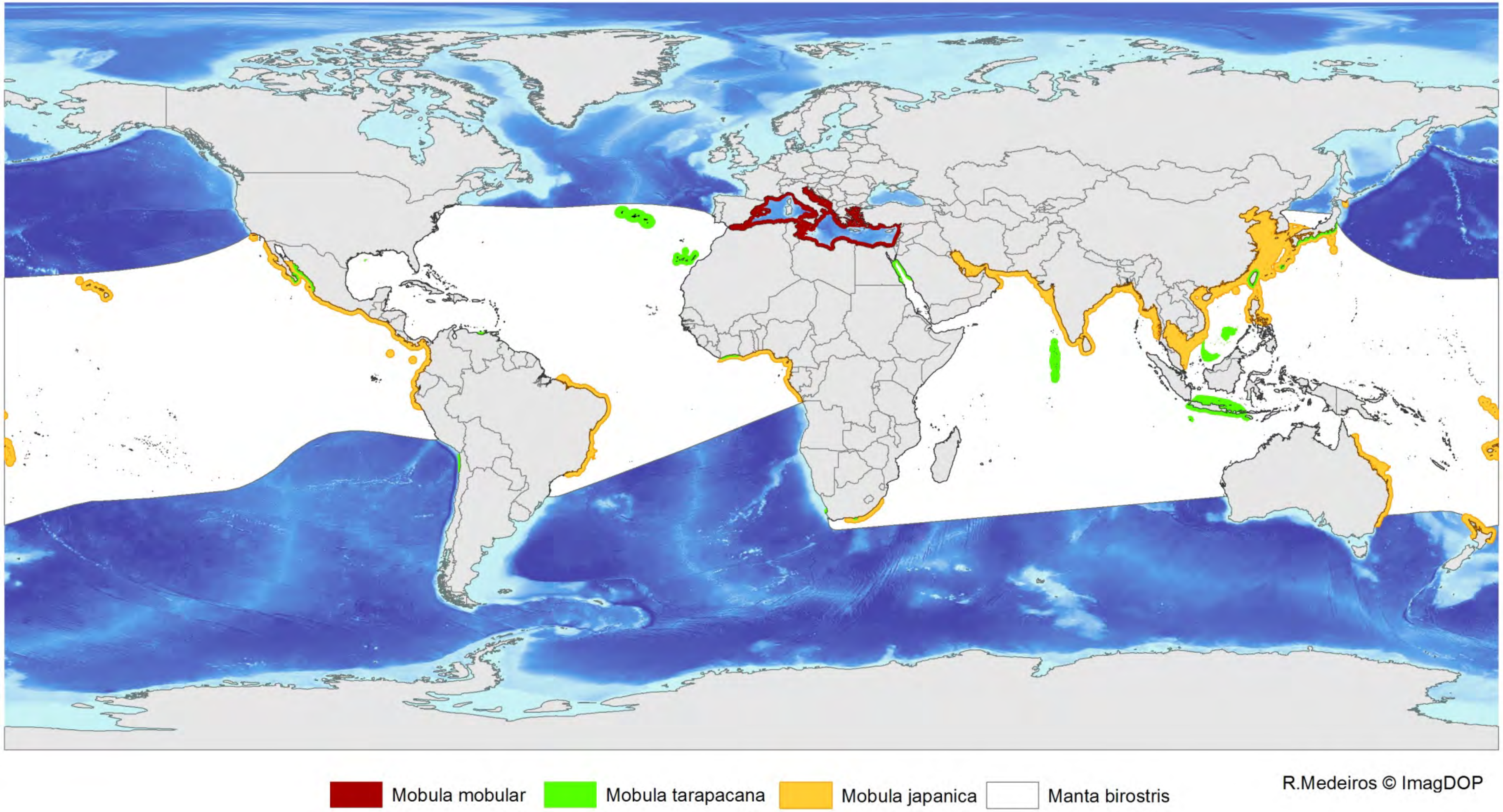
Appendix I - Map of the distribution ranges of *M. tarapacana*, *M. mobular*, *M. japanica* and *M. birostris*

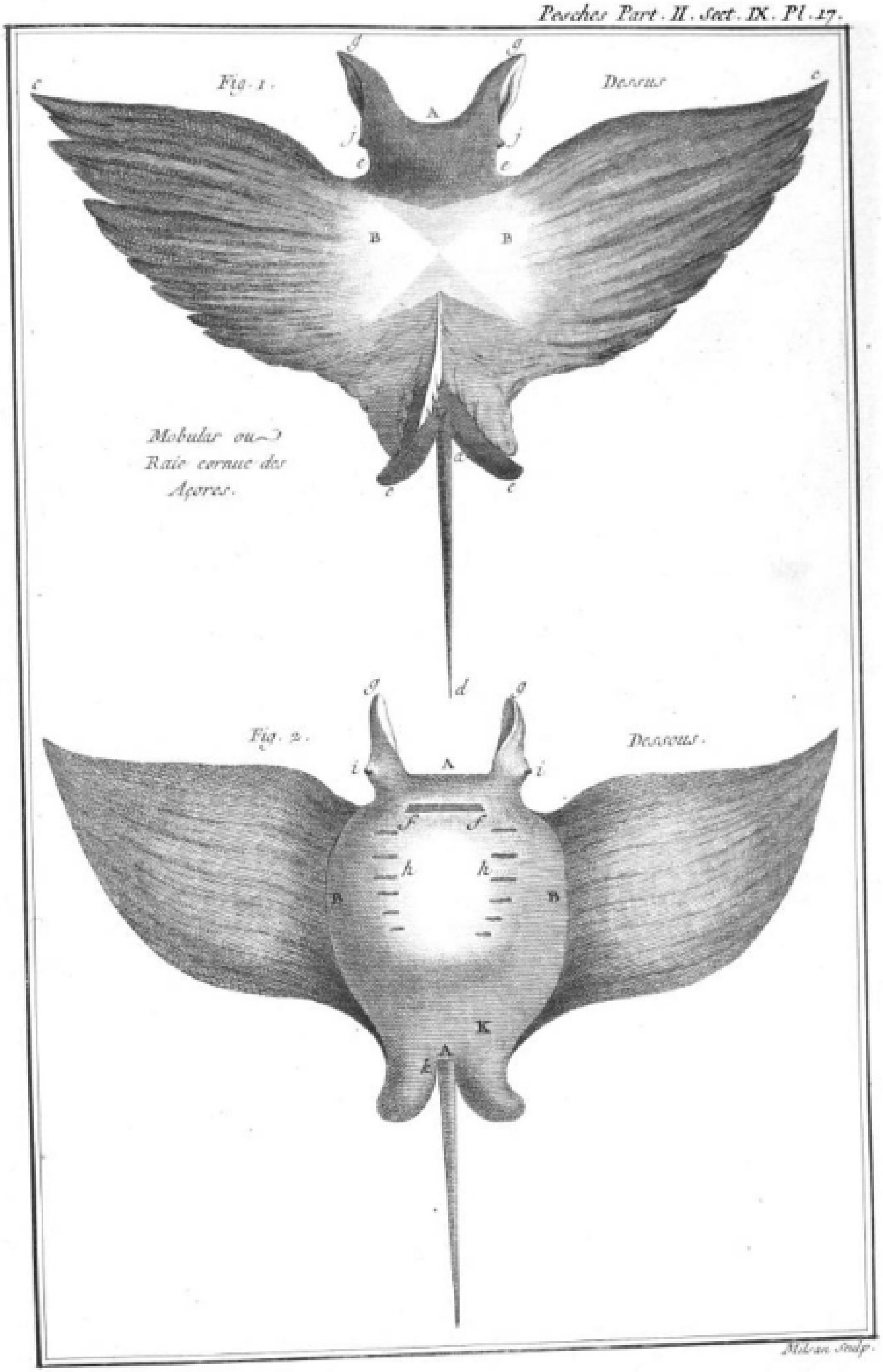


R.Medeiros © ImagDOP

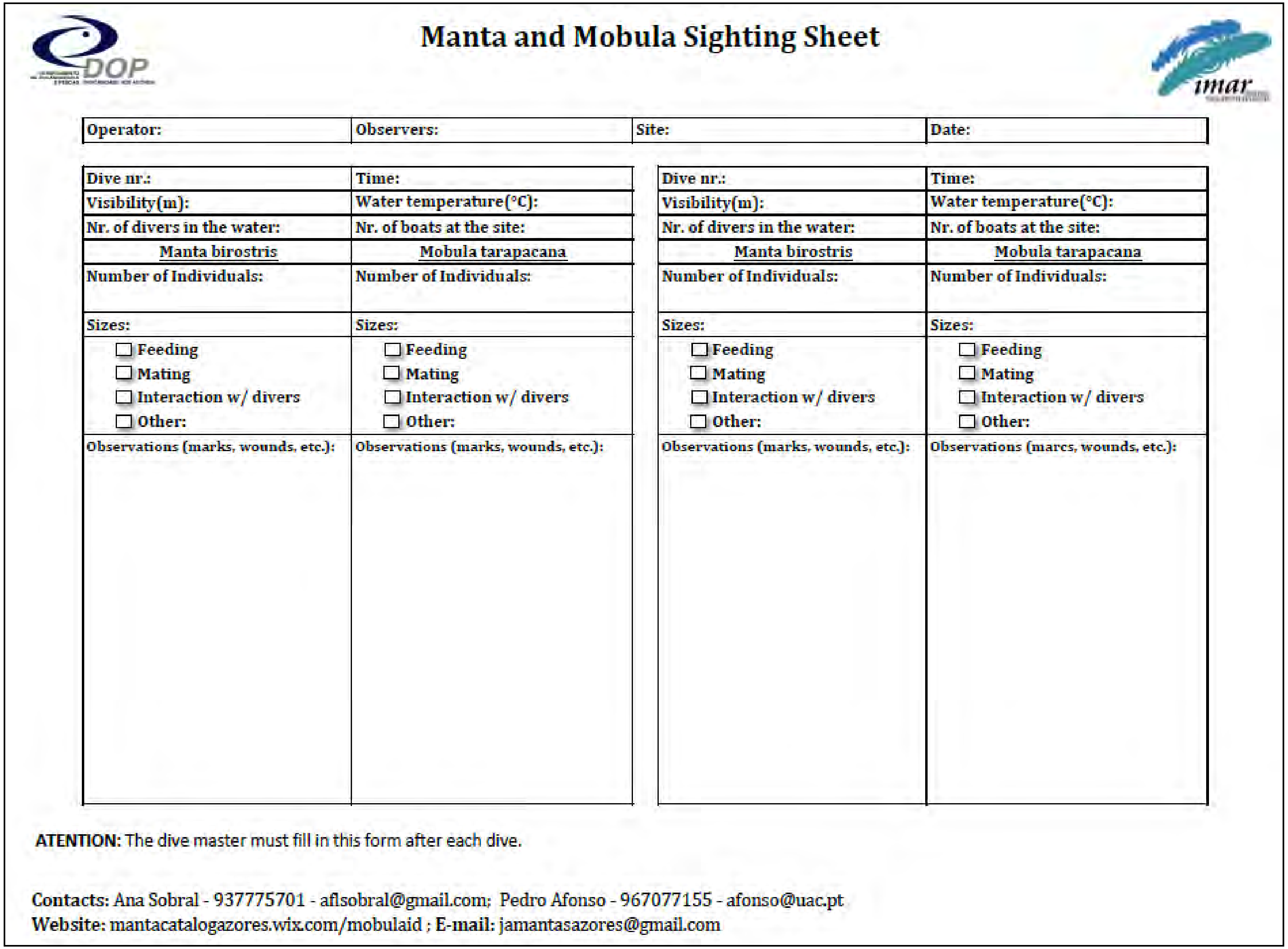
Derivative work based on IUCN Red List Data - IUCN Distribution maps (M.tarapacana, *M. mobular*, *M. japanica*, *M. birostris*) <www.iucnredlist.org>

Appendix II - Specimen of *Manta birostris* harpooned in Horta harbor in the 1950's

Appendix III - "Raie cornue" by Duhamel du Monceau (1777)



Appendix IV - Mobulid Sighting Sheet



Manta and Mobula Sighting Sheet

Operator:	**Observers:**	**Site:**	**Date:**

Dive nr.:	**Time:**	**Dive nr.:**	**Time:**
Visibility(m):	**Water temperature(°C):**	**Visibility(m):**	**Water temperature(°C):**
Nr. of divers in the water:	**Nr. of boats at the site:**	**Nr. of divers in the water:**	**Nr. of boats at the site:**
Manta birostris	**Mobula tarapacana**	**Manta birostris**	**Mobula tarapacana**
Number of Individuals:	**Number of Individuals:**	**Number of Individuals:**	**Number of Individuals:**
Sizes:	**Sizes:**	**Sizes:**	**Sizes:**
☐ Feeding ☐ Mating ☐ Interaction w/ divers ☐ Other:	☐ Feeding ☐ Mating ☐ Interaction w/ divers ☐ Other:	☐ Feeding ☐ Mating ☐ Interaction w/ divers ☐ Other:	☐ Feeding ☐ Mating ☐ Interaction w/ divers ☐ Other:
Observations (marks, wounds, etc.):	**Observations (marks, wounds, etc.):**	**Observations (marks, wounds, etc.):**	**Observations (marcs, wounds, etc.):**

ATENTION: The dive master must fill in this form after each dive.

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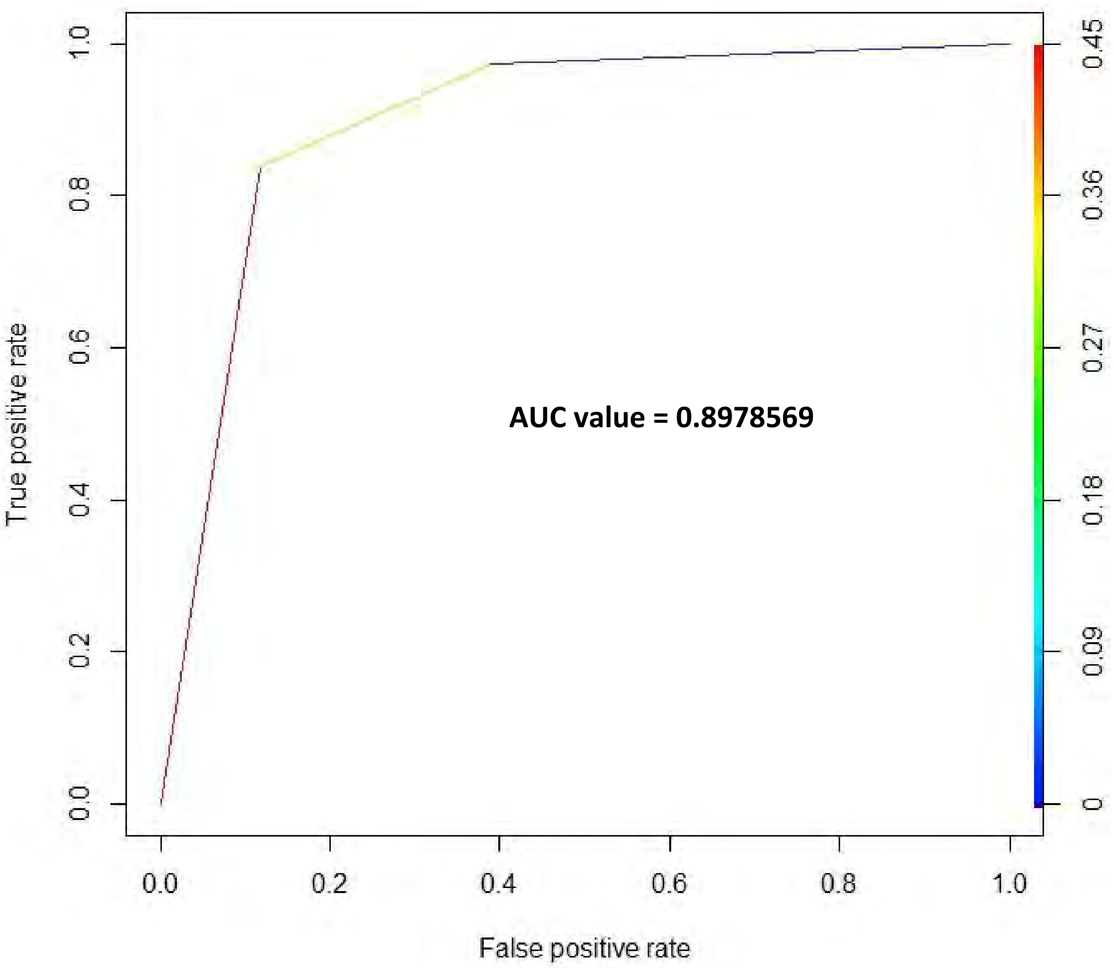
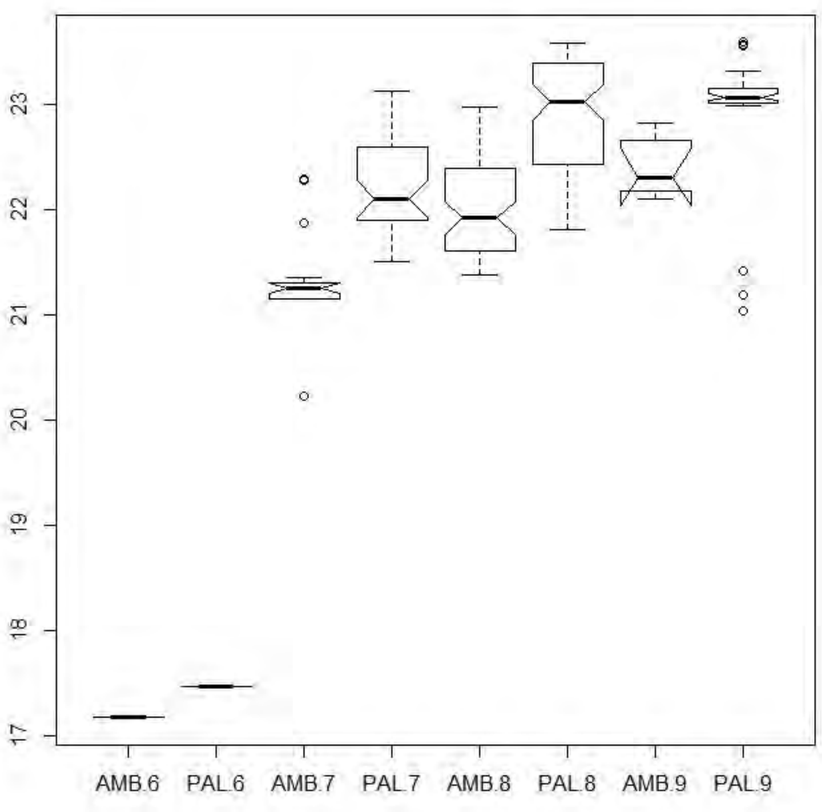
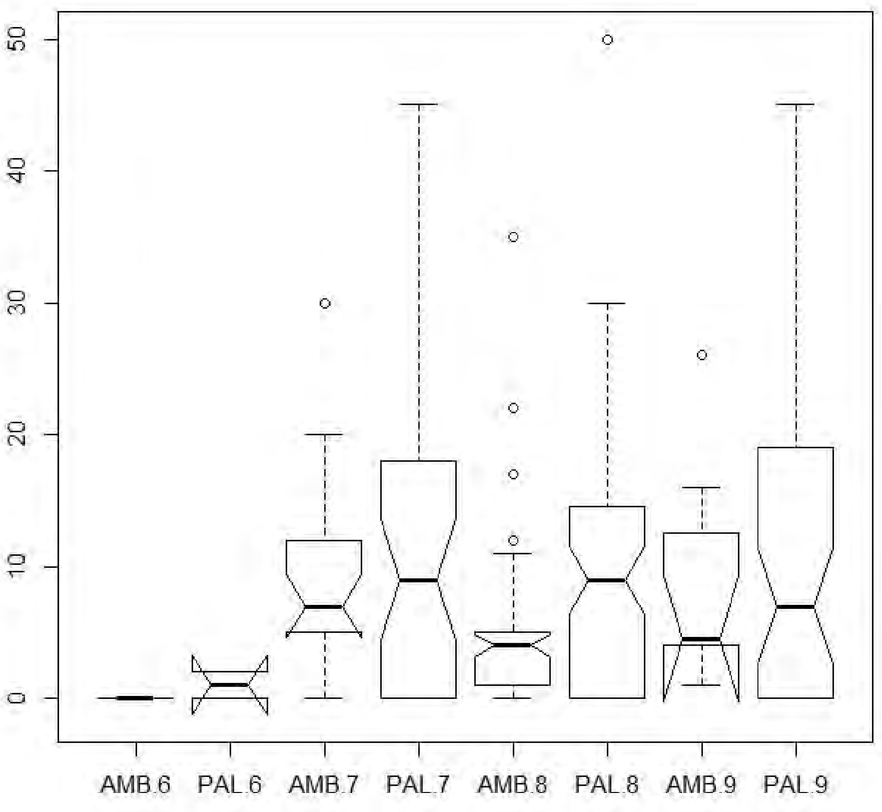
Website: mantacatalogazores.wix.com/mobulaid ; **E-mail:** jamantasazores@gmail.com

Appendix V - ROC curve and respective AUC value obtained in the model performance evaluation



Appendix VI - Notched Box-Plots comparison between the two main aggregation sites for temperature and mean of individuals per dive (2012/2013)





Legend:	**AMB - Ambrósio Reef**
	PAL - Princesa Alice Bank
	Numbers - Months